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Gut-Liver-Brain Axis and Alcohol Use Disorder: Treatment Potential of Fecal Microbiota Transplantation

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PURPOSE: Chronic alcohol use is a major cause of liver damage and death. In the United States, multiple factors have led to low utilization of pharmacotherapy for alcohol use disorder (AUD), including lack of provider knowledge and comfort in prescribing medications for AUD. Alcohol consumption has direct effects on the gut microbiota, altering the diversity of bacteria and leading to bacterial overgrowth. Growing evidence suggests that alcohol's effects on the gut microbiome may contribute to increased alcohol consumption and progression of alcohol-associated liver disease (ALD). This article reviews human and preclinical studies investigating the role of fecal microbiota transplantation (FMT) in ameliorating alcohol-associated alterations to the liver, gut, and brain resulting in altered behavior; it also discusses the therapeutic potential of FMT.

SEARCH METHODS: For this narrative review, a literature search was conducted in September 2022 of PubMed, Web of Science Core Collection, and Google Scholar to identify studies published between January 2012 and September 2022. Search terms used included "fecal microbiota transplantation" and "alcohol."

SEARCH RESULTS: Most results of the literature search were review articles or articles on nonalcoholic fatty liver disease; these were excluded. Of the remaining empirical manuscripts, very few described clinical or preclinical studies that were directly investigating the effects of FMT on alcohol drinking or related behaviors. Ultimately, 16 studies were included in the review.

DISCUSSION AND CONCLUSIONS: The literature search identified only a few studies that were directly investigating the effect of FMT on ALD or alcohol drinking and related behaviors. Largely proof-of-concept studies, these findings demonstrate that alcohol can alter the gut microbiome and that the microbiome can be transferred between humans and rodents to alter affective behaviors frequently associated with increased alcohol use. Other studies have shown promise of FMT or other probiotic supplementation in alleviating some of the symptoms associated with ALD and drinking. These results show that the implementation of FMT as a therapeutic approach is still in the investigatory stages.

KEYWORDS: alcohol; fecal microbiota transplant; alcohol-associated liver disease; gut-brain axis; gastrointestinal microbiome; microbiota; probiotics; behavior

Alcohol-associated liver disease (ALD) is a leading cause of morbidity and mortality in people with alcohol use disorder (AUD).¹ Alcohol exerts its effect on the liver through both direct and indirect pathways and can eventually lead to steatosis, steatohepatitis, fibrosis, hepatocellular carcinoma, and cirrhosis.² However, only approximately 10% to 20% of patients with ALD develop cirrhosis.² When decompensated cirrhosis develops, liver transplantation should be considered; however, a transplant may not be a feasible option for certain patients. Transplant eligibility is determined in a multidisciplinary fashion that includes a vigorous medical, psychosocial, surgical, and financial evaluation. Furthermore, the peri- and post-transplant periods can pose unique challenges to patients with underlying AUD. Individuals with chronic AUD are at risk for nutrient deficiencies, malnourishment, and sarcopenia.³ As such, they can enter transplant in a frail state that can predispose patients to infection, impaired wound healing, and sarcopenia (loss of muscle mass and function). In addition, transplant committees often require that patients engage in post-transplant alcohol cessation programs. To obviate the need for liver transplants, efforts to treat AUD and reduce craving should begin earlier in the disease course. In the United States, currently approved pharmacologic therapies for AUD include disulfiram, acamprosate, and naltrexone.⁴

Although pharmacological treatments exist, the treatment gap for AUD is higher than for any other mental disorder,⁵ and these treatments are prescribed only for a small percentage of patients with AUD. Several factors may contribute to the underuse of pharmacologic treatments for AUD, including lack of provider knowledge and comfort in prescribing these medications, low compliance with treatment among patients, and patient heterogeneity combined with the availability of only three approved medications. Thus, most patients with AUD—especially those with advanced AUD—are left untreated, and there is a need for additional, more effective therapies.

Newer therapeutic regimens include gut microbiome manipulation, which may modulate alcohol intake and drinking behavior.^{2,6} Growing evidence suggests that alteration of intestinal microbiota—which include not only bacteria but also fungi and viruses—contributes to the progression of excessive alcohol consumption and ALD, and this may form a therapeutic target.^{2,6} Alcohol consumption has both direct and indirect effects on the gut microbiota via alcohol metabolism, activation of inflammatory cascades, and alterations in the enteric nervous system.^{2,6} This suggests that by altering the gut microbiota, alcohol consumption may be modulated, slowing the progression of ALD.^{2,6}

The Impact of Alcohol on the Gut-Liver Axis

Gut-liver communication occurs both through the hepatic portal vein and the hepatic biliary system and can be influenced by the gut microbiota.⁶ Dietary nutrients absorbed from the gut can be carried directly to the liver via the portal vein. However, if the gut microbiota composition or gut barrier function is disrupted, other mediators or toxins can take the same route to disrupt liver homeostasis.⁷ The hepatic biliary system along with systemic circulation allows the liver to provide feedback to the gut via release of bile acids and other bioactive molecules.⁶

Alcohol consumption induces gut dysbiosis, an imbalance in gut microbiota, through several mechanisms. Chronic alcohol exposure decreases the production of mucus and antimicrobial peptides such as alpha-defensins and disrupts the intestinal barrier.^{2,8,9} This allows for translocation of lipopolysaccharide (LPS) and other endotoxins into the liver via the portal vein.¹⁰ LPS is produced by gram-negative bacteria and is one of the main factors in the pathogenesis of ALD. LPS activates toll-like receptors on the surface of Kupffer cells and induces pro-inflammatory signaling cascades, the release of cytokines, and, ultimately, hepatocyte damage.⁶ People with ALD often show higher levels of circulating pro-inflammatory mediators, such as LPS, interleukin 8 (IL-8), and IL-17.¹¹ Pro-inflammatory circulating cytokines were found to positively correlate with scores of depression, anxiety, and alcohol craving in active drinkers.¹² Moreover, inflammation markers were found to correlate with ALD severity.^{7,13}

Alcohol use could also alter gut microbiota by reducing production of short-chain fatty acids (SCFAs), which are beneficial fermentation products.¹⁴ SCFAs have anti-inflammatory and immune-modulatory activity and help maintain the intestinal barrier.⁶ Alcohol has been shown to decrease SCFA production, reflected in the fecal content of patients with alcohol-associated cirrhosis.¹⁵ This alcohol-induced disruption of bacterial metabolites (such as SCFAs, and bile acids among others) is a consequence of altered gut microbiota composition.

Alcohol use has been shown to result in bacterial overgrowth and dysbiosis. In general, alcohol reduces *Bacteroidetes*, *Clostridia*, and *Verrucomicrobiae* and leads to increases in *Proteobacteria*, *Gammaproteobacteria*, and *Bacilli*.¹⁶ Alcohol also has direct cytotoxic effect on hepatocytes; its metabolite acetaldehyde triggers pro-inflammatory signaling cascades and damages the epithelial barrier.⁹

The Impact of Alcohol on the Gut-Brain Axis

The gut microbiome also influences brain function and behavior through a variety of mechanisms and thus may be involved in the onset and severity of some psychiatric disorders, such as AUD.⁶ Research has suggested that bacterial metabolites can cross the blood-brain barrier via sensory nerves that innervate the gut.⁶ In patients with AUD, chronic low-grade inflammation leads to changes in pro-inflammatory mediators that can cross the blood-brain barrier to activate nuclear factor kappa B (NF- κ B) in glial cells, leading to neuronal damage.¹⁷ This concept was further confirmed in a study demonstrating that a single injection of LPS led to increases in tumor necrosis factor-alpha (TNF-alpha) in the liver and brain, promoted microglial activation, and induced degeneration of dopamine-secreting neurons.¹⁷ Although some bacterial species can produce neurotransmitters, such as gamma-aminobutyric acid (GABA) and dopamine, it is debated whether these neurotransmitters can cross the blood-brain barrier.⁶ It may be that signaling by the vagal nerve influences neurotransmitter production, which could impact behaviors associated with AUD, such as anxiety.⁶ However, anti-inflammatory cytokines such as IL-10 have been shown to reverse anxiety-like behavior related to substance use.¹⁸ Thus, multiple factors can influence the development of mood disorders. Vagal signaling may play a critical role in the onset and severity of AUD, as significant reduction in voluntary drinking was seen in rats that underwent vagotomy.¹⁹

Microbiota-derived ammonia can also impact the central nervous system.⁶ Due to poor hepatic clearance, high levels of ammonia are seen in some patients with ALD, which can reach the brain and lead to astrocyte death, brain damage, and cognitive alterations. Another potential mechanism how gut microbiota may affect brain function is through the previously discussed alcohol-related decrease in levels of SCFAs, such as butyrate.⁶ Butyrate is a potent inhibitor of histone deacetylases and thus can lead to epigenetic changes such as modulation of histone modifications.²⁰ Such epigenetic changes in the brain have the potential to impact current and future substance use by modulating addiction and reward networks.²¹ One study reported correlations between the gut microbiome and behavioral and neurophysiological traits that define AUD, such as measures of impulsivity and augmentations in striatal dopamine receptor expression.²²

This review presents the growing number of clinical and preclinical studies that are beginning to investigate the therapeutic role and mechanisms underlying fecal microbiota transplantation (FMT) in ALD and AUD (see Table 1). It is

important to note that not all patients with AUD have dysbiosis and/or increased intestinal permeability; the reason for this is unclear. A literature search using the terms “fecal microbiota transplantation” AND “alcohol” found very few studies that directly investigated the effect of FMT on alcohol drinking behavior. In addition, only a small number of articles showed the impact of FMT on affective behaviors that are frequently associated with excessive alcohol use. Some studies have shown promise in using gut microbial manipulation for alleviating some of the symptoms associated with ALD. Using these studies, the review outlines the interplay between the modulation of the gut microbiome, the gut-liver-brain axis, and AUD. The article also discusses why microbiome manipulation may be a promising therapeutic for ALD and proposes future directions.

Search Methods

A September 2022 search of the PubMed database using the search terms “fecal microbiota transplantation AND alcohol, NOT review” identified 71 articles that were published between January 1996 and September 2022. Among these articles, 16 were preclinical studies that used alcohol in their model (e.g., animals treated with alcohol, or animals treated with FMT from alcohol-exposed subjects). Most of the excluded articles described studies of non-alcohol-associated liver disease. Of the 16 included preclinical publications, six assessed the effects of FMT or the modulation of the microbiome on ALD. Six other articles investigated the role of modulation of the gut microbiome on alcohol-associated behaviors (e.g., sociability, anxiety, and depression) or drinking behavior, with some reporting changes in gene or protein expression in the brains of recipient animals. The other four articles not directly discussed below were excluded for the following reasons: one article was a commentary, and three were focused on alcohol's role on innate and adaptive immunity or pulmonary infection, not the gut-liver-brain axis. The 71 identified articles included 11 human/clinical studies, but four were excluded because they were either not related to alcohol or were not focused on microbial therapeutics. The remaining seven articles were human/clinical studies related to alcohol or cirrhosis (see Table 1).

A similar search strategy was employed in the Web of Science Core Collection database and Google Scholar. These searches identified 32 publications, and these were also contained in the PubMed dataset. Of note, none of these publications were published prior to 2016.

Table 1: Summary of Preclinical and Clinical Studies Assessing the Effects of Fecal Microbiota Transplant (FMT) on Alcohol-Related Outcomes

Study*	Subjects	Model	Main Finding
Ferrere et al. (2017) ²³	Mice	Signs of ALD lesions after Lieber-DeCarli diet	FMT prevented the development of alcohol-induced liver lesions, but the effect depended on the host microbiome.
Wrzosek et al. (2021) ³⁰	Mice	Signs of ALD after FMT from SAH patients	Pectin-FMT beneficially reshaped the GM, in an AhR-dependent manner.
Yu et al. (2020) ³¹	Mice	Signs of ALD lesions after Lieber-DeCarli diet with ethanol	FMT or LRP6-CRISPR improved GM diversity and composition to ameliorate ALD symptoms.
Yan et al. (2021) ³²	Mice	Signs of ALD lesions after Lieber-DeCarli diet with ethanol	TQE supplementation or TQE-FMT alleviated chronic alcohol-induced liver injury and markers of gut barrier dysfunction.
Yan et al. (2021) ³³	Mice	Signs of ALD lesions after Lieber-DeCarli diet with ethanol	UA had hepatoprotective effects and suppressed alcohol-induced oxidative stress and intestinal barrier disruption.
Guo et al. (2022) ³⁴	Mice	Acute ALD signs by ethanol lavage	Goji berries restored intestinal epithelial cell integrity and prevented acute liver injury induced by alcohol intake.
Xiao et al. (2018) ³⁹	Mice	FMT from noncontingent drinking mice	Alc-FMT transferred negative affective behaviors following withdrawal, altered brain gene expression, and reduced GM diversity.
Segovia-Rodriguez et al. (2022) ⁴⁰	Rats	FMT from ethanol-exposed rats (10 g/kg for 10 days)	Alc-FMT increased drinking and reduced locomotor activity, but this was dependent on antibiotics pretreatment.
Ezquer et al. (2022) ⁴²	Alcohol-preferring rats	Alcohol relapse drinking and LGG treatment	LGG modified the GM, reduced alcohol intake, and altered brain protein expression in a model of relapse drinking.
Bajaj et al. (2021) ⁵⁶	Humans	Patients with alcohol-associated cirrhosis and AUD	FMT reduced alcohol consumption and cravings and increased microbial diversity.
Philips et al. (2022) ⁵⁸	Humans	SAH hepatitis patients	FMT decreased alcohol relapse rates and increased time to relapse, increased beneficial GM diversity, and lowered rates of infections and hospitalizations with higher survival rates.
Philips et al. (2017) ⁵⁹	Humans	Open-label study of patients ineligible for steroid therapy	FMT recipients had higher transplant-free survival associated with reduction in pathogenic bacteria.
Sharma et al. (2022) ⁶⁰	Humans	Open-lab nonrandomized trial with severe alcohol-associated hepatitis with ACLF	FMT significantly reduced 28- and 90-day mortality and inflammatory cytokines.
Bajaj et al. (2017) ⁶²	Humans	Open-label randomized trial: outpatient men with cirrhosis and recurrent HE received FMT enema	Improved cognition along with increased microbial diversity.
Bajaj et al. (2019) ⁶⁵	Humans	Randomized, single-blind study: cirrhosis with recurrent HE receiving FMT capsules vs. placebo	FMT capsules were safe and improved duodenal mucosal diversity, dysbiosis, and objective measures of encephalopathy.
Philips et al. (2018) ⁶⁸	Humans	Comparative study between pentoxifylline, corticosteroid, nutritional therapy, and FMT	FMT had highest survival rates at 3-month follow-up by modulating GM composition and function and decreasing inflammatory pathways.
Zhao et al. (2020) ³⁸	Humans to mice	Cross-species Alc-FMT	Human to mouse Alc-FMT increased alcohol preference and negative affective behaviors and altered brain gene expression.
Wolstenholme et al. (2022) ⁴¹	Humans to mice	Cross-species Alc-FMT and treated Alc-FMT	Alcohol preference and intake were reduced in patients with AUD after receiving FMT, and this behavior was transmissible to mice; liver, intestine, and brain gene expression was altered in mice.
Leclercq et al. (2020) ⁴³	Humans to mice	Cross-species Alc-FMT	Human-to-mouse Alc-FMT increased depression-like behavior and lowered sociability; brain neurotransmitter and myelin gene expression were altered.

*Studies are ordered by citation number within each subject type.

Note: ACLF, acute-on-chronic liver failure; AhR, aryl hydrocarbon receptor; Alc, alcohol; ALD, alcohol-associated liver disease; AUD, alcohol use disorder; CRISPR, clustered regularly interspaced short palindromic repeats; FMT, fecal microbiota transplant; GM, gut microbiota; HE, hepatic encephalopathy; LGG, *Lactobacillus rhamnosus* Gorbach-Goldin; LRP6, low-density lipoprotein-related protein 6; SAH, severe alcohol-associated hepatitis; TQE, *Thymus quinquecostatus* Celak extract; UA, ursolic acid.

Results

Gut Microbiome and ALD: Preclinical Studies

In one of the seminal preclinical studies to investigate whether manipulation of the intestinal microbiome can prevent the development of ALD, Ferrere et al. showed that factors other than alcohol exposure are involved in the development of ALD.²³ In this study that compared mice raised in two different institutions and that were fed the same Lieber-DeCarli diet—a liquid diet for rodents that contains all dietary and hydration needs as well as alcohol to induce the pathogenesis of early-stage ALD—mice consumed similar amounts of alcohol, had similar liver weights, and initially had similar fecal microbiota composition. However, mice from one facility developed early signs of ALD while mice from the other facility did not. Following 10 days of the Lieber-DeCarli diet supplemented with 5% ethanol, the animals exhibited specific microbiota profiles that were associated with susceptibility or resistance to ALD symptoms. In the ALD-sensitive mice, the alcohol diet induced a decrease of cecal *Bacteroidetes* and *Proteobacteria* and an increase of *Actinobacteria* and *Firmicutes*. Thus, the ALD-sensitive mice had 50% less *Bacteroides* than did the ALD-resistant mice at the end of the 10-day period. To prove that the microbiota were likely responsible for ALD sensitivity or resistance, the researchers performed FMT by transferring fecal matter from ALD-resistant mice to ALD-sensitive mice. FMT or pectin (complex heteropolysaccharides that can modulate the growth of gut microbiota) treatment protected the susceptible mice from alcohol-induced depletion of *Bacteroides*, and the microbiomes of FMT-treated mice were similar to the microbiomes of ALD-resistant mice. Moreover, FMT prevented the development of alcohol-induced liver lesions.²³ This study was an important first step in showing that the endogenous microbiome influences an individual's susceptibility to ALD and that manipulation of the intestinal microbiome can prevent the development of alcohol-induced liver lesions and may be a strong therapeutic treatment strategy.

Following this seminal study, additional research groups investigated whether probiotics or dietary supplements that alter the microbiome can also reduce ALD symptoms.^{6,19,24-29} These studies generally demonstrated a positive outcome of treatment with probiotics on liver outcomes; however, as they did not use FMT, a detailed discussion is beyond the scope of this article. To mechanistically understand how pectin alters the intestinal microbiome and therapeutically treats ALD, mice received an FMT from patients with severe alcohol-associated hepatitis to establish alcohol-induced liver lesions in the context of the human microbiota.³⁰ The animals were then treated with pectin via FMT. Compared with control animals, pectin-treated mice showed a higher number of bacterial genes involved in carbohydrate, lipid, and amino-acid metabolism. Metabolomic analyses identified alterations in bacterial tryptophan

metabolism and increased indole derivatives, suggesting activation of the aryl hydrocarbon receptor (AhR) signaling system. AhR agonists simulated the effects of pectin in liver tissue and reversed the signs of ALD. Conversely, knock-out of the AhR gene in mice reduced the effects of beneficial microbiota on alcohol-induced liver injury. Finally, the researchers found decreased level of AhR agonists in patients with severe alcohol-associated hepatitis, suggesting that AhR may be a new therapeutic target in ALD.³⁰ These findings indicate that pectin reshapes the microbiome in the context of the human microbiota and not only prevents, but reverses, alcohol-induced liver injury in mice.

In another study, Yu et al. directly compared FMT to clustered interspaced short palindromic repeats (CRISPR) inactivation of low-density lipoprotein receptor-related protein 6 (LRP6), a co-receptor of the canonical Wnt/beta-catenin pathway, in their ability to ameliorate ALD symptoms.³¹ Knock-down of LRP6 by CRISPR, they hypothesized, would reduce Wnt signaling in hemopoietic stem cells to reduce their activation and, thus, improve the effects of liver fibrogenesis in their model of ALD. Rats fed an ethanol-containing Lieber-DeCarli diet to induce liver fibrosis and model early-stage ALD were then administered FMT from healthy rats or treated with LRP6-CRISPR. Histological and molecular assays revealed moderately improved liver histological markers in the FMT-treated rats that were accompanied by similar changes in fibrosis biomarkers. LRP6-CRISPR-treated mice showed similar improvements in liver histology and molecular markers, but with a greater effect size. Both LRP6-CRISPR and FMT treatment partially restored the composition of the gut microbiome and increased gut microflora diversity. Compared with untreated ALD-rats, LRP6-CRISPR and FMT both increased gut microbiota richness and diversity and resulted in a similar microbiota composition structure. Thus, principal coordinate analysis indicated that the gut microbiome of rats treated with LRP6-CRISPR and FMT overlapped and intersected with each other and with the control group. Specifically, LRP6-CRISPR and FMT each increased abundance of *Lactobacillus*. Thus, targeting the gut microbiome using samples from healthy rats or directly inactivating a member of the Wnt signaling pathway can improve the diversity and composition of the microbiome to ameliorate ALD symptoms.³¹

Three studies have used FMT procedures to show that gut microbiome remodeling may be a causal mechanism underlying the hepatoprotective effects and reductions in alcohol-induced liver injury of specific dietary enhancements, such as ursolic acid (UA) or Goji berries.³²⁻³⁴ UA, a bioactive constituent in teas, fruits, edible plants, and herbs, also has hepatoprotective activity.³⁵⁻³⁶ Using a model of chronic alcohol exposure to induce liver injury, Yan et al. showed that UA had not only hepatoprotective effects, but also suppressed alcohol-induced oxidative stress and intestinal barrier disruption.³³ An FMT study was performed to investigate the possible contribution

of gut microbiota manipulation in the beneficial effects of UA on alcohol-induced liver injury. Compared to mice receiving control-FMT, recipients of FMT from UA-consuming donors had a remodeled gut microbiome, less alcohol-induced gut dysbiosis, and reduced oxidative stress.³³ Alcohol-induced liver injury was also partly alleviated in UA-FMT recipient mice, suggesting the hepatoprotective activity of UA is transferable and can be partly attributed to gut dysbiosis correction.³³ Using a traditional Chinese medicinal plant, Goji berries, Guo et al. were able to restore the intestinal epithelial cell integrity and prevent acute liver injury induced by alcohol intake in mice.³⁴ To examine whether the Goji-modulated gut microbiota played a causal role on liver protection, an FMT experiment was performed in mice pretreated with antibiotics. FMT from donors that consumed Goji berries also protected against elevations in markers of acute alcohol-induced liver injury in recipient mice.³⁴ *Thymus quinquecostatus* Celak extract (TQE) is a species of thyme, widely used as food additive in Asia, that possesses hepatoprotective activity.³⁷ To investigate the mechanisms of TQE's liver protective effects in vivo, TQE supplementation alleviated chronic alcohol-induced liver injury and markers of gut barrier dysfunction in mice, likely through suppression of toll-like receptor 4-mediated inflammatory response and overproduction of reactive oxygen species.³² FMT studies using material from TQE-exposed donors also counteracted the alcohol-induced gut dysbiosis and partially ameliorated liver injury in the recipient mice, suggesting a causal role of the gut-liver axis in the hepatoprotective effects of TQE.³² Together, these studies show hepatoprotective effects of dietary supplements on acute or chronic alcohol-induced liver disease. FMT was used to show that these hepatoprotective effects can be transferrable and show causal role of the gut-liver axis in models of ALD.

Gut Microbiome and Alcohol Consumption: Preclinical Studies

Few studies have used preclinical models to directly investigate the role of the gut microbiome on alcohol drinking or alcohol-related phenotypes such as anxiety and depression.³⁸⁻⁴³ Some of these studies used cross-species FMT to establish causality of the gut microbiome on alcohol drinking and related behavior.^{38,40-42} Most of these six studies investigated the effect of microbiomes after alcohol exposure on similar outcomes and on gene or protein expression within the brain.^{38,39,41-43} In one of the first studies directly assessing the ability of the gut microbiome to contribute to the development of alcohol-related behaviors, transplantation of gut microbiota from alcohol-fed mice facilitated the development of depressive-like behavior in alcohol-naïve recipients.³⁹ In this model of noncontingent voluntary alcohol consumption, 4 weeks of escalating ethanol concentrations in the drinking water did not alter bacterial abundance but did change gut microbiota composition. Alcohol-exposed mice displayed signs of negative affective behavior

following alcohol withdrawal in two rodent models of depression (i.e., the forced swim and tail suspension tasks). Additionally, they exhibited decreased expression of the brain-derived neurotrophic factor (*Bdnf*) and corticotropin-releasing hormone receptor 1 (*Crhr1*) genes, as well as increased expression of the mu opioid receptor (*Oprm1*) gene in the hippocampus. Fourteen days of daily FMT from alcohol-drinking mice into alcohol-naïve recipients (Alc-FMT) increased their depression-like behavior, similar to that of the alcohol-drinking donors. These findings were interpreted as transference of behavioral signs of alcohol withdrawal-induced negative affect. Additionally, similar gene expression changes in *Bdnf*, *Crhr1*, and *Oprm1* found in alcohol-exposed mice were seen in the hippocampus of Alc-FMT mice. Finally, as seen in previous studies, both alcohol consumption and alcohol-FMT decreased the relative abundance of *Lactobacillus* and increased *Allobaculum* abundance.³⁹

To investigate whether changes in the gut microbiome are a cause or a consequence of alcohol drinking, Segovia-Rodriguez et al. treated alcohol-naïve rats with FMT from rats exposed to high (10 g/kg) ethanol doses (Alc-FMT), control-FMT, or phosphate-buffered saline control for 10 days.⁴⁰ Antibiotic pretreatment was also tested in each group given the known effects of antibiotics on gut microbiome diversity and alcohol intake. Alc-FMT rats without antibiotic pretreatment increased their alcohol intake as compared to rats given control buffer via oral gavage, while control-FMT mice had decreased alcohol intake in the drinking in the dark multiple scheduled access model. The increased intake in Alc-FMT rats occurred 2 weeks after the last fecal transplant. The researchers suggested that this could be due to an interaction between the new Alc-FMT microbiota received and alcohol consumption, producing a synergistic effect that favored bacteria most benefited by alcohol consumption. Antibiotic pretreatment caused a significant reduction in alcohol consumption, and neither Alc-FMT nor control-FMT had an effect on intake. Additionally, spontaneous locomotor activity was reduced in the Alc-FMT mice, and antibiotic pretreatment abolished this effect.⁴⁰ The findings suggest that, similar to the study by Ferrere et al.,²³ alcohol preference may be dependent on the content of the gut microbiome since antibiotic pretreatment abolished the effects of both control-FMT and Alc-FMT.⁴⁰

In another study not involving FMT, a dietary probiotic (*Lactobacillus rhamnosus* Gorbach-Goldin [LGG]) was used to modify the gut microbiota and assess alcohol intake in a rat model of alcohol relapse drinking.⁴² Rats selectively bred for alcohol drinking consumed alcohol for 5 weeks before they were administered antibiotics followed by daily LGG during a forced deprivation period. Antibiotic treatment alone led to a reduction (30%–40%) of early alcohol relapse drinking (i.e., within 60 minutes of restored access to alcohol), which increased to a 20% decrease of relapse drinking with 24-hour access. LGG treatment inhibited relapse drinking by 66% to

80%, as did administration of *N*-acetylcysteine + acetylsalicylic acid (NAC+ASA), which inhibits the alcohol-induced hyperglutamatergic condition. However, the combination of LGG and NAC+ASA during the deprivation period showed additive effects and virtually suppressed (90% inhibition) binge-like drinking after renewed access to alcohol. The reductions in alcohol deprivation effect were accompanied by differential alterations in protein levels in the nucleus accumbens. LGG treatment increased dopamine transporters, while NAC+ASA increased glutamate transporter levels (xCT and GLT-1), suggesting these dietary supplements are acting through different mechanisms to reduce alcohol relapse.⁴²

Role of Gut Microbiome in ALD: Clinical Studies

The gut microbiome—including bacteria, fungi, and viruses—has been implicated in the progression of liver disease in patients with underlying AUD; however, the few clinical studies that exist offer variable results.

Bacteria

A study by Maccioni et al. compared patients with ALD to healthy controls in an analysis of microbiota from feces and duodenal mucosa.⁴⁴ In this study, patients with hepatic inflammation and fibrosis had increases in potentially pathogenic bacterial taxa, including *Streptococcus*, *Shuttleworthia*, and *Rothia*. This supports the notion that alcohol exposure increases intestinal permeability and that this can potentially contribute to ALD development, though further studies are warranted. Patients with alcohol-associated cirrhosis exhibit an increase in oral microbial species (*Lactobacillus salivarius*, *Veillonella parvula*, *Streptococcus salivarius*, and *Bifidobacterium*) in stool compared to controls and patients with alcohol use disorder without cirrhosis.⁴⁵ Furthermore, pro-inflammatory bacteria such as *Enterobacteriaceae* were increased in patients with alcohol dependence, whereas butyrate-producing species (*Clostridiales*) were decreased.⁴⁵ Specifically, cirrhosis was significantly associated with the presence of *Bifidobacterium*. The *B. dentium* species, linked to alcohol-associated cirrhosis, has been shown to play an important role in GABA production.⁴

Another study analyzed microbiota in the colons of healthy controls as well as 48 patients with AUD with and without liver disease.⁴⁶ Mutlu et al. suggested that dysbiosis was worse in patients with alcohol-associated cirrhosis than in those with cirrhosis from other causes. Their study demonstrated that even in the early stages of ALD (without cirrhosis), changes in the gut microbiome occurred, such as reduced *Bacteroidetes* and increased *Proteobacteria*, and that levels of endotoxin were higher in patients who consumed alcohol.⁴⁶ Alcohol also has been shown to decrease commensal taxa in patients consuming alcohol, irrespective of their cirrhosis status.⁴⁷ It is suspected that increases in oral microbiota in the stool of patients with cirrhosis could be a result of the higher rate of oral infections, changes in

salivary microbiome, and use of acid-lowering medications in this population. One study also suggested that increasing severity of liver disease is associated with a relative decrease in *Akkermansia muciniphila*.⁴⁸ Therefore, changes to the gut microbiome may be influenced by the severity of liver disease.

Fungi

Studies in people with ALD have identified an increase in *Candida* species and a decrease in *Epicoccum*, *Galactomyces*, and *Debaryomyces*. Lower fungal diversity was observed in patients with ALD compared to healthy controls. In addition, these changes to the intestinal mycobiota were consistent among patients with varying degrees of ALD.^{49,50}

Viruses

The link between viruses and ALD is complex, and current knowledge is limited.^{51,52} In patients diagnosed with alcohol-associated hepatitis, phages with hosts as varied as *Escherichia*, *Enterobacteria*, and *Enterococcus* were increased, as were viruses such as Parvoviridae and Herpesviridae. Specifically, the severity of ALD was associated with the presence of *Staphylococcus* phages and Herpesviridae.⁵²

Effects of gut microbiota modulation

Several studies have assessed the effects of modulation of the gut microbiota on ALD. In a double-blind, placebo-controlled study, Amadiou et al. assigned a prebiotic (inulin) versus placebo for 17 days to 50 patients with ALD.⁵³ Patients receiving inulin had significantly higher markers of hepatic inflammation. In the subset of patients who had early ALD (as defined based on FibroScan and serum values), inulin administration was linked to an increase in *Bifidobacterium* and a decrease in *Bacteroides*, and again, higher levels of hepatic inflammation. These findings suggest that inulin may be able to alter the gut microbiome but not necessarily lead to clinically apparent changes to inflammation and that prebiotics may not be successful or beneficial for improvement in liver parameters. This study was limited, however, by sample size and a relatively short duration of inulin administration. Another study assessed the effects of LGG use in patients with moderately severe alcohol-associated hepatitis. LGG was associated with reduced short-term liver injury and reduction of alcohol consumption to abstinence levels at 6 months.⁵⁴

The role of SCFAs also has been explored in patients with ALD. A metabolomics analysis of fecal specimens demonstrated changes in tetradecane, reduced antioxidant fatty alcohols, and reduced SCFAs.⁵⁵ These alterations promote an environment prone to oxidative stress and increased gut permeability.

Role of FMT in AUD Treatment

Another area of interest has been the role of FMT in AUD treatment. Bajaj et al. demonstrated the safety of FMT in patients with alcohol-associated cirrhosis.⁵⁶ They concluded that FMT was associated with reduced alcohol consumption

and craving, with higher SCFA and microbial diversity. There was also a nonsignificant trend toward abstinence in the FMT group. Wolstenholme et al. further explored these mechanisms in a cross-species FMT design, mentioned below.⁴¹ A larger trial studying the clinical efficacy of FMT (NCT05548452) is currently enrolling.⁵⁷

To extend these findings, Philips et al. treated patients with severe alcohol-associated hepatitis with FMT and prospectively analyzed stool samples.⁵⁸ During a follow-up period of up to 3 years, patients who underwent FMT had lower rates of ascites, encephalopathy, infections, and hospitalizations with higher survival rates. Moreover, the FMT group demonstrated decreased alcohol relapse rates and longer time to relapse when compared to the standard-of-care group. Regarding microbiota composition, the FMT group demonstrated an increase in *Bifidobacterium* and a decrease in *Acinetobacter*, thus favoring a nonpathogenic milieu.

In patients with severe alcohol-associated hepatitis refractory to steroid therapy, liver transplantation, with the limitations described above, typically is the next treatment option. To address this, an open-label study was conducted with eight patients who were ineligible for steroid therapy and were treated with nasojejunal FMT for 1 week.⁵⁹ Patients treated with FMT were found to have higher transplant-free survival, associated with reduction in pathogenic bacteria, as compared to historical patients with steroid-refractory alcohol-associated hepatitis (87% vs. 33%). Specifically, at the 1-year follow-up, patients treated with FMT had fewer *Proteobacteria* and more *Actinobacteria*. Furthermore, they exhibited a relative increase in nonpathogenic bacteria such as *Enterococcus villorum* and *Bifidobacterium longum*. Notably, there was coexistence of recipient and donor species at 6 and 12 months after FMT.⁵⁹

The benefit of steroid treatments for severe alcohol-associated hepatitis is modest and limited to 28-day survival. Patients with alcohol-associated hepatitis have microbiota changes characterized by predominance of pathogenic species leading to immune dysregulation. Another study comparing FMT in 13 patients with standard of care (without steroids) in 20 patients reported a statistically significant increase in 90-day survival with FMT (54% vs. 25%, $p = 0.02$).⁶⁰ In an extension of these two studies,^{59,60} Pande et al. compared the safety and efficacy of healthy-donor FMT versus prednisolone therapy in patients with severe alcohol-associated hepatitis in an open-label study; each group included 60 patients.⁶¹ There was a statistically significant improvement in 90-day survival in the FMT group compared to the prednisolone group (75% vs. 57%, $p = .044$). Moreover, there were significantly fewer deaths related to infections in the FMT group, suggesting that FMT can be a safe alternative in patients with severe alcohol-associated hepatitis. However, further studies are needed with differing formulations.

FMT and Gut-Brain Axis Changes in ALD: Clinical Studies

A randomized controlled trial of FMT enema of men with cirrhosis and recurrent hepatic encephalopathy found that FMT increased microbiota diversity and improved cognition compared with standard of care.⁶² Using a rationally derived stool donor that was enriched in SCFA-producing *Lachnospiraceae* and *Ruminococcaceae*, this open-label randomized controlled trial with a follow-up period of 5 months found that with antibiotic pretreatment and administration of an FMT enema, the FMT was significantly better tolerated than the standard of care treatment.⁶² Whereas five patients in the standard of care group developed hepatic encephalopathy, none of the patients who had received FMT did. Other benefits associated with FMT included improved cognitive performance and changes in the microbiome, such as relative reduction in nonpathogenic taxa and increased microbial diversity.⁶² A subanalysis of the data showed that improvement in microbial function was linked to cognitive improvement.⁶³ Long-term follow-up of participants in this trial showed a continued relative increase in *Burkholderiaceae* and decrease in *Acidaminococcaceae* in the FMT group.⁶⁴ Furthermore, the FMT group had decreased rates of liver-related hospitalizations and hepatic encephalopathy recurrence, suggesting that FMT could significantly improve the clinical course of patients with cirrhosis and have a positive impact on quality of life as well as reduce the economic burden of hospitalization.⁶⁴

The effect of orally administered FMT on the gut-brain axis in cirrhosis also was studied in a phase I, randomized, placebo-controlled trial. Cognitive function improved after FMT, as measured by performance using the EncephalApp.⁶⁵ The study also confirmed the primary endpoint of safety and tolerability of the oral FMT capsules.⁶⁵ FMT also improved mucosal diversity, dysbiosis, and microbial function.⁶⁶

Cross-species studies of microbiota and AUD

In one of the first cross-species studies, the gut microbiota from patients with AUD increased alcohol preference, induced changes in anxiety-like and depression-like behaviors, and altered brain gene expression of recipient mice.³⁸ The fecal microbiome of men hospitalized for AUD (Alc-FMT), enriched in *Firmicutes* and *Bacteroidetes*, or of the control group of men who had abstained from alcohol for at least a year (control-FMT) was transplanted over 13 days into male mice that had been pretreated with antibiotics. Alcohol intake and preference for 4% or 8% alcohol in a two-bottle choice model were increased in the Alc-FMT mice compared to control-FMT mice. Alc-FMT mice also showed decreased anxiety-like behavior (indicated by increased time in the open arms of the elevated plus maze or in the center of an open field), increased depression-like behavior (indicated by immobility in the tail suspension test), and fewer

social interactions compared to control-FMT mice. With respect to gene expression, Alc-FMT mice showed reduced expression of the metabotropic glutamate receptor 1 (*mGluR1*) and *PKCε* mRNA in the nucleus accumbens and reduced *Bdnf* and GABA_A receptor (alpha-1GABA_AR) expression in the medial prefrontal cortex. Of note, antibiotic treatment prior to FMT modified some behaviors (e.g., decreased anxiety-like behavior) and increased locomotor activity in some tasks; however, social interactions and depressive-like behavior were not altered. Overall, the findings demonstrated that the gut microbiome of heavy drinkers can transmit some behavioral phenotypes similar to those seen in human drinkers.³⁸

A separate study extended these cross-species findings by investigating the effects of an alcohol-FMT on addiction-associated behaviors such as sociability, anxiety-like and depression-like behavior; on brain functions such as myelination, neurotransmission, and inflammation; and on intestinal bacterial load and permeability.⁴³ Mice that received an FMT from patients with AUD with severe symptoms of gut dysbiosis; high depression, anxiety, and alcohol craving; and low sociability also displayed deficits in a social preference task and higher depressive-like behavior; however, no differences were found in models of anxiety-like behavior.⁴³ This was accompanied by increased corticosterone levels compared to mice that received control FMT. Within the brains of Alc-FMT mice, expression of several neurotransmitter subunits and myelin-associated genes was altered, but pro-inflammatory cytokines, chemokines, and markers of microglial activation were increased in the striatum, but not the prefrontal cortex, suggesting a local inflammatory response. Total bacterial load in the intestine was reduced in Alc-FMT mice, suggesting a lower bacterial count. The relative abundance of *Bacteroidetes* was decreased, while the abundance of *Firmicutes* was increased, similar to what is found in patients with AUD. This was accompanied by indicators of increased intestinal permeability, including decreased expression of markers of defense immune mechanisms, loss of intestinal homeostasis (reduced expression of *Reg3g* and *Lcn2*), modification of tight junction expression, and atrophy of the mucosal structure (reduced villous height and crypt depth in the ileum). Interestingly, the study suggested that the behavioral changes may not have been induced through a peripheral inflammatory response, but rather may have been a result of blood metabolite changes. Although the FMT-treated mice were not exposed to alcohol, increased portal vein ethanol concentrations were found in Alc-FMT mice. This suggests that the Alc-FMT mice likely were colonized by higher amounts of alcohol-producing bacteria such as *Clostridium*, *Lactococcus*, *Turicibacter*, and *Akkermansia*.

In a third study using a cross-species FMT design, changes in alcohol preference and intake that occurred in patients with AUD after receiving a fecal transplant were transmissible by

FMT to germ-free mice (i.e., which had been treated to lack any microorganisms).⁴¹ The study used fecal samples from a randomized clinical trial that demonstrated reduced alcohol craving and consumption after fecal transplantation in patients with severe AUD. Germ-free male mice then received either stool or sterile supernatants (the nonmicrobial buffer collected from around the stool pellet) collected from trial participants pre-/post-fecal transplant. Mice colonized with postfecal transplant stool but not supernatants exhibited reduced alcohol acceptance, intake, and preference compared with mice receiving pre-fecal transplant stool. Analyses of gene expression in the liver, intestine, and prefrontal cortex revealed that a majority of the differentially expressed genes—which were related to immune response, inflammation, oxidative stress response, and epithelial cell proliferation—occurred in the intestine rather than in the liver or prefrontal cortex.⁴¹ These findings suggest a potential for therapeutically targeting gut microbiota and the microbial-intestinal interface to alter gut-liver-brain axis and reduce alcohol consumption in humans.

Conclusions and Future Directions

The studies reviewed here demonstrate the role of the gut microbiome in AUD and ALD. They suggest that the use of probiotics, prebiotics, or FMT warrants further investigation as therapeutic approaches for these conditions. In clinical and preclinical studies, excessive drinking or exposure to high levels of alcohol was associated with dysbiosis, intestinal permeability, and changes in immune response (see Table 1). Clinical studies have suggested that use of FMT in patients with AUD improved SCFA levels, which may reduce inflammation and aid in preventing additional liver damage.⁵⁶ FMT also has recently been used in preclinical models to manipulate the gut liver axis with certain dietary supplements to alleviate signs of acute or chronic alcohol-induced liver disease.³²⁻³⁴

Preclinical studies have used probiotics, prebiotics, or FMT from animals that had consumed those substances to improve alcohol-related behaviors such as alcohol consumption, providing evidence that gut microbiome manipulation may improve not only inflammation-related markers, but alcohol-related behaviors as well.^{41,42} Several of the preclinical studies identified in this narrative review were proof-of-concept FMT studies to show that behaviors such as anxiety-like and depression-like phenotypes and alcohol drinking can be induced by FMT from a donor with a history of alcohol exposure.^{38-40,67} However, the body of evidence in regards to FMT studies currently is still limited.

Clinical data suggest that with strict donor screening protocols, FMT appears to be safe, with low incidence of

reported adverse events; however, long-term prospective data are still lacking.⁵⁶ Currently, FMT only is indicated for recurrent *Clostridium difficile* infection, but the mounting evidence from preclinical and clinical studies suggests that it may be a therapeutic option for ALD as well.⁴⁷ Several challenges exist, however, including the need to define a healthy stool donor, determine the optimal route of FMT administration, and find effective ways to validate endpoints. Changes in the microbiome can lead to progression of ALD by maintaining a state of localized and systemic inflammation.⁴⁶ Also, although human studies support the role of healthy-donor FMT in improving transplant-free survival, reducing rates of infections, and even ameliorating craving for alcohol in patients with AUD, clinical data are limited by small sample sizes. Moreover, these studies often have focused on advanced ALD, and the benefit of FMT intervention on the liver and on psychological parameters in patients with less advanced forms of ALD remains unknown.

A study that compared pentoxifylline, corticosteroid, and nutritional therapy with FMT found that patients who received FMT via nasojunal route had the highest survival rates of all groups at 3-month follow-up, suggesting a possible mortality benefit for FMT. FMT also led to improvement in clinical parameters while modulating and targeting inflammatory pathways such as LPS.⁶⁸ Therefore, when compared to other medical interventions such as steroids that have side effects, FMT could potentially serve as a relatively benign treatment modality. However, a major limitation to this study was inclusion of only male patients, which raises the question of generalizability.

Understanding of the role of the microbiome in progression of ALD is growing rapidly. However, questions remain regarding its exact role in the pathophysiology of liver disease and in therapeutic strategies. Although abstinence remains the cornerstone of therapy for AUD, the point at which abstinence can modulate changes at the microbiome level is poorly understood. Future studies should focus on the composition and function of the microbiome and its byproducts at the various stages of the ALD spectrum. This will require large, prospective clinical trials with a diverse population sample. Although preclinical studies have suggested that manipulation of the gut microbiome may alter drinking behavior, few clinical trials of microbiome-targeted interventions have assessed drinking behavior as an endpoint. Such studies would be important in assessing the impacts of FMT on AUD outcomes outside of ALD. The gut-brain axis also is known to play a critical role in AUD, as demonstrated by individuals with AUD having increased gut permeability that leads to higher rates of depression, anxiety, and alcohol craving after a short period of abstinence.¹² These observations suggest the microbiota can modulate cravings and other psychiatric comorbidities associated with addictive behaviors.

Dysbiosis occurs in some patients across the spectrum of liver disease severity, and changes in the microbiome are evident at the bacterial, viral, and fungal community level. Probiotics may address these changes; however, although probiotics have been associated with improvements in direct and indirect markers of disease severity in patients with ALD, most studies only had a small sample size, had a heterogeneous trial design, and were rarely reproduced. Targeting bacterial metabolites also could be promising, and given that patients with ALD have reduced levels of total fecal bile acids and SCFAs, addressing these changes could be a potential therapeutic target.

In summary, this review highlights the fact that, to date, few studies have evaluated FMT as a therapeutic option for reducing symptoms associated with excessive alcohol use. However, the number of such investigations is growing, and early studies have shown remarkable potential with a good safety profile. Although additional, larger clinical studies still are needed to determine whether FMT is an effective therapeutic strategy, evidence to date suggests that targeting the gut microbiome could be a promising treatment option for decreasing the risk of relapse in AUD patients and ameliorating the severity of ALD.

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Alcohol and Skeletal Muscle in Health and Disease

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PURPOSE: Alcohol-related myopathy is one of the earliest alcohol-associated pathological tissue changes that is progressively exacerbated by cumulative long-term alcohol misuse. Acute and chronic alcohol use leads to changes in skeletal muscle mass and function. As discussed in this evidence-based review, alcohol-mediated mechanisms are multifactorial with effects on anabolic and catabolic signaling, mitochondrial bioenergetics, extracellular matrix remodeling, and epigenomic alterations. However, systematic studies are limited, especially regarding the acute effects of alcohol on skeletal muscle.

SEARCH METHODS: This review focuses on peer-reviewed manuscripts published between January 2012 and November 2022 using the search terms “alcohol” or “ethanol” and “skeletal muscle” in MEDLINE, PubMed, and Web of Science using EndNote reference management software.

SEARCH RESULTS: Eligible manuscripts included full-length research papers that discussed acute and chronic effects of alcohol on skeletal muscle mass and function in both clinical and preclinical studies. The review also includes alcohol-mediated skeletal muscle effects in the context of comorbidities. The three databases together yielded 708 manuscripts. Of these, the authors excluded from this review 548 papers that did not have “alcohol” or “muscle” in the title and 64 papers that were duplicates or did not discuss skeletal muscle. Thus, of all the manuscripts considered for this review, 96 are included and 612 are excluded. Additionally, relevant papers published earlier than 2012 are included to provide context to the review.

DISCUSSION AND CONCLUSIONS: Both acute and chronic alcohol use decrease protein synthesis and increase protein degradation. Alcohol also impairs mitochondrial function and extracellular matrix remodeling. However, there is a gap in the literature on the known alcohol-mediated mechanisms, including senescence, role of immune activation, and interorgan communication, on the development of alcohol-related myopathy. With increased life expectancy, changing alcohol use patterns, and increasing frequency of alcohol use among females, current observational studies are needed on the prevalence of alcohol-related myopathy. Additionally, the compounding effects of acute and chronic alcohol use on skeletal muscle with aging or exercise, in response to injury or disuse, and in the context of comorbidities including diabetes and human immunodeficiency virus (HIV), call for further investigation. Though evidence suggests that abstinence or reducing alcohol use can improve muscle mass and function, they are not restored to normal levels. Hence, understanding the pathophysiological mechanisms can help in the design of therapeutic strategies to improve skeletal muscle health.

KEYWORDS: alcohol; muscles, skeletal; comorbidity; protein synthesis; proteolysis; metabolism; mitochondria

Alcohol misuse is the most common form of substance misuse and is associated with liver, cardiovascular, and metabolic diseases as well as with infections and cancers.¹ Although an estimated 20% to 25% of people who drink heavily develop alcohol-related liver disease,² 40% to 60% of people with alcohol misuse have alcohol-related myopathy.³ Evidence that alcohol use leads to skeletal muscle (SKM) weakness, even in the absence of neuropathology, was independently documented in the 1800s by James Jackson⁴ and Magnus Huss.⁵ More empirical reports that alcohol or its metabolites could directly or indirectly lead to adaptations of SKM mass and function and that there are differences with acute and chronic alcohol misuse were formulated in the 1950s and 1960s.⁶⁻⁸

SKM mass is maintained by the balance of anabolic (protein synthesis) and catabolic (protein breakdown) signaling. Major anabolic stimuli—including amino acids, insulin, insulin-like growth factor 1 (IGF-1)—and mechanical loading promote protein synthesis by converging on the mechanistic/mammalian target of rapamycin (mTOR) signaling pathway (reviewed by Bourgeois et al.⁹ and Steiner et al.¹⁰). SKM protein breakdown occurs through activation of the ubiquitin proteasome pathway (UPP) and SKM-specific ubiquitin ligases or atrogenes; atrogen-1 (also known as muscle atrophy F-box, or MAFbx) and muscle RING-finger protein-1 (MuRF-1) are often used as markers of UPP activation. The second major protein breakdown pathway is activation of the autophagic-lysosomal system that degrades misfolded proteins by formation of a phagophore followed by engulfing of degraded proteins.⁹⁻¹² Myofibers, their structural components, and the extracellular matrix intricately communicate to maintain SKM structure. Though most adult muscle growth is driven by hypertrophy of existing myofibers, muscle stem cells (satellite cells) contribute to myofiber regeneration, especially in response to injury or atrophy.^{13,14} Finally, being highly dynamic and with high energy demands, SKM relies heavily on mitochondria for bioenergetic demands, redox balance, and programmed death signaling (reviewed by Bourgeois et al.⁹). Evidence suggests that alcohol significantly affects all these major attributes of SKM mass and function, as discussed in this review.

Most current knowledge of the systematic cellular and molecular alterations seen with alcohol-related myopathy is from preclinical rodent studies.¹⁵ However, the pathophysiological mechanisms of alcohol misuse are complex and are influenced by genetics, sex, lifestyle factors, psychosocial determinants, health comorbidities, and patterns of alcohol use.¹⁶ Published literature in the 1990s and early 2000s provided epidemiological evidence for the prevalence of alcohol-related myopathy.^{17,18} With the changing patterns of alcohol use,^{19,20} changes in dietary and lifestyle choices,²¹ increase in life expectancy,²² and increasing frequency of

alcohol misuse among females,¹⁹ there is a need for recent studies on the prevalence and the disease course of alcohol-related myopathy. Moreover, despite evidence for the high prevalence of alcohol-related myopathy, there is limited literature on its effects on aging, whole body metabolism, response to injury or atrophy, and exercise.

One of the challenges in assessing the effects of alcohol consumption on skeletal muscle and other systems is the sometimes-inconsistent definition of drink sizes and drinking levels, particularly when comparing studies conducted in different countries (see Table 1). The National Institute on Alcohol Abuse and Alcoholism provides information about drinking patterns for adults and defines a standard drink in the United States,²³ although standard drink definitions sometimes differ in other countries.²⁴ The World Health Organization maintains a global database that provides information on several alcohol-related topics, including levels and patterns of alcohol use.¹⁶

Search Methods

This review is based on a literature search of three databases—PubMed, MEDLINE (OvidSP), and Web of Science's Core Collection (Thomas Reuters)—using the EndNote program. The literature search included articles published between January 2012 and November 2022. The search terms used were “ethanol” and “skeletal muscle” as MeSH terms in PubMed and title, abstract, and keywords for MEDLINE and Web of Science.

Results of the Literature Search

The three databases yielded 708 papers, and titles were screened to include only papers that had the terms alcohol or ethanol, and muscle in the title. With this, 548 manuscripts were excluded. Also excluded were 64 manuscripts that were either duplicates across the three databases or manuscripts that did not discuss SKM. Thus, 96 manuscripts are included in the discussion. Eligible studies were those that included acute or chronic effects of alcohol on SKM mass and function in both clinical and preclinical studies.

This review briefly discusses the salient literature related to alcohol effects on SKM prior to 2012 to provide context. Following this, database search results are organized based on acute and chronic effects of alcohol on SKM metabolic signaling pathways as well as structural and functional adaptations. The review also discusses the effects of alcohol on SKM in the context of some comorbidities, including HIV, pain, cancer, and disuse. Finally, the review discusses gaps in literature and identifies some of the salient future directions that can be pursued.

Table 1. Alcohol Consumption: Drink Sizes and Drinking Levels in Select Countries

Defining Drinking Levels				
	United States	Iceland and United Kingdom	China, France, Ireland, and Spain	Austria
Standard drink ²³	0.6 fluid oz or 14 g pure alcohol (12 oz regular beer, 5 oz wine, or 1.5 oz distilled spirits)	8 g pure alcohol	10 g pure alcohol	20 g pure alcohol
Binge drinking ²³	Pattern of drinking that brings blood alcohol concentration to 0.08% or higher in 2 hours (four or more U.S. standard drinks in women; five or more U.S. standard drinks in men)			
Heavy drinking ²³	<i>Women:</i> Consuming more than three drinks per day or more than seven drinks per week <i>Men:</i> Consuming more than four drinks per day or more than 14 drinks per week			
World Health Organization: Alcohol Consumption Categories ¹⁶				
Abstainer	Consumed no alcohol in lifetime or in past 12 months			
Former drinker	Previously drank alcohol but no consumption in the past 12 months			
Consumer	Consumed alcohol in the past 12 months			
Heavy episodic drinker	Consumed 60 g or more alcohol on at least one occasion in the past 30 days			

Results of the Reviewed Studies

Overview

The effect of alcohol misuse on SKM mass and function is referred to as acute and chronic alcohol-related myopathy. Acute alcohol-related myopathy presents clinically as breakdown of damaged muscle tissue (rhabdomyolysis)²⁵ and is the most frequent cause of nontraumatic rhabdomyolysis.²⁶ This can occur even with a single binge-drinking session (blood alcohol concentration 0.08 g/dL), and symptoms generally resolve after 1 to 2 weeks of abstinence.²⁷ Alcohol-related rhabdomyolysis predominantly affects muscles of the pelvic and shoulder girdles and is associated with increased circulating levels of creatine kinase and myoglobin, compartment syndrome particularly of the lower extremities, and, in severe cases, acute renal failure.^{25,28} Evidence also indicates that people who have chronic alcohol-related myopathy can be prone to rhabdomyolysis following an alcohol binge and can show signs of episodic myalgia, muscle weakness, and dark-colored urine.²⁶ An estimated 0.5% to 2.0% of people with alcohol misuse present with acute alcohol-related myopathy.²⁶ Apart from rhabdomyolysis, acute alcohol affects SKM anabolic protein synthesis pathways²⁹⁻³⁴ and catabolic pathways.³⁵

Chronic alcohol-related myopathy (CAM) is the most frequent form of alcohol-related myopathy with an overall prevalence of 2,000 per 100,000 people with alcohol misuse.¹⁷ Clinical signs associated with CAM are progressive proximal muscle weakness, type II fiber (fast twitch glycolytic fibers) atrophy, pain, and myotonia.^{26,36} Onset of CAM is associated

with cumulative lifetime or long-term high-dose alcohol consumption.^{17,26} Thus, clinical manifestations of CAM are seen in older individuals (ages 40 to 60) and are more common in people with other comorbidities, with ~ 50% of people with alcohol-related liver cirrhosis and ~ 80% of people with alcohol-related cardiomyopathy presenting with CAM.^{26,37,38} CAM is characteristically marked by decreased protein synthesis, dysregulation of proteins in the insulin signaling pathway and the mTOR complex 1 (mTORC1) pathway, and dysregulation of myofibrillar and sarcoplasmic proteins.³⁹⁻⁴¹ In addition, chronic alcohol intake increases SKM catabolic signaling.^{39,42,43} The effects of chronic alcohol use on SKM mitochondrial function are not clear. Early studies in people with CAM showed a lack of association with mitochondrial energy supply.⁴⁴ However, other studies indicate that chronic alcohol consumption increases SKM glycogen and lipid storage, with megamitochondria and dilated sarcoplasmic reticulum.⁴⁰

These alcohol-associated molecular changes could potentially affect muscle strength. In people with a history of alcohol misuse, mean strength increased from baseline over a 5-year abstinence period, but remained significantly weaker than age-matched controls, with more than half of them still showing histological signs of myopathy.³ People who consume a single drink (1 g ethanol/kg body weight) showed a significant decrease in peak strength even 36 or 60 hours post-exercise, indicating that alcohol use accentuates the loss of both dynamic and static strength seen with eccentric exercise.⁴⁵ However, consumption of low-dose alcohol (0.5 g ethanol/kg body weight) after eccentric muscle exercise does not affect muscle force.⁴⁶

Thus, epidemiological and molecular data from seminal work provide evidence that both acute and chronic alcohol use adversely affect SKM and clinically manifest as rhabdomyolysis or weakness of the proximal muscles, respectively. Building on this clinical and preclinical research, and with advances in cellular and molecular assays over the past decade, significant strides have been achieved on elucidating the pathophysiological adaptations that lead to acute and chronic alcohol-related myopathy.

Acute Effects of Alcohol on SKM

Acute alcohol effects on SKM anabolic signaling

The major SKM anabolic pathway is the mTORC1 pathway leading to muscle protein synthesis (Figure 1). Anabolic stimuli (e.g., insulin, IGF-1) activate phosphoinositide 3 kinase, which phosphorylates and activates protein kinase B (Akt). Akt phosphorylates and inactivates tuberous sclerosis complex 1 and 2 (TSC1, TSC2) through inhibition of TSC2 guanosine-triphosphate hydrolase (GTPase) activity. TSC2 GTPase removes GTP from Ras homolog enriched in brain (Rheb). TSC2 inactivity allows GTP-bound Rheb to accumulate, which stimulates mTORC1 activity. Downstream of mTORC1, S6 kinase 1 (S6K1) is activated, allowing for the activation of ribosomal protein S6. Additionally, eukaryotic initiation factor 4E-binding protein (4E-BP1) is inactivated downstream of mTORC1. Both activation of S6K1 and inactivation of 4E-BP1 increase translational machinery, allowing protein synthesis to increase. Amino acids also regulate the mTORC1 pathway. When amino acids (e.g., leucine) bind to sestrin 1/2, sestrin dissociates from GTPase activating proteins (GAP) toward Rags complex 2 (GATOR2). This decreases the inhibitory effect of GATOR2 on GATOR1. Together, there is a conformational change to Ragulator, which has late endosomal/lysosomal adaptor and MAPK and mTORC1 activators (LAMTOR) increasing GTP-bound RagA and guanosine diphosphate-bound RagC that ultimately activates mTORC1. mTORC1 is also regulated by several other upstream proteins, including AMP-activated protein kinase (AMPK) and regulated in development and DNA damage response 1 (REDD1).^{10,47}

In vitro studies in C2C12 myotubes showed that 100 mM ethanol modulates Rag and AMPK/TSC2/Rheb signaling, decreasing the anabolic effects of leucine.⁴⁸ A single intraperitoneal alcohol injection (3 g/kg) in either fasted or fed male mice prevented the increase in fed-state protein synthesis and phosphorylation of ribosomal protein S6 kinase at threonine 389 (S6K1^{Thr389}).^{49,50} In the fed state, alcohol administration decreased the association of Raptor and RagC with immunoprecipitated LAMTOR1 and increased sestrin1–GATOR2 and vacuolar-type ATPase V1 association with LAMTOR1 within 1 hour of administration, dysregulating protein–protein interactions of the Rag-Ragulator complex.⁵⁰ However, a study using the same alcohol administration paradigm in REDD1 knockout mice indicated that REDD1 may not play a

role in alcohol-mediated decreased protein synthesis, but may be involved in UPP-mediated protein breakdown.⁵¹

Male mice that were injected with 3 g/kg alcohol intraperitoneally and administered electrically stimulated muscle contractions decreased stimulation-induced total rate of protein synthesis and blunted the phosphorylation of S6K1 (Thr⁴²¹/Ser⁴²⁴ and Thr³⁸⁹) and its substrate rpS6 (Ser^{240/244}), indicating that acute alcohol administration dysregulates stimulation-induced changes in protein synthesis and mTORC1 signaling.⁵² A small clinical study among trained male and female participants who were administered alcohol (1.09 g/kg fat-free body mass) 10 to 20 minutes after an acute heavy resistance exercise trial found decreased exercise-induced phosphorylation of the mTOR(Ser²⁴⁴⁸) and S6K1 (Thr³⁸⁹) in males, with no changes in females.⁵³ Other studies also showed that alcohol (1.5 g/kg body mass) consumed after exercise decreased mTORC1 signaling and protein synthesis.⁵⁴

Acute alcohol-mediated alterations on the endocrine profile are thought to contribute to impaired SKM anabolic signaling. An alcohol binge post-exercise decreased testosterone to cortisol ratio⁵⁵ or maintained increased testosterone levels post-exercise in healthy young males.⁵⁶ These changes in the hormonal profile can potentially lead to decreased anabolic and increased catabolic signaling, thus affecting SKM protein balance; however, definitive studies are needed to prove this. Moreover, the effects of alcohol on estrogen signaling and their impact on SKM mass and function in both males and females remain largely unknown.

Acute alcohol effects on SKM catabolic signaling

A single intraperitoneal injection of alcohol (3 g/kg) blunted the expected fed-state decrease in autophagy in mice, with no significant effects on UPP.⁴⁹ A single intraperitoneal injection of alcohol (5 g/kg) in female mice demonstrated altered expression of genes implicated in fatty acid oxidation, including peroxisome proliferator-activated receptor (PPAR) alpha and PPAR-beta, AMPK, and cluster of differentiation 36 (CD36), as well as of genes involved in protein breakdown, such as MuRF1, Krüppel-like factor 15 (Klf15), and branched chain amino acid transaminase 2 (Bcat2). These changes were associated with increased circulating corticosterone levels and dysregulation of energy substrate metabolism.⁵⁷ C2C12 myoblasts treated with 100 mM of alcohol showed increased proteolysis. An inhibitor of autophagy (3-methyladenine) prevented the increase in proteolysis while a proteasome inhibitor (MG132) did not affect proteolysis, highlighting the relevance of activation of autophagic-lysosomal pathway to alcohol-induced catabolic responses in SKM.³⁷

An acute intraperitoneal alcohol injection (5 g/kg) to female mice significantly disrupted mRNA expression of gastrocnemius clock genes as well as clock-controlled genes implicated in SKM function. Alcohol also increased circulating corticosterone levels and one of its target genes, REDD1, in SKM.⁵⁸ The disruption of circadian clocks in different tissues is a possible mechanism of

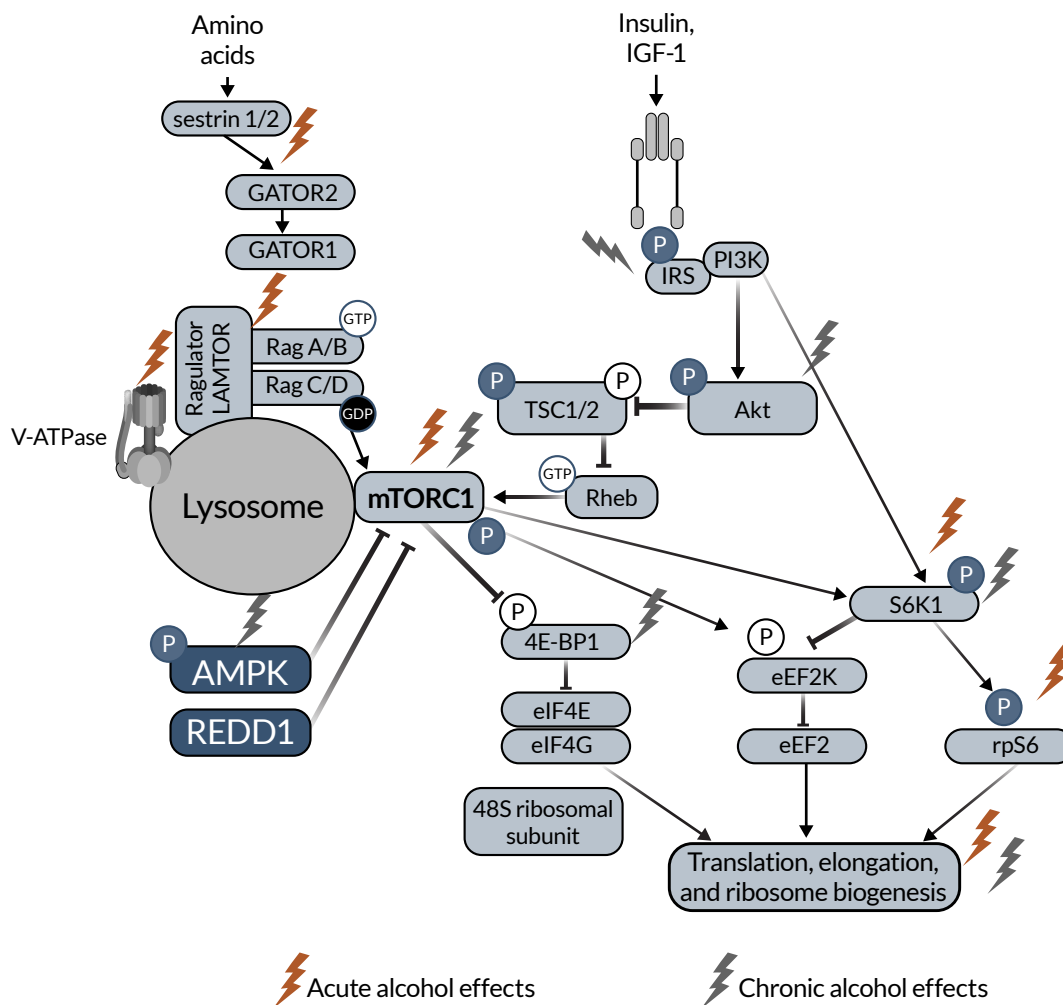


Figure 1. Schematic representation of mTOR anabolic signaling in skeletal muscle. The schematic highlights proteins that have been shown to be affected by acute (red lightning bolt icon) and chronic (light gray lightning bolt icon) alcohol intake in the skeletal muscle. Note: 4E-BP1, eukaryotic initiation factor 4E-binding protein; Akt, protein kinase B; AMPK, AMP-activated protein kinase; eEF2k, eukaryotic elongation factor 2 kinase; GAP, GTPase activating proteins; GATOR2, toward Rags complex 2; IGF-1, insulin-like growth factor 1; IRS, insulin receptor substrate; LAMTOR, late endosomal/lysosomal adaptor and MAPK and mTORC1 activators; mTORC1, mechanistic/mammalian target of rapamycin complex 1; PI3K, phosphoinositide 3 kinase; REDD1, regulated in development and DNA damage response 1; Rheb, Ras homolog enriched in brain; S6K1, S6 kinase 1; TSC1, tuberous sclerosis complex 1; TSC2, tuberous sclerosis complex 2.

alcohol-induced tissue injury.⁵⁹⁻⁶¹ It remains to be determined whether disruption of the clock genes and circadian rhythm is a mechanism of alcohol-mediated SKM dysfunction and whether there are sex- or age-specific effects.

Effects of acute alcohol exposure on SKM function

There are limited studies on the effects of acute alcohol exposure on SKM function. Studies in both male and female mice show that a single intraperitoneal injection of 5 g/kg alcohol decreased absolute and normalized peak-isometric (no change in length) tetanic (continuous muscle contraction) force generated in the triceps surae (gastrocnemius, soleus, and plantaris) muscles and increased fatigue within 1 hour of alcohol administration. These deficits were still present at

24 hours post-alcohol administration in male mice but not in female mice.⁶² In male mice administered 3 g/kg ethanol, there were no differences in twitch or tetanic force in the extensor digitorum longus muscle.⁶³ Whether differential kinetics of alcohol clearance or sex hormone differences contribute to the sex-dependent effects warrants further investigation.

In a clinical study from the United States, individuals who consumed alcohol (1.09 g/kg lean mass) had decreased time to exhaustion when using a cycle ergometer 18 to 24 hours post-alcohol consumption, suggesting an alcohol-mediated detrimental impact on severe-intensity exercise performance. However, the single alcohol dose did not affect muscle power, strength, or fatigability (decrease in maximal force in response

to contractile activity).⁶⁴ Similarly, heavy episodic drinking (six to 20 or more standard drinks [defined as 8 g of alcohol]) on the previous day in male rugby players in New Zealand decreased lower body performance with no effect on isometric strength and sprint performance.⁶⁵ In Australia, rugby players given 1 g ethanol/kg body weight 4 hours after a rugby match did not have statistically significant differences in maximal voluntary contraction, voluntary activation, or changes in creatine kinase, testosterone, or cortisol the morning after the match.⁶⁶ In the United States, among females who performed two bouts of maximal single leg eccentric extension followed by a single drink (1.09 g/kg fat-free body mass), isometric torque was fully recovered and eccentric torque partially recovered after 48 hours. Using the same exercise regimen, females who consumed a single drink (0.88 g/kg body weight) had no effect on strength recovery.^{67,68} Though the exact mechanisms are not known, it is possible that estrogens play a protective role in muscle recovery in females.^{69,70}

One possible explanation for the impaired strength with acute alcohol use is impaired glycolytic function, as severe intensity exercise relies heavily on anaerobic ATP production. In vitro exposure of primary male and female myoblasts to alcohol decreased glycolytic function.⁷¹ Alternatively, it is possible that the effects of alcohol are indirectly mediated by alterations in endocrine mediators. Acute alcohol binge drinking after resistance exercise increases cortisol levels, a known SKM catabolic mediator.^{55,72} Moreover, alcohol can interfere with SKM regeneration. Normally, muscle-damaging exercises activate regenerative processes with an initial inflammatory response to activate satellite cells.⁷³ Acute binge alcohol intake during resistance exercise decreases the early SKM inflammatory response in both trained males and females,^{67,74} providing evidence that it can adversely affect SKM regenerative processes. Moreover, these studies and others have shown marked alcohol-induced decreased myoblast differentiation, suggesting impaired recovery from exercise could be due to decreased regeneration capacity.^{75,76}

It should be noted that these effects of single, predetermined alcohol bouts on SKM function were examined in healthy and young males or females; however, whether this holds true in the general population, where the pattern and amount of alcohol consumed is variable, must be considered. Moreover, nutritional state and type of exercise can be confounding factors. In addition, the effect of acute alcohol binges on SKM function during exercise training in people with chronic alcohol misuse also necessitates investigation. In the BEER-High-Intensity Interval Training (BEER-HIIT) study conducted in Spain, females who consumed 12–24 g alcohol per day and males who consumed 24–36 g alcohol per day throughout the 10-week training period did not exhibit lower exercise-mediated increases in lean mass, aerobic fitness, and muscle strength.^{77,78} However, people in treatment for alcohol misuse have decreased isokinetic torque (strength), work, power, and isometric and isotonic

muscle loading even after detoxification.⁷⁹ This is compounded by the fact that ~ 15% of people with alcohol misuse have significant mobility impairment.^{80–82}

In summary, acute alcohol exposure dysregulates multiple proteins in the mTORC1 pathway and decreases muscle protein synthesis. There is also evidence that acute alcohol use increases catabolic signaling by activating both UPP and autophagy. Acute alcohol intake before or after exercise affects SKM function and is influenced by gender, type of exercise strength, and amount of alcohol. However, the role of other confounders (e.g., age, chronic alcohol misuse) warrants further investigation.

Chronic Effects of Alcohol on SKM

Studies to identify when CAM develops are difficult and confounded by underreporting of its overall prevalence. A clinical observational study among males from Russia reports that CAM takes about 10 years to develop, with proximal paresis occurring only in people who have muscle atrophy.⁸³

Some of the common causes that lead to dysfunctional SKM mass, especially with chronic alcohol use, are increased inflammation and oxidative stress. Zebrafish exposed to 0.5% alcohol for 8 weeks had decreased body weight and muscle fiber cross-sectional area with increased expression of the pro-inflammatory cytokines interleukin 1-beta (IL-1-beta) and tumor necrosis factor alpha (TNF-alpha) and increased expression of high-mobility cassette-1/toll-like receptor 4/nuclear factor-kappa B (HMGB1/TLR4/NF-kappa B) signaling proteins.⁸⁴ Similarly, chronic alcohol-fed rats showed increased SKM TNF-alpha and IL-6 expression and activation of the Janus kinase (JNK) pathway.⁸⁵ Clinical studies also confirmed that TNF-alpha expression was negatively associated with lean muscle mass in people with chronic alcohol misuse.⁸⁶ The increase in inflammation has also been linked to SKM oxidative stress and tissue dysfunction.⁸⁷ Rats with chronic exposure to alcohol had decreased antioxidant enzyme activity and increased malondialdehyde content.⁸⁸ The contribution of oxidative stress to increased protein degradation and SKM dysfunction was demonstrated in C2C12 myoblasts and mitochondria-targeted Mito-TEMPO attenuated alcohol-mediated increase in autophagy.⁸⁹

Chronic alcohol effects on SKM anabolic signaling

Chronic alcohol intake upregulates IGF binding protein-1 and myostatin, leading to decreased SKM protein synthesis.¹¹ Chronic alcohol feeding of rats for 14 weeks decreased SKM protein synthesis and prevented the anabolic effects of leucine administration irrespective of sex, indicating that there are no sex-specific effects of alcohol on SKM protein synthesis. However, the study also showed that at 6 weeks, males had decreased SKM protein synthesis, but there were no changes in females.⁹⁰ Alcohol administration for 4 weeks in female mice found dephosphorylation of mTORC1 and AMPK, which was mechanistically linked to an increase in protein

phosphatase 2A.⁹¹ Additionally, chronic alcohol administration in mice, ethanol treatment of C2C12 myoblasts, and analyses in people with alcohol-related cirrhosis all demonstrated that alcohol had synergistic effects with increased ammonia to impair SKM protein synthesis and increase protein breakdown.⁹² Multiomics analyses of alcohol-treated C2C12 cells and SKM from ethanol-fed mice identified several beta-hydroxymethylbutyrate-responsive targets. Moreover, beta-hydroxymethylbutyrate restored ethanol-induced decreased mTORC1 signaling, protein synthesis, and mitochondrial respiration as well as decreased sarcopenic phenotype.⁹³ In a study in Russia, females who reported consuming about 11 units of ethanol per day (1 unit is 10 ml of pure [96%] ethanol) for an average of 5.6 ± 0.6 years had decreased plasma IGF1 levels and decreased SKM expression of insulin receptor substrate (IRS-1), p-AktB, and p-4E-BP1. This was also associated with decrease in cross-sectional fiber area of both type I (slow oxidative) and II (fast glycolytic) fibers.⁹⁴ Similarly, middle-aged males with chronic alcohol misuse had decreased circulating IGF-1 levels and reduced SKM expression of IRS-1 and p-4E-BP1. This was also associated with increased mRNA expression of heat shock proteins and atrogenes and a relative increase in the proportion of fast glycolytic muscle fibers,^{95,96} indicating both a decrease in anabolic signaling and an increase in catabolic signaling.

Studies suggest that chronic alcohol consumption does not affect SKM glucose uptake,⁹⁷ despite the fact that chronic ethanol administration in rats reduced gastrocnemius expression of IRS-1, Akt, and p70S6K.⁹⁸ Chronic alcohol intake increased triglyceride deposition and decreased glucose uptake in SKM, which can lead to metabolic dysregulation.⁹⁹ Additionally, pigs fed a hypercaloric high-fat diet and alcohol diet for 7 weeks showed increased expression of proteins in the insulin signaling pathway and hyperglycemia.¹⁰⁰ In addition, PPAR-delta activation protected against alcohol-induced decreased Akt phosphorylation and increased mitochondrial uncoupling, indicating that PPAR-delta activation can protect against alcohol-induced SKM lipotoxicity and insulin resistance.⁹⁹

Physical activity or structured exercise generally is beneficial to anabolic signaling. However, female mice that were provided access to running wheels for 5 weeks and access to 20% ethanol in water during the last 5 days had decreased exercise-induced SKM p70S6K phosphorylation and increased MAFbx expression, suggesting that perhaps physical activity alone might not be sufficient to counteract the effects of alcohol-mediated SKM changes.¹⁰¹

Chronic alcohol effects on SKM catabolic signaling

Zebrafish exposed to 0.5% alcohol for 8 weeks had increased expression of markers of SKM atrophy and autophagy. This was associated with concomitant increases in reactive oxygen species content, decreased mRNA expression of antioxidant enzymes, and protein expression of Nox2,⁸⁴ linking the catabolic changes to increased oxidative stress. Similarly, rats fed 3 g/kg

body weight of alcohol for 4 weeks showed increased SKM MuRF1 expression, decreased pAkt/Akt ratio, and increased p-FoxO/FoxO ratio¹⁰² indicating UPP activation. In contrast, chronic alcohol feeding did not result in increased SKM expression of atrogenes in older rats.¹⁰³ However, there are some discrepancies regarding the activation of SKM autophagy with chronic alcohol misuse. For example, some research showed increased expression of autophagy markers in people with alcohol-related cirrhosis and chronic alcohol-fed mice.³⁷ However, this was not observed in other studies of chronic alcohol-fed mice¹¹ or in primary myoblasts isolated from in vivo chronic binge alcohol administered to macaques.⁷⁶

Chronic alcohol effects on SKM structural characteristics

A clinical study from Brazil showed that people who consumed more than 80 g alcohol per day and followed a sedentary lifestyle had reduced SKM index and phase angle (SKM specific indicator of cellular membrane integrity).¹⁰⁴ Similarly, in the United States, people with chronic alcohol misuse had significantly lower whole SKM area³⁷ and lower femoral and gluteal muscle areas.¹⁰⁵ However, in a large 12-year observational study among Korean people, high protein intake compared to low protein intake was protective against the development of low SKM mass index. Alcohol consumption in females but not males reduced the protective effect of high protein intake. Among the total participant population who consumed diets with high protein content, heavy drinking was not associated with development of low SKM mass index,¹⁰⁶ suggesting that dietary and lifestyle modifications potentially can prevent the imbalance in protein turnover with alcohol use.¹⁰⁷ In an observational study from Russia, females who reported consuming 11 ± 1 units of ethanol/day for an average of 5.6 ± 0.6 years had decreased cross-sectional area of both type I and type II fibers; decreased expression of titin and nebulin, two large proteins involved in maintaining the sarcomere structure; and increased expression of the protease calpain-1 and ubiquitinated proteins.⁹⁴ These findings are consistent with those from preclinical studies showing that chronic alcohol administration for 6 months in rats increased autolysis of mu-calpain; decreased titin, nebulin, and titin hyperphosphorylation; and led to the development of hindlimb muscle atrophy.¹⁰⁸ Chronic alcohol also decreased expression of myosin heavy chain (MHC), a major motor protein in the thick filament, and troponin-T, which is necessary for myosin and actin positioning.⁶³ Adult fish maintained at 0.5% ethanol for 8 weeks showed decreased SKM cross-sectional area, and this was associated with decreased SKM miR-140 expression and increased miR-146a expression. miR-140 targets the Notch signaling pathway, whereas miR-146a targets the Notch antagonist Numb, and these changes in miRNAs are implicated as mechanisms for the observed decreased SKM cross-sectional area.¹⁰⁹

In addition to the contractile and structural SKM proteins, extracellular matrix (ECM) remodeling plays a critical role in

regeneration, anabolic signaling, and mitochondrial function. Adult male rats on an alcohol-containing liquid diet for 24 weeks increased expression of collagens, hydroxyproline, and alpha-smooth muscle actin (marker of myofibroblast activation). This was associated with an increase in other matrix proteins, including integrin-alpha-5, L-selectin, platelet endothelial cell adhesion molecule (PECAM), secreted protein acidic and rich in cysteine (SPARC), and ADAM metalloproteinase with thrombospondin type 1 motif 2 (ADAMTS2). Alcohol also increased the inflammatory cytokines TNF-alpha, IL-12, and IL-6, and decreased IL-10 mRNA expression,¹¹⁰ likely contributing to the observed increase in ECM deposition. Finally, chronic alcohol feeding in rats increased expression of transforming growth factor-beta 1 (TGF-beta) and associated receptors along with downstream signaling components,¹¹¹ as well as expression of matrix metalloproteinase 9,¹¹² providing evidence for ECM remodeling and promotion of an SKM profibrotic phenotype.

Chronic alcohol effects on SKM functional characteristics

In a longitudinal study among Japanese men and women, alcohol use was positively associated with decreased grip strength, and this association did not change over a 2-year period.¹¹³ Similarly, in a cross-sectional study among people living in China, men who consumed more than 25 g of alcohol per day had increased risk of low muscle mass and grip strength.¹¹⁴ In animal studies, female mice consuming 20% alcohol in water for 40 weeks had decreased grip strength and decreased lean muscle mass, without major neuromuscular junction changes, suggesting that muscle weakness is potentially driven by muscle atrophy.¹¹⁵ In other studies, fatigability and alterations in twitch and tetanic tension were seen with chronic alcohol intake.⁶³ Evidence from studies using alpha-E83K mutant mice suggested that acute alcohol increased force and that this was due to direct actions of alcohol on the extracellular region of the neuromuscular nicotinic acetylcholine receptor (nAChR).¹¹⁶

Chronic alcohol effects on mitochondrial function

Caenorhabditis elegans exposed to ethanol had decreased expression of mitochondrial fission factor dynamin-related protein 1 (DRP-1), and mitochondrial network fragmentation leading to mitochondrial unfolded protein response, which was mechanistically linked to SKM weakness.¹¹⁷ Similarly, rats fed a chronic alcohol diet had decreased levels of mitofusin-1, dysregulation of mitochondrial topoisomerase, and decreased mitochondrial membrane integrity.¹¹⁸ Chronic alcohol administration also increased SKM 4-hydroxy-2-nonenal, decreased mitochondrial Complex IV and V activity, and decreased acetylcholinesterase expression, potentially indicating that mitochondrial dyshomeostasis was associated with inhibition of acetylcholinesterase, leading to myofiber atrophy.⁹⁸ In addition, chronic alcohol feeding in the context of high-fat diet for 6 weeks decreased SKM Complex I and III activity, antioxidant activity, as well as increased lipid peroxidation in

both male and female mice.¹¹⁹ A study using parkin-knockout mice fed an alcohol diet for 12 weeks showed that parkin was critical for alcohol-mediated disruption of mitochondrial complex activity, autophagy/mitophagy balance, and apoptosis.¹²⁰ Finally, ethanol treatment of primary myoblasts isolated from male and female macaques during 5 days of differentiation decreased extracellular acidification rate, an indicator of glycolysis, and increased maximal oxygen consumption rate. These changes were associated with decreased differentiation, suggesting that bioenergetic alterations regulate alcohol-mediated impaired myogenesis.⁷¹ Despite these indications of alcohol-mediated mitochondrial changes, few systematic studies have focused on mitochondrial bioenergetics and function with acute and chronic alcohol intake.

In summary, chronic alcohol-mediated increases in inflammation and oxidative stress contribute to CAM. Chronic alcohol exposure dysregulates multiple proteins in the mTORC1 signaling pathway and decreases SKM protein synthesis. As seen with acute alcohol exposure, chronic alcohol exposure increases protein breakdown by impacting autophagy and UPP. Preclinical and clinical studies provide evidence for decreased SKM mass with chronic alcohol exposure. Though the exact mechanisms of alcohol's effect on SKM structural and functional characteristics are not known, dysregulation of contractile proteins, muscle regulatory factors, and ion channels may be implicated.¹²¹ Evidence also suggests that physical exercise and protein-rich diets can potentially reduce the adverse effects of chronic alcohol intake on SKM.

Alcohol Effects on SKM in the Context of Comorbidities

SIV/HIV

With effective antiretroviral therapy (ART), people with HIV (PWH) have a near-normal life expectancy that has increased the earlier occurrence of age-associated comorbidities, including impaired SKM mass and function as well as frailty. Alcohol misuse is a maladaptive coping behavior among PWH¹²² that can exacerbate HIV-specific effects on SKM. Significant knowledge of the effects of chronic alcohol intake on SKM in the context of HIV has been derived from the rhesus macaque model. Chronic binge alcohol (CBA) administration in ART-naïve macaques infected with simian immunodeficiency virus (SIV), a clinically relevant preclinical model of HIV, increased SKM expression of pro-inflammatory cytokines and decreased antioxidant capacity.^{123,124} CBA produced dysregulation of epigenomic networks, including those of transcriptomic, DNA methylation, and miRNA implicated in ECM remodeling, pro-inflammatory milieu, protein homeostasis, calcium and ion homeostasis, neuromuscular junction signaling, and satellite cell growth and survival, providing evidence for mechanisms leading to CBA-mediated SKM loss at end-stage SIV infection.¹²⁵ ECM remodeling was confirmed by increases in SKM hydroxy proline

content and collagen expression, as well as by upregulation of expression of TGF-beta, tissue inhibitor of metalloproteinase (TIMP-1), and matrix metalloproteinase 2 and 9 (MMP2 and 9).¹²⁶ CBA also activated UPP, increasing catabolic signaling.¹²³

With effective ART regimens and controlled infection, the prevalence of overt SKM wasting in PWH has significantly decreased, especially during the asymptomatic stage of HIV disease. However, there are cellular and molecular changes that contribute to dysfunctional SKM mass. CBA administration decreased myoblast differentiation, myogenic gene expression, and SKM enriched miRs (myomiR) expression.¹²⁷ Data also suggested that miR-206 targeted Class IIA histone deacetylase (HDAC4), and that an HDAC inhibitor could partially ameliorate CBA-mediated decrease in myoblast differentiation.¹²⁸ Ongoing studies have explored the possibility of extracellular vesicles as mediators of intercellular communication contributing to decreased myoblast differentiation. Results showed significant alterations in expression of myomiRs in extracellular vesicles derived from myotubes formed from myoblasts isolated from CBA animals; however, no significant differences existed in extracellular vesicle size or concentration.¹²⁹ CBA also dysregulated expression of genes implicated in mitochondrial homeostasis (e.g., peroxisome proliferator-activated receptor gamma, coactivator 1 beta [PGC-1b]; PPAR-alpha; estrogen-related receptor alpha; and superoxide dismutase) in SKM of ART-naïve SIV-infected male macaques at end-stage disease.¹³⁰ In animals that were treated with ART and with controlled infection, CBA decreased succinate dehydrogenase activity (complex II of the electron transport chain) in type 1 and type 2 fibers, as well as myoblast maximal oxygen consumption rate. Results also indicated that formoterol, a beta-adrenergic agonist, increased myoblast PGC-1b expression and mitochondrial DNA, and improved maximal oxygen consumption rate,¹³¹ suggesting that exercise training or increased physical activity may help alleviate alcohol-related myopathy.

In a study among PWH with increased fasting plasma glucose but no diagnosed diabetes, negative indicators of myoblast bioenergetic health (proton leak, nonmitochondrial oxygen consumption rate, and bioenergetic health index) were higher among people with higher scores on the Alcohol Use Disorders Identification Test (AUDIT). This was also associated with increased mitochondrial volume and decreased expression of genes implicated in mitochondrial health.¹³² In the same cohort of PWH, circulating miR-206 was decreased in people with recent alcohol use as indicated by positive phosphatidylethanol (PEth).¹³³ In another PWH cohort, body composition had significant modulatory effects on frailty, with higher fat-free mass index, body fat, and body mass index associated with decreased frailty risk. The study also indicated a negative association of frailty with fat-free mass index among people with detectable PEth, indicating that increased SKM mass is protective in PWH with alcohol use.¹³⁴

SKM pain

SKM pain is associated with several comorbid conditions, including diabetes and HIV. A study in the United States that examined whether people wanted to drink more alcohol when they experienced SKM pain observed that men had a higher risk than women to self-medicate with alcohol.¹³⁵ Preclinical evidence suggests that chronic alcohol administration in mice promotes SKM mechanical hyperalgesia, and that probiotics can significantly reduce this alcohol-induced SKM mechanical hyperalgesia.¹³⁶ Moreover, in an SKM disuse atrophy model, chronic alcohol feeding of rats for 10 weeks produced mechanical hyperalgesia and associated pain-related neuroadaptations, indicating that chronic alcohol misuse could exacerbate complex pain regional syndrome.¹³⁷

Cancer cachexia

The association among alcohol, muscle pain, and muscle functional mass also extends to other comorbidities. In mice injected with melanoma cells, those fed 20% alcohol (weight/volume) in water for 3 months had increased SKM inflammation, apoptosis, and protein degradation. There was also a significant decrease in satellite cell numbers and impaired myogenesis, indicating that alcohol exacerbated cancer-associated cachexia.¹³⁸ In a Lewis lung carcinoma mouse model, chronic alcohol administration decreased expression of proteins in the mTORC1 pathway and increased expression of proteins of both UPP and autophagy, indicating a shift to increased SKM catabolism. Moreover, there was increased phosphorylation of Smad and extracellular signal-regulated kinase signaling proteins as well as increased expression of SKM and circulating myostatin, negative regulators of SKM mass.¹³⁹ These studies suggest that alcohol use can exacerbate cancer-associated cachexia.

Disuse Atrophy and Injury

Another condition where alcohol can produce detrimental SKM changes is in response to disuse or injury. Alcohol misuse puts people at increased risk for injury and falls due to motor incoordination and peripheral neuropathies.^{140,141} In a model of barium chloride-induced injury of the tibialis anterior muscle, chronic alcohol exposure increased inflammation and fibrosis and decreased the cross-sectional area of regenerated muscle fibers.¹⁰³ In a model of cryoinjury of tibialis anterior muscle, chronic alcohol administration increased inflammation, and low-level laser therapy decreased inflammation and improved recovery.¹⁴² In a model of SKM disuse, chronic alcohol feeding for 10 weeks in ovariectomized or intact female rats indicated that alcohol dysregulated genes implicated in regeneration and increased TGF-beta expression.¹³⁴ In addition, primary macaque myoblasts isolated from in vivo CBA-administered macaques had decreased differentiation potential and concomitant decrease in myogenic gene expression,⁷⁶ indicating that alcohol could affect SKM regenerative potential. In a model of disuse atrophy, repeated binge alcohol administration activated UPP

and decreased protein synthesis.¹⁴³ Based on evidence that alcohol can affect satellite cell regenerative function, exacerbate catabolic signaling, and curb protein synthesis, further studies on the effects of alcohol on muscle injury or disuse atrophy are warranted.

Alcohol, SKM, and Aging

Alcohol misuse is disproportionately on the rise among older individuals.²⁰ Approximately 45% of current drinkers age 60 and older consume more than seven drinks per week, and 25% consume more than 14 drinks per week in the United States.¹⁴⁴ Alcohol use among older individuals potentially decreases SKM mass and function,¹⁴⁵ increasing the risk of morbidity.¹⁴⁶⁻¹⁴⁸ Thus, alcohol-related myopathy potentially can exacerbate age-related declines in SKM mass and function. However, there are few published studies on alcohol's effects on SKM function in aging, and this warrants attention. Aged female F344 rats fed an alcohol diet for 20 weeks showed decreased lean mass and SKM protein synthesis with dysregulation of multiple proteins in the mTORC1 pathway but with no significant effects on catabolic pathways.¹⁴⁹ In a large observational study of older males in Japan, alcohol misuse and liver fibrosis led to greater loss of SKM mass¹⁵⁰ and increased intramuscular adipose tissue accumulation.¹⁵¹ A study among postmenopausal women found increased risk for sarcopenia among those with high-risk alcohol use.¹⁵² Similarly, in another large study among elderly Korean women, binge drinking once or more per week was associated with a higher risk for sarcopenia.^{152,153} However, there are some indications that alcohol consumption may not be associated with sarcopenia in older adults.¹⁵⁴ The increased aging of the population and the rising frequency of alcohol use in both male and female aged individuals make this an important area in need of further investigation.

Summary and Recommendations for Future Work

As indicated in this review, although there is a large focus on the effects of both acute and chronic alcohol intake on the mTORC1 pathway and protein synthesis, gaps remain in the literature on alcohol's effects on mitochondrial bioenergetics, protein degradation, and satellite cell function. Additionally, most of the published clinical and preclinical studies are either observational or descriptive, and future studies that are mechanistic and prove causality are highly warranted. This is particularly relevant with respect to acute effects of alcohol and in the context of exercise training, injuries, atrophy, and aging. There is also a gap in literature on additional alcohol-mediated mechanisms such as epigenomic alterations, role of senescence, effects of immune activation, and circadian signaling that can lead to impaired

SKM mass and function. In addition, both clinical and preclinical studies on alcohol-mediated SKM effects in the presence of comorbidities are limited.

Few studies have identified whether the effects of alcohol are directly mediated or the result of alcohol metabolism and generation of metabolites such as acetaldehyde.³⁷ Although there is evidence of alcohol directly affecting SKM function, especially in ex vivo systems, it is debatable whether significant alcohol metabolism occurs in SKM. It is possible that acetaldehyde can cause adverse effects in SKM. Similarly, the possibility that alcohol-associated SKM effects result from actions of soluble factors or extracellular vesicles released from distant organs, in a true interorgan communication, remains to be explored.^{155,156}

Regarding published clinical studies on alcohol-related myopathy, the lack of objective measures of alcohol use, with few studies including biomarkers of alcohol use, confound the ability to draw conclusions and may explain the sometimes discrepant reports in the literature. Most clinical studies rely on self-report of alcohol use instead of validated questionnaires for assessing alcohol use, making it difficult to draw conclusions and comparisons across populations. With the mission of research and health care institutions, and the community at large, for rigor and reproducibility, it is recommended that peer-reviewed studies use standardized alcohol use questionnaires or biomarkers of alcohol use. For example, AUDIT, timeline follow-back, and lifetime drinking history are established self-report questionnaires that can be used, and PEth is a reliable biomarker of recent alcohol use.^{157,158}

Abstinence is the most effective treatment for alcohol-related myopathy. Abstinence results in significant improvement in muscle strength,^{3,159} but fails to reach similar levels to those of age-matched controls. Reducing alcohol use is also a good strategy to improve functional SKM mass.^{3,159} An ideal strategy to improve SKM function in persons with alcohol misuse is to increase physical activity or structured physical exercise while reducing alcohol consumption. The efficacy of exercise-induced beneficial effects on SKM function in subjects with varying levels of alcohol use remains to be determined. Similarly, diet modifications also may help improve SKM function and remain to be systematically studied. Overall, elucidating specific mechanisms and increasing fundamental knowledge of alcohol-mediated effects on SKM can help design therapeutic targets to improve SKM health and overall quality of life.

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NIAAA 50th ANNIVERSARY FESTSCHRIFT

Alcohol Use Disorder and Alcohol-Associated Liver Disease

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Publisher's Note

This article was based on a presentation at the NIAAA 50th Anniversary Science Symposium, "Alcohol Across the Lifespan: 50 Years of Evidence-Based Diagnosis, Prevention, and Treatment Research," held on November 30–December 1, 2020. Links to the videocast are available on the [NIAAA 50th Anniversary Science Symposium agenda](#) webpage.

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This article is part of a Festschrift commemorating the 50th anniversary of the National Institute on Alcohol Abuse and Alcoholism (NIAAA). Established in 1970, first as part of the National Institute of Mental Health and later as an independent institute of the National Institutes of Health, NIAAA today is the world's largest funding agency for alcohol research. In addition to its own intramural research program, NIAAA supports the entire spectrum of innovative basic, translational, and clinical research to advance the diagnosis, prevention, and treatment of alcohol use disorder and alcohol-related problems. To celebrate the anniversary, NIAAA hosted a 2-day symposium, "Alcohol Across the Lifespan: 50 Years of Evidence-Based Diagnosis, Prevention, and Treatment Research," devoted to key topics within the field of alcohol research. This article is based on Dr. Shah's presentation at the event. NIAAA Director George F. Koob, Ph.D., serves as editor of the Festschrift.

KEYWORDS: alcohol; alcohol-associated liver disease; screening; prevention; mortality; patient readmission; policy; liver diseases

Alcohol use disorder (AUD) is prevalent worldwide, and the burden of heavy alcohol consumption has been increasing over time. An important complication of prolonged, heavy alcohol use is alcohol-associated liver disease (ALD), which can progress from liver steatosis to fibrosis and cirrhosis and frequently involves alcohol-associated hepatitis. In particular, cirrhosis—the most severe type of ALD—can be associated with fatal and resource-intensive complications and impose a significant social and financial burden on families, hospitals, and communities.

This article summarizes the epidemiology of alcohol use and ALD and describes the outcomes and mortality associated with ALD. This is followed by a review of screening and prevention approaches for AUD and ALD, as well as of current treatment strategies for both conditions, including integrated treatment approaches. Policy measures to mitigate the impact of alcohol misuse are also discussed.

Epidemiology of Alcohol Use and ALD

There is currently a very high burden of alcohol use and misuse globally. In 2016, an estimated 2.4 billion people worldwide consumed alcohol, including 1.5 billion men and 900 million women.¹ Furthermore, nearly 40% of people who consume alcohol reported heavy, episodic drinking in 2016 (defined as 60 or more grams of pure alcohol on at least one single occasion at least once per month).²

According to the fifth edition of the *Diagnostic and Statistical Manual of Mental Disorders*, AUD is a maladaptive pattern of alcohol use characterized by two or more from a list of symptoms, such as increasing alcohol use despite negative consequences; persistent, unsuccessful attempts to quit drinking; craving; tolerance; or withdrawal.³ The estimated global prevalence of

AUD is currently 9% and continues to rise.⁴ Of note, psychiatric comorbidities are often present in individuals with AUD and may precede the onset of heavy alcohol use.⁵

ALD is a common complication associated with long-term alcohol misuse and AUD, and clinicians may encounter a spectrum of ALD in practice (Figure 1). Hepatic steatosis occurs in 90% to 95% of patients with chronic, heavy alcohol use. Steatosis causes inflammation of the liver, known as steatohepatitis, and progression to liver fibrosis occurs in 20% to 40% of patients. Liver fibrosis can continue to progress and result in cirrhosis in 8% to 20% of patients. Hepatocellular carcinoma is a primary liver neoplasm that is a complication of cirrhosis, occurring in 3% to 10% of these patients.⁶ Alcohol-associated hepatitis is a specific clinical entity that occurs with long-term heavy alcohol use and may occur anywhere along the spectrum of ALD. There are several risk factors for progression of ALD, which include female sex, obesity, dietary factors, genetic polymorphisms, harmful patterns of alcohol consumption, and smoking. Clinicians should also consider and treat comorbidities that may contribute to disease progression, such as viral hepatitis, hemochromatosis, and human immunodeficiency virus (HIV).⁶

Cirrhosis is associated with chronic alcohol use, which accounts for 21% of physiologically compensated cirrhosis around the world. The global prevalence of alcohol-related, compensated cirrhosis remained relatively unchanged from 1990 (290/100,000 people) to 2017 (288/100,000 people). However, the global prevalence of decompensated cirrhosis rose from 1.1 million individuals in 1990 to 2.5 million individuals in 2017, with the greatest increases found in Western and Central Europe. Furthermore, ALD is the underlying cause of 30% of hepatocellular carcinoma cases.⁷ The overall burden of ALD is expected to increase over time. This prediction is based on multiple variables, including socioeconomic factors, changes in drinking patterns, and the rising prevalence of obesity and fatty liver disease.⁵

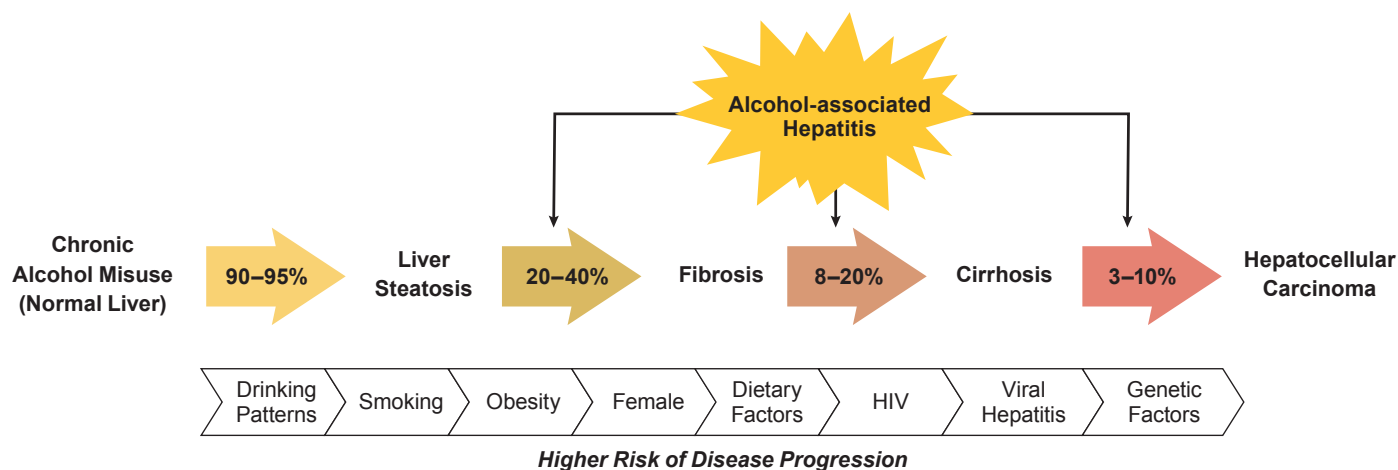


Figure 1. The spectrum of alcohol-associated liver disease, from steatosis to cirrhosis complicated by hepatocellular carcinoma. Alcohol-associated hepatitis can occur at any stage of disease. Numerous risk factors and comorbidities contribute to the risk of disease progression.⁶ Note: HIV, human immunodeficiency virus.

Mortality and Outcomes Associated With ALD

The incidence of cirrhosis is expected to triple by the year 2030 due to the rising prevalence of ALD as well as non-alcoholic fatty liver disease (NAFLD). Cirrhosis is associated with fatal complications, such as gastrointestinal hemorrhage, renal failure, and hepatocellular carcinoma, which impose significant social and financial burdens on families, hospitals, and communities. Mortality rates from cirrhosis have risen in the United States from 2009 to 2016, with the greatest relative increase observed in young people (ages 25 to 34).⁸ This trend parallels increased mortality due to AUD. Compared with women, men had a higher age-adjusted mortality due to cirrhosis (2:1) and hepatocellular carcinoma (4:1). However, women experienced a more rapid increase in cirrhosis-related mortality than did men; the annual percentage increase in mortality was highest in women ages 25 to 34.⁸ Among different racial/ethnic groups in the United States, Native Americans and white Americans had the highest mortality due to cirrhosis, whereas Asians and Pacific Islanders had the highest mortality due to hepatocellular carcinoma. Furthermore, Hispanic individuals had a higher mortality from cirrhosis and hepatocellular carcinoma, compared with non-Hispanic individuals.⁸

The development of ALD may also be dependent on other factors related to the patient's health, such as obesity. Dietary guidelines by the U.S. government state that to minimize risks associated with drinking, adults of legal drinking age can choose not to drink or to drink in moderation by limiting intake to two drinks or less per day for men and one drink or less per day for women, on days when alcohol is consumed.⁹ However, the American College of Gastroenterology recommends that the obese population should avoid alcohol consumption entirely due to increased risk of hepatic steatosis—a condition characterized by lipid deposits within the liver that is caused by heavy alcohol consumption or metabolic syndrome and can lead to chronic liver disease and cirrhosis.¹⁰ A large cohort study using the Mayo Clinic Biobank examined the impact of alcohol consumption and obesity on the development of hepatic steatosis and mortality.¹¹ Moderate alcohol consumption (defined in the study as no more than two standard drinks per day) increased the risk of hepatic steatosis and all-cause mortality in obese individuals (body mass index [BMI] > 30 kg/m²), whereas heavy drinking (defined as more than two standard drinks per day) increased the risk of hepatic steatosis and all-cause mortality in all patients, regardless of BMI.¹¹ In individuals with a normal BMI (< 25 kg/m²), moderate alcohol consumption lowered the risk of hepatic steatosis and all-cause mortality. This effect was not observed in overweight individuals (BMI 25 kg/m² to 30 kg/m²).

Screening and Prevention Strategies for AUD and ALD

The key to mitigating the future burden of AUD and ALD is early detection and prevention. Unfortunately, ALD is often detected at a later stage of disease when patients present with decompensated cirrhosis. Improved screening modalities for liver fibrosis are needed to identify affected individuals before irreversible, decompensated liver disease develops. Technologies such as smartphone applications, telemedicine, or electronic medical records can be used to improve population screening for AUD and ALD and may prove useful in linking people with a diagnosis of AUD or ALD to treatment programs or support groups. Such tools have been well received by individuals who drink heavily and those who have cirrhosis.⁵

Not all people with AUD are identified through screening or receive treatment. Barriers to AUD treatment include a shortage of providers, limited insurance reimbursement, and patient attitude toward treatment. Most screening for AUD occurs in health care environments, usually when patients are evaluated for other medical issues. Individuals who have little to no contact with health care systems almost never receive screening.⁵

Accordingly, in-person screening for AUD and ALD should be expanded outside of traditional health care environments to nontraditional settings such as pharmacies, annual employee health screenings, or driver's license renewal appointments.⁵ A prime example for this approach is the effectiveness of screening for hypertension at community barbershops.¹²

Treatment of AUD

Once AUD or ALD has been identified, treatment and therapy should be initiated early to prevent disease progression or relapse to alcohol use. Treatment of AUD may involve nonpharmacological and pharmacological approaches.

Nonpharmacological Treatment

Nonpharmacological therapies for AUD, such as patient counseling and motivational interviewing, play a key role in achieving alcohol abstinence. These strategies are used universally and can be employed by any health care provider, including primary care providers.

Motivational interviewing is a form of nonconfrontational counseling that encourages patients to make choices consistent with their long-term goals and health. This technique is especially helpful in patients with heavy alcohol use that does not meet diagnostic criteria for AUD.¹³ Providing patients with feedback surrounding changes in liver tests is associated with decreased alcohol use in patients who have, or are at risk of, chronic liver

disease.¹⁴ Motivational interviewing can be used in combination with pharmacotherapy to help patients achieve alcohol abstinence.¹⁵ Other nonpharmacological treatment strategies for AUD include establishing a supportive patient-physician relationship, scheduling follow-up clinic visits, engaging family members for support, referring patients to 12-step programs, developing coping strategies to manage early relapse, and treating psychiatric comorbidities.¹⁵

Pharmacological Treatment

The U.S. Food and Drug Administration (FDA) has approved three medications to treat AUD; these include disulfiram, naltrexone, and acamprosate. Baclofen is another option for therapy; however, it has not been approved by FDA. Although these medications are well studied for AUD, few studies have examined their effectiveness in patients with cirrhosis. Any medication approved by FDA can be used in patients with mild forms of liver disease; however, the use of disulfiram and naltrexone is cautioned in patients with cirrhosis or any features suggestive of liver dysfunction.¹⁵

Disulfiram is an acetaldehyde dehydrogenase inhibitor that produces an acetaldehyde syndrome characterized by facial flushing, nausea, vomiting, tachycardia, and hypotension when consumed with alcohol. It is prescribed as a deterrent to alcohol consumption based on this reaction. A meta-analysis showed that disulfiram significantly helped with alcohol abstinence in six out of 11 clinical trials.¹⁶ Disulfiram is most effective in patients who are committed to abstinence or take it in a monitored fashion.¹⁶ Cirrhosis is a known contraindication to disulfiram use due to reported events of liver failure leading to death or liver transplantation. Liver toxicity also has been reported in patients without liver disease.

Naltrexone is an opioid receptor antagonist that affects alcohol use primarily by inhibiting mu-opioid receptors and reducing the rewarding and reinforcing effects of alcohol. Clinical trials have demonstrated that naltrexone therapy is associated with a reduced risk of relapse to alcohol use and longer abstinence compared to placebo.¹⁷ Naltrexone can result in elevated liver enzymes, especially at doses greater than 100 mg per day, and should be avoided in patients with acute hepatitis or acute liver failure. Providers should monitor for injection-site hematomas related to naltrexone injections in patients with coagulopathy of liver disease. Naltrexone is contraindicated in patients who are being treated for opioid use disorder with mu-opioid receptor agonists (i.e., methadone or buprenorphine).

Acamprosate can reduce the symptoms of alcohol craving during prolonged abstinence and reduces alcohol intake in patients with AUD.¹⁸ Its therapeutic effects on AUD are thought to be through antagonizing *N*-methyl-D-aspartate (NMDA) receptors, although it also has been reported that pharmacological effects could modulate gamma-aminobutyric

acid type A (GABA_A) receptor activity.¹⁵ Acamprosate can be used safely in patients undergoing treatment for opioid use disorder and has no hepatic metabolism. However, its safety and efficacy in patients with advanced liver disease has not been validated. Dose adjustments of acamprosate are required in patients with chronic kidney disease, especially when the creatinine clearance is below 30 mL per minute.

Baclofen is a selective GABA type B (GABA_B) receptor antagonist that is typically prescribed for muscle spasticity. Although it is not approved by FDA for treatment of AUD, baclofen is commonly used off-label in other countries. Several clinical trials and open-label studies using baclofen to treat AUD in patients with advanced liver disease have shown mixed results.^{19,20} Overall, baclofen use is not associated with liver toxicity and can be used safely in patients with ALD.¹⁵

Integrated Care of Patients With AUD and ALD

In patients with alcohol-associated hepatitis, the most important predictor of long-term mortality is alcohol relapse. In fact, recurrent episodes of alcohol-associated hepatitis in patients who relapse to alcohol use have a mortality of nearly 60%. Among patients with alcohol-associated hepatitis, 34% to 37% relapse to alcohol use, and approximately 30% are readmitted to hospitals. The most common reasons for readmission are recurrent alcohol-associated hepatitis (19%) and alcohol intoxication and/or alcohol withdrawal (8%).²¹

Integrated treatment that addresses not only the patients' liver disease but also alcohol use can improve outcomes. In patients with alcohol-associated hepatitis, alcohol rehabilitation—defined as residential or outpatient AUD treatment or mutual support group participation—after hospital discharge is associated with a 70% to 84% decrease in 30-day readmission rate, an 89% to 91% decrease in 30-day alcohol relapse, and an 80% reduction in mortality.²¹ Furthermore, alcohol rehabilitation plays a particularly important role in therapy of people with AUD and ALD because only a few medications to treat AUD can be used in individuals with recent or active alcohol-associated hepatitis. A large body of evidence suggests that psychosocial interventions, such as cognitive behavioral therapy and motivational interviewing, are effective tools for supporting alcohol abstinence.

Overall, there is a clear need for the implementation of alcohol rehabilitation in preventing undesirable patient outcomes.²¹ Currently, only 16% to 20% of patients with alcohol-associated hepatitis attend alcohol rehabilitation. However, patients who were seen by addiction specialists during hospitalization are twice as likely to attend alcohol rehabilitation after discharge.²¹ Implementing these strategies for the care

of patients with AUD can reduce the risk of alcohol relapse, recurrent alcohol-associated hepatitis, hospital readmission, and overall mortality. Therefore, it is strongly suggested that health care providers should arrange for alcohol rehabilitation at the index hospitalization, and referral should be used as a quality metric in the management of all patients with alcohol-associated hepatitis. In this manner, implementing quality metrics could lead to improved patient outcomes.²¹ Further integrated care can

include evaluation of alcohol biomarkers, validated screening tools, appropriate pharmacotherapy, multidisciplinary and telehealth care, as well as appropriate referral for specialty care (Figure 2).

Recent technological advances have improved health care delivery to patients with AUD and ALD. Biomonitoring (using wearable devices) and telehealth have revolutionized patients' access to health care. With these approaches, providers can

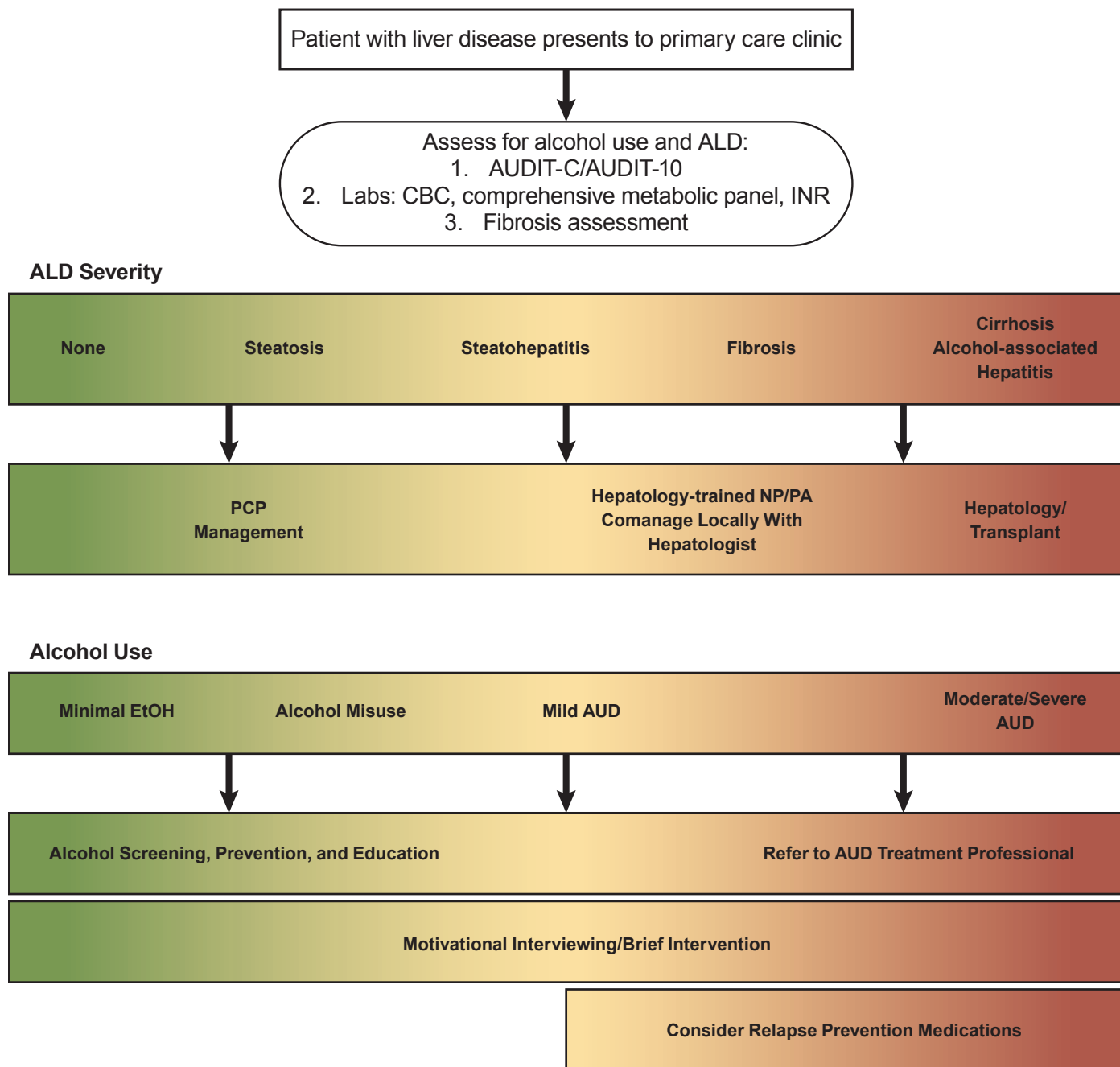


Figure 2. Treatment paradigm for patients with AUD and the spectrum of ALD. Reprinted with permission from Asrani et al., 2021.⁵ Note: ALD, alcohol-associated liver disease; AUD, alcohol use disorder; AUDIT-10, 10-item Alcohol Use Disorders Identification Test; AUDIT-C, three-item Alcohol Use Disorders Identification Test; CBC, complete blood count; EtOH, ethanol; INR, international normalized ratio; NP, nurse practitioner; PA, physician assistant; PCP, primary care provider.

obtain clinical information, such as blood alcohol levels or vital signs, and respond accordingly through smartphone applications and other technology. This advancement has allowed providers to reach more patients.²²

Treatment of ALD

The mainstay of treatment for patients with alcohol-associated hepatitis is therapy for AUD, either pharmacologic, nonpharmacologic, or a combination thereof. Alcohol-associated hepatitis is classified as either mild or severe based on the Maddrey discriminant function (mDF) or the model for end-stage liver disease (MELD) scores.²³ Therapy for patients with mild alcohol-associated hepatitis (mDF < 32 or MELD < 20) is centered around supportive care and AUD therapy. Nutritional support is essential as malnutrition and sarcopenia are common complications of ALD and have a negative impact on patient outcomes. Enteral nutrition supplementation, instead of intravenous administration, is preferred due to lower cost, greater safety, and lower risk of infection. Feeding tube insertion is safe in patients with nonbleeding, esophageal varices who have not undergone recent variceal band ligation. Fluid resuscitation, preferably with albumin, is also part of treatment.¹⁰

For patients with severe alcohol-associated hepatitis (mDF > 32 and MELD > 20), corticosteroids should be considered in addition to supportive therapy. If there are no contraindications to corticosteroids, prednisolone can be initiated to treat severe alcohol-associated hepatitis as its use modestly increases 1-month survival.²⁴ Corticosteroid use in clinical practice is often limited by concern about adverse reactions and high risk of infection. Once treatment has been started, clinicians should assess patient response using the Lille score, which is a calculated score on treatment day 7 to estimate if a patient is responding to corticosteroid therapy.²⁵ Clinicians can discontinue corticosteroids in nonresponders and avoid the increased risk of infection associated with their use. There is some indication that the Lille score on day 4 is as accurate as on day 7 in predicting treatment response.²⁵ There is currently an unmet need for alternative and safe medical therapy for severe alcohol-associated hepatitis.¹⁰

Liver Transplantation

Liver transplantation is a treatment option for patients with severe ALD, including those with severe alcohol-associated hepatitis that fails to respond to corticosteroids. ALD is the leading indication for liver transplant in the United States, accounting for 15% of liver transplants in the nation, as well as for 20% of liver transplants in Europe.^{10,26} The process starts with a referral to a liver transplant center, followed by a formal evaluation and listing for transplant. However, numerous barriers to receiving a liver transplant exist for patients with

ALD. For example, physicians may be biased against referral for a formal evaluation based on patient age or race, lack of empathy due to considering AUD a behavior rather than a disease, duration of alcohol use, and geographical area.²⁷

Relapse to alcohol use occurs in 17% to 30% of patients on a waiting list for a liver transplant and in 10% to 60% of post-transplant patients.²⁸ This emphasizes that a liver transplant cures liver disease but not the underlying AUD. Many transplant programs require patients to abstain from alcohol for a minimum of 6 months before considering a liver transplant; however, protracted abstinence is not a reliable predictor of recidivism. Instead, important predictors include age, social support, psychiatric comorbidities, polysubstance abuse, family history, and previous failed rehabilitation attempts. The Psychosocial Assessment of Candidacy for Transplantation scale is widely used to determine a patient's risk of recidivism and need for alcohol rehabilitation prior to liver transplantation.²⁹ Patients should be screened for recidivism at every clinic visit, as 10-year survival after liver transplantation is 45% to 71% in those with harmful alcohol use versus 75% to 93% in abstinent patients with occasional slips.³⁰ Self-reported alcohol use may not be reliable, and clinicians should consider using biomarkers to assess for ongoing alcohol consumption.¹⁰

Liver transplantation for ALD remains a controversial topic and requires careful consideration and expertise. Established criteria for transplant candidacy specify that patients should be presenting with liver disease for the first time, have failed medical therapy, and are without severe medical or psychosocial comorbidities. It is important to avoid liver transplantation in patients who will recover without it and in those with low predicted short-term survival. This will avoid creating a disparity in available liver grafts based on indication and socioeconomic factors. Transplant candidates with ALD should have a high likelihood of long-term abstinence, and treatment of AUD should be incorporated into pre- and post-transplant care.³¹

Recent Advances in ALD and Implications for Treatment

Alcohol-associated hepatitis is characterized by unrelenting inflammation that is a complex response to hepatocellular stress and death. Advancements in understanding the molecular biology of ALD have changed approaches to caring for patients. Heavy, long-term consumption of alcoholic beverages results in damage to hepatocytes, which respond by releasing extracellular vesicles (EVs). The release of EVs results in activation of inflammatory cells (e.g., macrophages), which release inflammatory cytokines such as tumor necrosis factor alpha (TNF-alpha), interleukin 1 beta (IL-1-beta), and IL-6.³² Research is now being conducted to investigate the interplay of other hepatic endothelial cells, hepatic stellate cells, and the patient's inflammatory cascade of lymphocytes. Further research also is needed to determine how alcohol's effects on

the intestine may result in mild intestinal injury, alter intestinal permeability, and affect the gut microbiome, which can result in the progression of ALD.³²

EV release from hepatocytes, which has been observed with *in vitro* studies, mouse models, and human subjects in response to liver injury, may be useful as a biomarker for ALD. Sehrawat et al. examined the quantity of EVs released and demonstrated that a high EV count was associated with a worse prognosis for ALD compared to a low EV count, and was predictive of disease severity and mortality.³³ Furthermore, detectable EVs in the blood were liver-specific and could be useful in the diagnosis of ALD and dynamic risk profiling.³³ Magnetic resonance elastography is also under investigation as a possible diagnostic tool for assessing inflammation, hepatic injury, and fibrosis in ALD.³⁴ This technology could be useful in clinical practice and avoid the need for a liver biopsy and its associated risks.

Enhanced understanding of the molecular biology of ALD has revealed targetable disease mechanisms for drug therapies and promising alternatives to corticosteroid therapy. Some therapies under current investigation include granulocyte colony stimulating factor (G-CSF), the IL-1 receptor antagonist anakinra, IL-22, and high-dose vitamin C. IL-22 therapy is of notable interest as it has already succeeded in a proof-of-concept study.³⁵ Thus, IL-22 reduced hepatocyte injury, promoted liver regeneration, reduced steatosis and fibrosis, and was not immunosuppressive. Recombinant IL-22 (termed F-652 in clinical trials) has demonstrated safety and efficacy in early, open-label studies with improved MELD scores and Lille scores, as well as reduced inflammatory markers. F-652 administered to patients with moderate to severe alcohol-associated hepatitis was associated with a reduction in patient MELD score at days 28 and 42.³⁵ Further studies are being conducted to evaluate the real-world efficacy of F-652. Other cytokines, such as TNF-alpha or transcription factor BRD4, also may be targeted to reduce hepatocellular injury.³⁵

Policies to Mitigate the Impact of AUD and ALD

The effects of AUD and ALD have major individual and societal impacts. National and regional interventions can help decrease the societal impact and reduce the number of individuals at risk. To lower the overall burden associated with AUD and ALD, medical societies have recommended community-wide alcohol reduction strategies as well as personalized treatment options for these conditions. Various initiatives led by the World Health Organization also aim to decrease the impact of alcohol use, for example, through appropriate taxation of alcohol, restricted alcohol availability, and restricted promotion to vulnerable populations.⁵

One of the strongest approaches to influencing alcohol consumption and, consequently, ALD risk at the population level is regulation of the unit price of alcohol through measures such as alcohol taxation. When alcohol prices increase, alcohol consumption and ALD burden notably decrease. Conversely, reduced alcohol prices are associated with increased alcohol consumption and alcohol-related deaths. However, the impact of these measures varies among population subgroups and is most prominent in groups with the highest amount of alcohol use and those with lower socioeconomic status.^{36,37} In addition to taxation, strategies such as adjusting for inflation and income, minimal pricing policies, volumetric taxes, and banning volume discounts can be employed to reduce alcohol consumption.⁵

Reducing availability is another strategy to decrease alcohol consumption and its consequences at the population level. Regulating hours of alcohol sales, controlling liquor licenses, and raising minimum legal purchasing age are examples of strategies to reduce alcohol availability. Educational initiatives also have proved effective in reducing the per-capita alcohol consumption. For example, over a period of 20 years, Iceland was able to reduce alcohol and drug use in young people from 42% to 5% by introducing a wide range of targeted policies involving families, schools, communities, and politicians.³⁸ Finally, limiting alcohol-related marketing, particularly to vulnerable populations such as youth, is an important strategy to reduce alcohol consumption.⁵

Conclusions

AUD and ALD are prevalent worldwide and are associated with significant morbidity and mortality. Currently, the individuals at highest risk of mortality are young people, women, as well as Native Americans and white Americans. Expanded screening approaches can reach individuals at high risk and those who have little contact with health care systems.

Treatment of the underlying AUD is essential for improving outcomes of patients with ALD. There are several approved medications for AUD; however, their use is cautioned in people with advanced liver disease. Alcohol rehabilitation significantly reduces 30-day hospital readmission, alcohol relapse, and mortality in individuals with ALD. Consulting addiction specialists and setting up alcohol rehabilitation at hospital discharge are quality metrics used when managing hospitalized patients with ALD.

Treatment of alcohol-associated hepatitis is centered around therapy for AUD, as well as supportive medical therapies and nutrition. Corticosteroids improve 1-month survival in alcohol-associated hepatitis, but the potential side effects limit their use. Additionally, liver transplantation is an option for patients with severe alcohol-associated hepatitis and advanced liver disease who have failed other therapies. Listing a patient for

transplant requires a formal evaluation at a liver transplant center; moreover, health care providers should screen for ongoing alcohol use at every clinic visit, both while patients are wait-listed and after liver transplantation. In recent years, several advancements in ALD research have led to improved diagnosis, prognostication, and treatment. For example, recombinant human IL-22 is an emerging therapy that is being tested in clinical trials for the treatment of alcohol-associated hepatitis. Additionally, policy makers have an opportunity to expand regulations to help reduce the burden of heavy alcohol consumption and, consequently, ALD.

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Hepatic Cannabinoid Signaling in the Regulation of Alcohol-Associated Liver Disease

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PURPOSE: The endocannabinoid system has emerged as a key regulatory signaling pathway in the pathophysiology of alcohol-associated liver disease (ALD). More than 30 years of research have established different roles of endocannabinoids and their receptors in various aspects of liver diseases, such as steatosis, inflammation, and fibrosis. However, pharmacological applications of the endocannabinoid system for the treatment of ALD have not been successful because of psychoactive side effects, despite some beneficial effects. Thus, a more delicate and detailed elucidation of the mechanism linking the endocannabinoid system and ALD may be of paramount significance in efforts to apply the system to the treatment of ALD.

SEARCH METHODS: Three electronic databases (PubMed, MEDLINE, and Cochrane Library) were used for literature search from November 1988 to April 2021. Major keywords used for literature searches were “cannabinoid,” “cannabinoid receptor,” “ALD,” “steatosis,” and “fibrosis.”

SEARCH RESULTS: According to the inclusion and exclusion criteria, the authors selected 47 eligible full-text articles out of 2,691 searched initially. Studies in the past 3 decades revealed the opposite effects of cannabinoid receptors CB1R and CB2R on steatosis, inflammation, and fibrosis in ALD.

DISCUSSION AND CONCLUSIONS: This review summarizes the endocannabinoid signaling in the general physiology of the liver, the pathogenesis of ALD, and some of the potential therapeutic implications of cannabinoid-based treatments for ALD.

KEYWORDS: alcohol; CB1R; CB2R; cell communication; endocannabinoid; fatty liver; metabotropic glutamate receptor 5; xCT

The prevalence of alcohol use disorder has been steadily rising around the world in recent years, and reducing the burden of alcohol-associated liver disease (ALD) caused by chronic alcohol consumption has become one of the most important global health issues.^{1,2} Excessive alcohol drinking (more than 40 g of pure alcohol per day) is closely associated with increased risk of all-cause mortality including chronic diseases, such as cancer, cardiovascular conditions, and neuronal diseases.³ ALD comprises a wide spectrum of liver injury including simple steatosis, steatohepatitis, liver cirrhosis, and hepatocellular carcinoma. The predominant cause of alcohol-associated liver disease, as evident by its name, is the persistent intake of alcohol, and yet the detailed mechanisms of ALD progression remain vague.^{4,5}

ALD develops through complex signaling pathways in the liver.⁶ Chronic alcohol consumption not only elicits various responses by innate immune cells in the liver, but also contributes to the metabolic dysfunction of hepatocytes, such as the production of reactive oxygen species (ROS), the abnormal lipogenesis induced by endoplasmic reticulum stress or mitochondrial dysfunction, and the secretion of inflammatory cytokines.⁶ Apart from alcohol-induced effects, endogenous cannabinoids (endocannabinoids), which are lipid mediators, also were found to play an important role in provoking ethanol-induced hepatic steatosis.⁷ The study of endocannabinoids began with the discovery that delta 9-tetrahydrocannabinol (THC), the major psychoactive component of cannabis, binds to G-protein-coupled receptors and exhibits diverse biological effects in the brain depending on the types of functioning cells affected.⁸ Over the past 3 decades, mounting evidence has shown that in peripheral organs, endocannabinoids modulate the progression of various diseases including nonalcoholic fatty liver disease (NAFLD), liver fibrosis, and ALD.⁹ However, the underlying mechanisms and the specifics of the cannabinoid signaling are yet to be elucidated. The authors of this review recently reported, however, that alcoholic steatosis is promoted by endocannabinoid production in hepatic stellate cells (HSCs), which is mediated by metabotropic glutamate receptor 5 (mGluR5).¹⁰ This review explores cannabinoid signaling in regard to the general physiology of hepatic function, the pathogenesis of ALD, and the potential therapeutic implications for ALD.

Methods and Results of the Literature Search

In-depth literature investigation was performed for this review article. Three online databases (PubMed, MEDLINE, and Cochrane Library) were used for literature search. The major

search terms used were “cannabinoid,” “endocannabinoid,” “cannabinoid receptor,” “alcoholic liver disease,” “steatosis,” and “fibrosis.” Among the initial search results retrieved from the online databases, articles published later than April 2021 and duplicate articles were removed, and articles written in English were screened first. Then, the authors included peer-reviewed original articles on animal experiments or clinical trials and well-organized review articles relevant to the subject. Research articles without peer review, abstracts of conferences or posters, and articles with unclear research processes or insufficient data were excluded. As a result, 47 eligible full-text articles were selected from a total of 2,691 searched initially. All authors independently conducted literature searches using the same online databases, and then selected appropriate references according to the inclusion and exclusion criteria.

Cannabinoid Signaling Systems and Hepatic Function

Endocannabinoid System

Marijuana (*Cannabis sativa*) has been widely used for medical applications (e.g., analgesic, antiemetic, appetite stimulant) since its discovery in ancient times.¹¹ Now it is better known to the public for its psychoactive effects such as euphoria, relaxation, increased awareness of sensation, and alteration of conscious perception.¹² Among the 60 different ingredients of marijuana, early research focused on THC, a phytocannabinoid, as it has the strongest psychoactive property. Because of its highly lipophilic and hydrophobic properties, THC was believed to provoke its effects nonspecifically by perturbing the membrane phospholipids. This misunderstanding persisted until the revelation of two cannabinoid receptors: type 1 (CB1R) and type 2 (CB2R).¹³

In comparison to their expression in the central nervous system (CNS), such as in the brain and spine, CB1R and CB2R are relatively less distributed and work differently in peripheral organs.^{14,15} For instance, CB1R and its ligands have critical roles in the pathogenesis of chronic liver diseases, such as steatosis and liver fibrosis.^{14,15} Meanwhile, CB2R is mainly distributed in immune cells or hematopoietic organs, where it functions as a protective responder to specific pathological conditions, especially in liver fibrosis.^{16,17} Like marijuana, endocannabinoids generally consist of analogs of long-chain polyunsaturated fatty acids and have an arachidonic acid moiety that confers a strong affinity with cannabinoid receptors.¹⁸ The two most extensively studied endocannabinoids are arachidonoyl ethanolamide (AEA) and 2-arachidonoyl glycerol (2-AG).¹⁸

The components and signaling pathways of the endocannabinoid system are similar in most organs throughout the body.¹⁸ As endogenous or exogenous cannabinoids arrive at target cells, both CB1R and CB2R are stimulated with heterotrimeric G-proteins and suppress adenylate cyclase to inhibit the phosphorylation of protein kinase A. In contrast, mitogen-activated protein kinase is stimulated to regulate additional gene expressions.^{14,18} In the case of CB1R, when heterodimeric G-protein is stimulated, it directly inhibits the membrane's calcium channels and stimulates the potassium channels to inhibit the release of neurotransmitters in neuronal cells.¹⁴ However, the activation of cannabinoid receptor-mediated signaling pathways may differ depending on the type of cells stimulated.¹⁸

Endocannabinoid Production and Degradation

Endocannabinoids are biosynthesized through various pathways from several precursors of phospholipids located in the cellular membrane. Figure 1 schematically summarizes the biosynthesis and degradation pathways of endocannabinoids AEA and 2-AG.^{11,14,19} N-arachidonoyl-phosphatidylethanolamine (NAPE), a phospholipid precursor located in the cell membrane, is preferentially synthesized from glycerophospholipid and phosphatidylethanolamine by N-acyltransferase (NAT) and sequentially hydrolyzed by the NAPE-specific phospholipase D (NAPE-PLD) in response to stimulation, subsequently resulting in the production of AEA (see Figure 1).¹⁹ Degradation of AEA involves its hydrolysis into arachidonic acid and ethanolamine by a number of enzymes, namely fatty acid amide hydrolase (FAAH) and N-acylethanolamine-hydrolyzing acid amidase (NAAA), in the intracellular space.^{20,21} As for 2-AG, *sn*-1-acyl-2-arachidonoyl-glycerol (DAG) is first produced from the intracellular glycerophospholipid by phospholipase C at the plasma membrane. Then, DAG is subsequently hydrolyzed by diacylglycerol lipase (DAGL) to 2-AG.²² Although the chemical structures of DAGL-alpha and DAGL-beta are slightly different, their preference for ligands is similar.¹⁴ Interestingly, a study has shown that DAGL-alpha has a more dominant role over DAGL-beta in regulating the levels of 2-AG in the brain, but the opposite was observed in the liver. In fact, only DAGL-beta, but not DAGL-alpha, has been reported to be expressed in HSCs of fatty mouse liver.^{7,10} Unlike AEA, 2-AG is believed to be degraded into arachidonic acid and glycerol by several enzymes, FAAH, and monoacylglycerol lipase (MAGL).²²

Generally, the activation of both NAPE-PLD and DAGL is triggered by changes in the intracellular calcium signaling.^{12,20} When calcium influx occurs in a cell by a specific stimulus, the intracellular concentration of AEA or 2-AG increases due to the activation of endocannabinoid-producing enzymes. The

newly synthesized endocannabinoids are then transported from the cytoplasm out of the cell by a specific transporter, the endocannabinoid membrane transporter.^{11,21} Because of their hydrophobic properties, the released endocannabinoids have high binding affinities to the membrane, enabling them to rapidly bind to their specific receptors and induce biological responses in the neighboring cells. For instance, the AEA and 2-AG generated by the activation of endocannabinoid-producing enzymes stimulate hepatic CB1R to induce *de novo* lipogenesis in nonalcoholic and alcoholic fatty liver.^{7,23} In general, 2-AG acts as a full agonist at these cannabinoid receptors, whereas AEA has a weaker potency as an agonist.¹³ Although levels of 2-AG and AEA in peripheral tissues vary, 2-AG (~ 0.8 pmol/mg tissue) is maintained at higher levels than AEA (~ 1.1 fmol/mg tissue) in the liver.⁷ In terms of alcohol-mediated endocannabinoid production, studies have demonstrated that chronic ethanol exposure or consumption induces 2-AG production in cerebellar granule neurons *in vitro* or in HSCs *in vivo*, respectively.^{7,10,24}

Cannabinoid Receptor Expression

In line with their differences in synthesis, AEA and 2-AG have different affinities for their respective cannabinoid receptors.¹² AEA has a stronger affinity for CB1R than for CB2R, whereas 2-AG has a similar affinity for both CB1R and CB2R. In addition, AEA and 2-AG are also known to bind receptors other than the cannabinoid receptors, such as the transient receptor potential vanilloid type 1 (TRPV-1) and the orphan G protein-coupled receptors 55 (GPR55) and 119 (GPR119).^{14,19} However, with little being known, the detailed physiological effect of endocannabinoid binding to these non-cannabinoid receptors on the cellular pathophysiology in the liver remains enigmatic.

Once the endocannabinoids, either synthetic or endogenous, bind to their cannabinoid receptors, both the CB1R and CB2R get stimulated enough to rapidly transduce extracellular signals into cells.^{25,26} With regards to their expression, they are widely distributed throughout our body as summarized in Figure 2. CB1R is predominantly distributed in the central and peripheral nervous system, including the sensorial peripheral and sympathetic nerves in humans and mice.²⁶ However, abundant evidence has confirmed that CB1R is also characteristically expressed in several peripheral tissues and organs, including liver, lung, gastrointestinal tract, urinary tract, thyroid, pancreas, heart, vascular endothelium, adipose tissue, reproductive organs, skeletal muscles, and immune system (see Figure 2).^{11,25} Unlike CB1R, CB2R is mainly expressed in cells and organs that are responsible for controlling peripheral hematopoiesis or immune functions (see Figure 2).^{25,26} For example, macrophages, neutrophils, monocytes, B lymphocytes, T lymphocytes, and microglial cells are representative of CB2R-expressing cells.

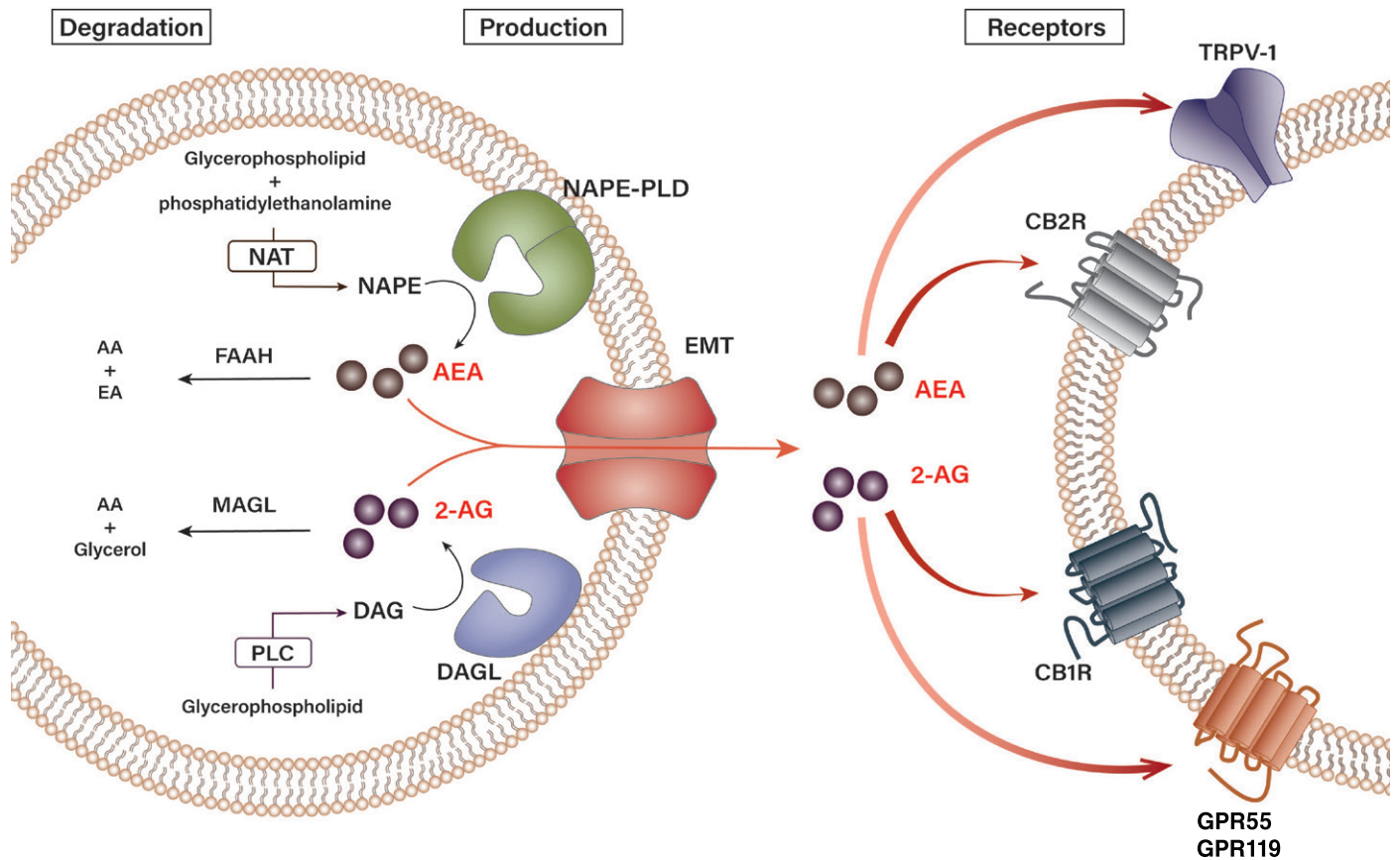


Figure 1. Biosynthesis and degradation pathways of endocannabinoids. Endogenous cannabinoids (endocannabinoids)—arachidonoyl ethanolamide (AEA) and 2-arachidonoyl glycerol (2-AG)—have distinct pathways of synthesis and degradation in cells. N-arachidonoyl-phosphatidylethanolamine (NAPE) is synthesized from glycerophospholipid and phosphatidylethanolamine by N-acyltransferase (NAT). Upon stimulation, NAPE subsequently gets hydrolyzed by NAPE-specific phospholipase D (NAPE-PLD) to produce AEA. Synthesis of 2-AG begins with the production of *sn*-1-acyl-2-arachidonoyl-glycerol (DAG) from glycerophospholipid by phospholipase C (PLC), which is then hydrolyzed by diacylglycerol lipase (DAGL) to 2-AG. The synthesized AEA and 2-AG are transported out of the cell by an endocannabinoid membrane transporter (EMT). The released AEA and 2-AG then bind their cannabinoid and noncannabinoid receptors in the neighboring cells to transduce extracellular signals. 2-AG binds both cannabinoid-1 receptor (CB1R) and cannabinoid-2 receptor (CB2R) with similar affinity, whereas AEA has a stronger affinity for CB1R. 2-AG and AEA also bind transient receptor potential vanilloid type-1 (TRPV-1) and orphan G protein-coupled receptors 55 (GPR55) and 119 (GPR119). AEA is hydrolyzed into arachidonic acid (AA) and ethanolamine (EA) by fatty acid amide hydrolase type-1 (FAAH-1) and type-2 (FAAH-2), and N-acyl ethanolamine-hydrolyzing acid amidase (NAAA), whereas 2-AG is degraded into AA and glycerol by monoacylglycerol lipase (MAGL) and FAAH.

Recently, an increasing number of reports have expanded the scope of peripheral tissue known to contain CB2R to include skin nerve fibers, keratinocytes, bone cells (i.e., osteoblasts, osteocytes, and osteoclasts), and somatostatin-secreting cells in the pancreas.²⁷

Cannabinoid Receptor Activation in the Liver

Early research on endocannabinoids focused on demonstrating the mechanism of psychoactive symptoms and their neurologic signals caused by the stimulation of CB1R in the brain.^{13,26} However, little attention was paid to the biological roles of the hepatic endocannabinoid system despite the discovery of

cannabinoid receptors in the liver.⁹ Nowadays, emerging lines of evidence have shown that diverse types of the hepatic cells not only express CB1R or CB2R but also employ them in the hepatic pathophysiology, drawing attention to the critical correlation between chronic liver diseases and cannabinoid receptor signaling.²⁸

Hepatocytes, the parenchymal cells of the liver, mainly express CB1R, but the level of expression is relatively low in the homeostatic condition (see Figure 2). However, CB1R expression is tremendously elevated in pathological conditions, such as alcoholic and nonalcoholic steatosis, primary biliary cirrhosis, and hepatocellular carcinoma.^{9,19,29} CB2R is rarely

expressed in the steady state of the liver, but its expression is elevated in immune cells during the occurrence of hepatic regeneration and diseases such as NAFLD, fibrosis, and hepatocellular carcinoma.^{29,30} As opposed to the hepatocytes, the cannabinoid signaling in hepatic nonparenchymal cells is relatively less explored. CB1R expression in HSCs was shown to have increased significantly in the rodent fibrosis model and cirrhotic human liver,^{11,21} suggesting that endocannabinoids can act as pro-fibrogenic mediators in the liver. Moreover, the authors' previous studies have demonstrated that alcoholic steatosis is exacerbated through CB1R activation in hepatocytes by 2-AG produced from HSCs.^{7,10} CB1R is also expressed in cholangiocytes, or bile duct epithelial cells, which are related to the pathophysiology of liver cirrhosis and primary biliary cirrhosis.³¹ Furthermore, several studies have identified the close association of CB2R expressions in hepatic nonparenchymal cells and NAFLD progression, but detailed mechanisms have yet to

be investigated. The distribution of the cannabinoid receptors in hepatic cells is briefly described in Figure 2.

Cannabinoid Signaling in the Pathogenesis of ALD

Alcohol Exposure and the Endocannabinoid System in ALD

Because alcohol exposure is considered a critical factor in causing complex physiological or pathological changes in the endocannabinoid system, curiosity about the biological function of cannabinoid receptors in ALD began to arise.^{9,28} Consequently, the endocannabinoid system and its receptors were found to be involved in the pathophysiological mechanisms of ALD by regulating immune function, metabolic modulation, and

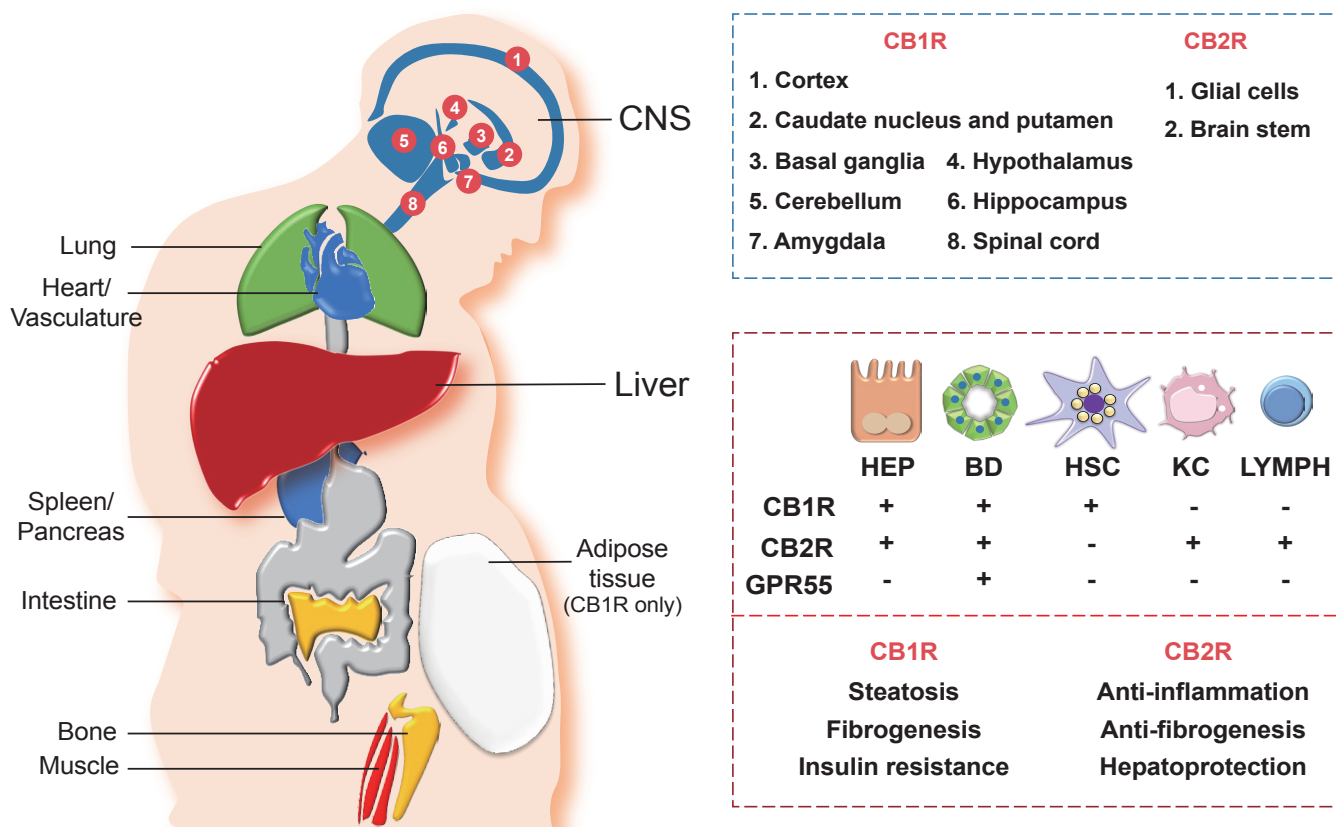


Figure 2. Distribution of cannabinoid receptors in various organs and hepatic cells. Cannabinoid receptors, cannabinoid-1 receptor (CB1R) and cannabinoid-2 receptor (CB2R), are expressed in various central and peripheral organs. CB1R and CB2R are most abundantly expressed in the central nervous system (CNS), where different parts of the CNS express either CB1R or CB2R (blue box). Both CB1R and CB2R are also expressed in peripheral organs including the heart, lung, spleen, pancreas, intestine, bone, muscle, and liver, as well as in the vascular system. Adipose tissues only express CB1R. In the liver, diverse types of cells—including hepatocytes (HEP), cholangiocytes (bile duct [BD] epithelial cells), hepatic stellate cells (HSC), Kupffer cells (KC), and lymphocytes (LYMPH)—differentially express cannabinoid receptors (CB1R and CB2R) and orphan G protein-coupled receptor 55 (GPR55), a noncannabinoid receptor that binds with endocannabinoids 2-AG and AEA (red box, top). Different functions of CB1R and CB2R in the liver are also indicated (red box, bottom).

inflammatory response in the onset and progression of ALD.^{29,32} Because the expression of CB1R and CB2R is well identified in hepatocytes and various nonparenchymal cells in the liver, accurate comprehension of the regulatory mechanisms by which alcohol exposure generates or stimulates the production of endocannabinoids—as well as the effects of alcohol on the activation of cannabinoid receptors—could lead to a breakthrough in understanding the exact pathophysiology of ALD and in discovering potential therapeutic targets.

Alcoholic Liver Injury Through Cannabinoid Signaling

The pathological changes in the endocannabinoid system can lead to the development of several chronic liver diseases. Because the expressions of CB1R and CB2R increase in pathological conditions such as NAFLD, primary biliary cirrhosis, liver cirrhosis, and hepatocellular carcinoma, the hepatic endocannabinoid system is most likely to affect the onset of ALD.^{9,28,29}

With the liver as the principal organ of alcohol metabolism, the majority of the alcohol consumed enters the liver to be metabolized, consequently activating the stress responses such as the production of ROS, inflammatory cytokines, or endoplasmic reticulum stress. These responses result in reduced fatty acid oxidation and enhanced hepatic lipogenesis.⁶ Several animal experiments have established that chronic alcohol consumption could exacerbate alcoholic fatty liver by triggering abnormal CB1R-mediated signaling.^{7,10} However, the authors' recent studies have clearly demonstrated that chronic alcohol consumption induces oxidative stress-mediated glutamate excretion from hepatocytes, which triggers the activation of mGluR5 to produce 2-AG, but not AEA, in HSCs via DAGL-beta. This, in turn, stimulates paracrine activation of hepatic CB1R,^{7,10} which leads to the subsequent elevation of the expression of sterol regulatory element-binding protein-1c (SREBP1c), a representative lipogenic transcription factor located downstream of the CB1R signaling pathway.^{7,30} As a result, the expression of target proteins of SREBP1c—namely acetyl coenzyme A (CoA) carboxylase and fatty acid synthase—are elevated, thereby inducing de novo lipogenesis in hepatocytes (see Figure 3).^{23,33} This study served as a crucial opportunity to identify the involvement of the endocannabinoid system in metabolic regulation through bidirectional interaction between hepatocytes and HSCs in the liver. The fatty acids produced are then converted into triglyceride (TG), which should be excreted from the liver in the form of TG-rich very-low-density lipoprotein (VLDL). However, pharmacological blockade of CB1R (AM6545 and rimonabant) decreases the hepatocytes' ability to clear TG-rich VLDL, significantly reducing hepatic TG levels and markedly increasing the release of TG-rich VLDL in alcoholic and nonalcoholic fatty liver.^{7,34}

In alcoholic liver injury and inflammation, the various types of ROS are one of the most important influential factors in the progression of ALD. The ROS is mainly generated through two metabolizing pathways that utilize different enzymes or proteins: alcohol dehydrogenase and cytochrome P450 2E1 (CYP2E1), which is a membrane protein that forms the cytochrome P450-dependent microsomal ethanol oxidizing system.⁶ The importance of ROS in alcoholic liver injury has been portrayed in a study that reported the close relationship between the endocannabinoid system and ROS-induced liver injury in the pathophysiology of chronic alcohol consumption.³⁵ In this study, ethanol-induced 2-AG preferentially induced CB1R activation, followed by an upregulation in gene expression of estrogen-related receptor gamma (ERR-gamma), an orphan nuclear receptor. The authors explained that the increased expression of ERR-gamma enhances CYP2E1 induction, resulting in ROS-induced alcoholic liver injury. In addition, when ethanol was fed chronically to CB1R knockout mice, the expression of ERR-gamma and CYP2E1 decreased and alcoholic liver injury was significantly attenuated. Furthermore, administration of GSK5182, which is a selective inverse agonist of ERR-gamma, ameliorated alcoholic liver injury by reducing oxidative stress, confirming the criticality of cannabinoid receptor signaling in ROS-induced alcoholic liver injury.³⁵ Among the various inflammatory pathways activated in ALD, Kupffer cells, which are macrophages that reside in liver tissue, execute a crucial role in the onset of hepatic inflammation.⁶ Currently, the most well-known mechanism of Kupffer cell activation is via lipopolysaccharide (LPS)/toll-like receptor 4 (TLR4) stimulation, by which the Kupffer cells acquire a pro-inflammatory phenotype.⁶ Like other cells in the immune system, Kupffer cells mainly express CB2R rather than CB1R, and activation of CB2R exerts an anti-inflammatory property on Kupffer cells in the development of ALD.²⁹ In fact, when wild-type mice were fed with alcohol, Kupffer cells were polarized to the anti-inflammatory (M2) phenotype, whereas the pro-inflammatory (M1) phenotype was amplified in CB2R-deficient Kupffer cells in response to LPS stimulation.³⁶ In line with this observation, Kupffer cells also have been shown to acquire a protective property via the activation of their CB2R as regulated by an autophagy-dependent pathway, which further supports the essential role of CB2R in Kupffer cells.³⁷ Moreover, chronic alcohol consumption instigates the disruption of the intestinal epithelium, causing changes in gut permeability and increasing the level of LPS in the hepatic portal flow. Consequently, Kupffer cells become activated by TLR4. A study by Szabady et al. suggested a conceivable interplay between intestinal endocannabinoids and ALD. The authors demonstrated that intestinal endocannabinoids produced by epithelial cells could prevent inflammation and maintain homeostasis in a healthy gut by modulating neutrophil influx.³⁸ Thus, intestinal

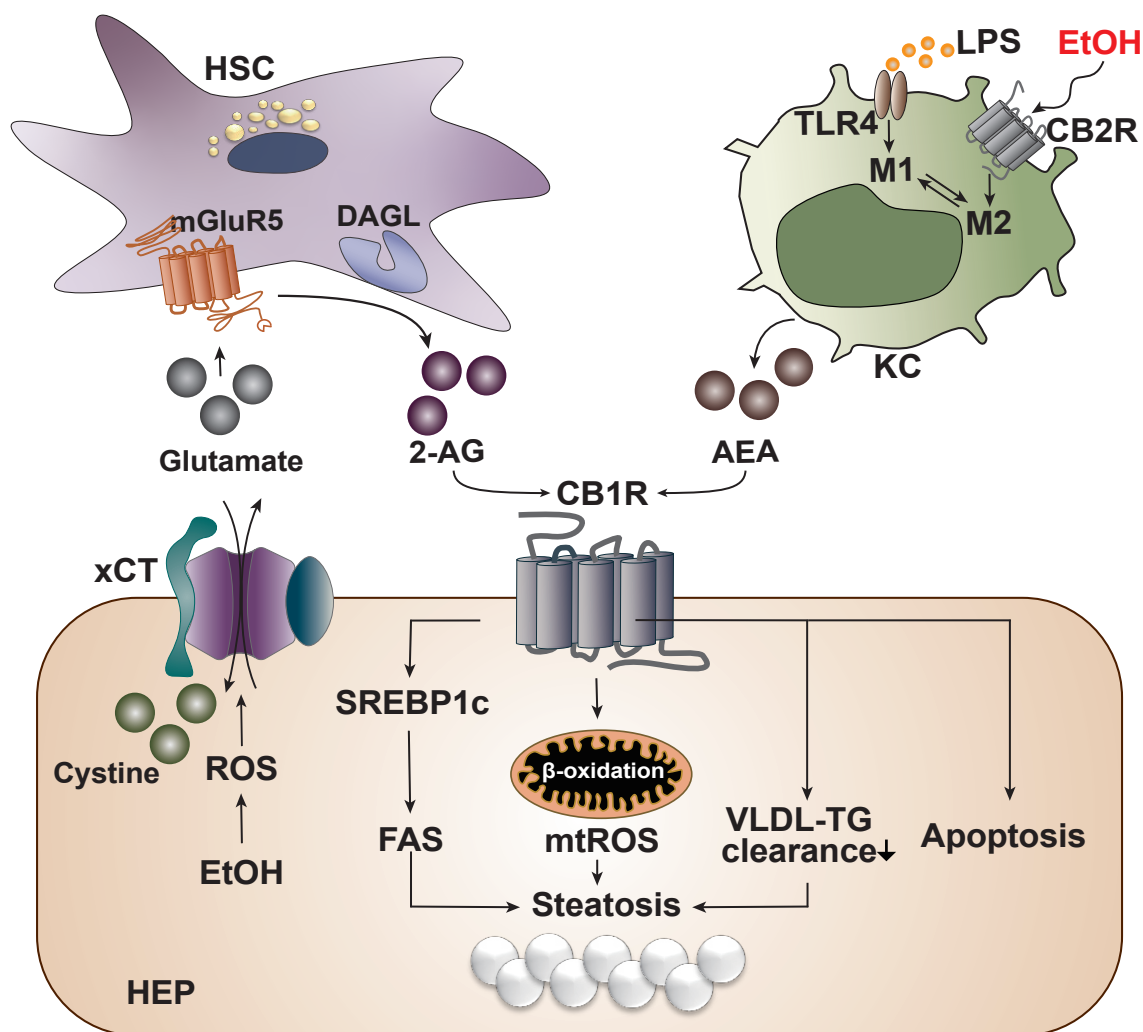


Figure 3. Cannabinoid signaling in the pathogenesis of alcohol-associated liver disease. Alcohol is mainly metabolized in hepatocytes (HEP) of the liver during which reactive oxygen species (ROS) is generated as a cellular stress response. The generated ROS stimulates and activates a cystine/glutamate antiporter (xCT) for the influx of cystine in exchange for the efflux of glutamate. The excreted glutamate then binds to a metabotropic glutamate receptor 5 (mGluR5) expressed in the neighboring hepatic stellate cells (HSC), inducing the production of 2-arachidonoyl glycerol (2-AG) by diacylglycerol lipase (DAGL). 2-AG produced in the HSC binds to cannabinoid-1 receptors (CB1R) expressed in the plasma membrane of neighboring HEP to induce de novo lipogenesis via the upregulation of sterol regulatory element-binding protein 1c (SREBP1c) and fatty acid synthase (FAS). This forms a bidirectional paracrine loop pathway through which HEP and HSC in close proximity can metabolically regulate each other. Activation of CB1R can also induce β -oxidation of fatty acids in mitochondria, generating mitochondrial ROS (mtROS), which ultimately contributes to the accumulation of fat, or steatosis. Activated CB1R perturbs the excretion of triglyceride (TG) in the form of TG-rich very low-density lipoprotein (VLDL), further contributing to hepatic steatosis. CB1R activation is also known to induce apoptosis of cells. Kupffer cells (KC) normally become activated via the lipopolysaccharide (LPS)/toll-like receptor 4 (TLR4) stimulation and acquire a pro-inflammatory (M1) phenotype. However, when the CB2R expressed in Kupffer cells are stimulated by ethanol, they obtain an anti-inflammatory (M2) phenotype. Activated Kupffer cells then produce arachidonoyl ethanolamide (AEA), which also binds and activates CB1R in the neighboring HEP.

endocannabinoids might play beneficial roles in ALD-mediated gut leakage and the subsequent translocation of LPS to the liver.

CB1R also was found to modulate alcohol-induced liver fibrosis.³⁹ A study conducted by Patsenker et al. observed a strong expression of CB1R in the fibrotic septa of patients with

alcohol-associated liver cirrhosis, and genetic and pharmacologic inhibition of CB1R attenuated both the hepatic inflammation and the alcoholic liver fibrosis by suppressing HSC activation.³⁹ Although it is well established that CB1R is involved in the development of hepatic steatosis and fibrosis, relatively few

studies have examined the role of CB2R in the pathophysiology of ALD. In a comparison study for the severity of hepatic steatosis, inflammation, and fibrosis using CB1R and CB2R knockout mice, the CB2R knockout mice showed severe fibrosis with aggravated steatosis and inflammation compared to those of the wild-type and CB1R knockout mice. This observation could be explained by the fact that the collagen production in activated HSCs was amplified in CB2R knockout mice,⁴⁰ indicating the protective role of CB2R in the progression of alcoholic liver fibrosis.

In brief, endocannabinoids have been found to have diverse effects on the pathophysiology of chronic liver disease, and various *in vivo* and *in vitro* experiments have been performed to investigate the characteristics of CB1R and CB2R in different types of ALD. To date, it is known that CB1R activation aggravates inflammation, steatosis, and fibrosis through the reduction of fatty acid oxidation and TG-VLDL secretion, enhanced *de novo* lipogenesis, and activation of HSCs, whereas CB2R inhibits inflammation and steatosis and has anti-fibrotic properties by exerting anti-inflammatory functions on Kupffer cells.^{29,32} Figure 3 summarizes the opposite roles of CB1R and CB2R in the progression of ALD.

Glutamate-Mediated Endocannabinoid Production

As described earlier, one of the key mechanisms underlying the development of alcoholic fatty liver is the CB1R-mediated *de novo* lipogenesis in hepatocytes via the metabolic loop pathway.⁷ However, questions remain as to which metabolic triggers lead to increased production of 2-AG in HSCs. Recently, the authors of this review substantiated that oxidative stress mediates the excretion of glutamate from the hepatocyte, stimulating the activation of mGluR5, which binds to glutamate, in nearby HSCs and leading to increased 2-AG production (see Figure 3).¹⁰ Similar to other reports, this report also found that chronic alcohol consumption depleted antioxidant glutathione through the inhibition of the methionine cycle and the transsulfuration system, resulting in a shortage of cysteine. However, this study had a more striking discovery. First, the CYP2E1-mediated ROS production in hepatocytes significantly increased the xCT (cystine/glutamate antiporter)-mediated uptake of extracellular cystine, in exchange for the excretion of cytosolic glutamate, to compensate for the glutathione deficiency. Second, this parallel release of glutamate stimulated activation of mGluR5 in HSCs, which led to the production of 2-AG through mediation by DAGL-beta. As a result, the 2-AG produced activated CB1R in neighboring hepatocytes, inducing *de novo* lipogenesis. These findings suggest a bidirectional paracrine loop between hepatocytes and HSCs, named the “metabolic loop pathway,” where both hepatocytes and HSCs regulate each other by

either producing a neurotransmitter or expressing its receptor. Thus, the authors proposed a novel view of concept through this bidirectional signaling that utilizes a neurotransmitter, an endocannabinoid, and their respective receptors to operate at a metabolic synapse between hepatocytes and HSCs. *In vivo* experiments using genetic or pharmacologic inhibition of xCT or mGluR5 showed an improvement in alcohol-induced hepatic steatosis. More interestingly, plasma levels of glutamate were found to be elevated in ALD patients with hepatic steatosis and hepatitis but not in patients with fibrosis and cirrhosis, which suggests that the function of glutamate is not limited to the hepatic steatosis and further studies are strongly required to address this curiosity. In summary, the discovery of a bidirectional loop pathway between hepatocytes and HSCs suggested a new mechanism for the development of ALD, proposing the possibility of its application as a novel pharmacological target or an opportunity for glutamate as a prospective diagnostic marker in ALD.

Therapeutic Implications for ALD

Past and Current Pharmacological Approaches

Various animal experiments have established that hepatic endocannabinoids and their receptors play fundamental roles in the pathophysiology of chronic liver diseases, and pharmacological targeting of CB1R and CB2R for the treatment of liver diseases has been attempted.²⁹ Table 1 summarizes the effects of cannabinoid receptor-modulating drugs and their targets in animal models of ALD to date. Unfortunately, most clinical trials have been performed on patients with obesity, metabolic syndrome, and NAFLD, and only a few studies have explored and reported the beneficial efficacies of CB1R antagonists in the progression of hepatic steatosis, inflammation, and fibrosis.^{21,25} In fact, clinical trials of cannabinoid receptor inhibitors have not been carried out in patients with ALD owing to the side effects of the drugs. For example, in a meta-analysis of nine clinical trials, adverse events, such as depression, anxiety, and nausea, were commonly observed with rimonabant at a dose of 20 mg per day for 6 to 24 months even though it had clinically meaningful results in metabolic disorders.⁴¹

Recently, a chemical compound that acts as a peripherally restricted antagonist of CB1R has been developed, which showed negligible CNS penetration and remarkable attenuation of alcoholic steatosis in mice.⁴² Thus, there is a silver lining in the possibility that with refinement and adjustment, this chemical might be a profound lead compound that could undergo clinical trials as a novel therapeutic target. In short, a growing number of experimental findings on the involvement of hepatic endocannabinoids in the pathophysiology of ALD has enabled the development of endocannabinoid-based or cannabinoid

Table 1 Effects of Various Cannabinoid Receptor–Modulating Drugs and Their Target Cells in Different Animal Models of Alcohol-Associated Liver Disease, by Pharmacological Trial

Trial	Reagent	Receptor	Target Cell	Action	Research Model	Effect and Results
Jeong et al. (2008) ⁷	Rimonabant	CB1R	Hepatocyte	Antagonist	Alcoholic fatty liver	Reduce steatosis (Lipogenesis↓, fatty acid oxidation↑)
Patsenker et al. (2016) ¹⁹	Rimonabant	CB1R	HSC	Antagonist	In vitro experiment	Induce apoptosis Reduce pro-fibrotic property
Louvet et al. (2011) ³⁶	JWH-133	CB2R	Kupffer cell	Agonist	Alcoholic fatty liver	M2 polarization of Kupffer cell (Steatosis↓, inflammation↓)
Kim et al. (2013) ³⁵	GSK5182	ERR-gamma	Hepatocyte	Antagonist	Alcoholic fatty liver and inflammation	Reduce oxidative stress (CYP2E1 expression↓, hepatocyte apoptosis↓)
Amato et al. (2018) ⁴²	Compounds 25	CB1R	Hepatocyte	Antagonist	Alcoholic fatty liver	Peripherally restricted purine antagonist
Choi et al. (2019) ¹⁰	CTEP	mGluR5	HSC	Antagonist	Alcoholic fatty liver	Inhibit mGluR5 and reduce steatosis (Lipogenesis↓, CB1R expression↓)
Choi et al. (2019) ¹⁰	Sulfasalazine	xCT	Hepatocyte	Antagonist	Alcoholic fatty liver	Inhibit xCT and reduce steatosis (Lipogenesis↓, CB1R expression↓)

Note: The upward arrow (↑) indicates an increase, and the downward arrow (↓) indicates a decrease. CB1R, cannabinoid-1 receptor; CB2R, cannabinoid-2 receptor; CYP2E1, cytochrome P450 family 2 subfamily E member 1; ERR-gamma, estrogen-related receptor-gamma; HSC, hepatic stellate cell; mGluR5, metabotropic glutamate receptor 5; xCT, cystine/glutamate antiporter.

receptor-based pharmacological approaches that, it is hoped, could become a novel therapeutic strategy for ALD.

Limitation of the Current Cannabinoid-Based Treatment

Until now, there have been several clinical trials and reports in which a CB1R antagonist has been administered as treatment for obesity or metabolic risk factors.⁴³⁻⁴⁵ The two most notable clinical trials are the ADAGIO-Lipids Trial and the Rimonabant in Obesity (RIO)-Europe study. In these clinical trials, cardiometabolic risk markers, such as body weight and lipid profiles, improved significantly when rimonabant, a well-known CB1R-selective antagonist, was administered to obese patients for 1 or 2 years, but the treatments were discontinued because of the psychiatric side effects including anxiety and depression.⁴⁵ Since then, the development of drugs with a mode of action restricted to the endocannabinoid system in the periphery has been undertaken. For example, peripheral organ-specific CB1R inverse agonist and antagonist (i.e., JD5037 and AM6545) were developed to reduce neuropsychiatric side effects, which were successful in

reducing and improving cardiometabolic risks and hepatic steatosis in animal experiments.^{34,46}

Apart from the CB1R antagonist, the pharmacological potential of the CB2R agonist, which is known to have hepatoprotective effects, also has been reevaluated.³⁶ Although only observed in mice, a study has confirmed that administration of JWH-133 (a CB2R agonist) exhibited improved alcoholic liver injury in mice by inducing the polarization of Kupffer cells into an M2 phenotype.^{36,37} Interestingly, according to a recent cross-sectional study, cannabis users showed a significantly reduced prevalence of ALD of all spectrums (alcoholic steatosis, alcoholic steatohepatitis, alcohol-associated cirrhosis, and hepatocellular carcinoma). However, the underlying mechanism remains in question.⁴⁷ Based on the description above, one could speculate that the cannabis absorbed might activate CB2 receptors in immune cells or prevent intestinal leakage of endotoxins including LPS. Therefore, to date, no drugs targeting the endocannabinoid system are available for direct application to clinical trials in ALD patients, and further studies are required to study underlying mechanisms and to develop a treatment specifically effective for ALD.

Conclusions

Endocannabinoids are membranous lipid mediators that regulate diverse physiological functions in both the CNS and the peripheral organs, including the liver. Over the past 30 years, it has been found that the endocannabinoid system is involved in a variety of pathways associated with the onset, or the progression, of several diseases, including ALD. The endocannabinoid system has been observed in both the hepatocytes and various nonparenchymal cells in the liver, in which the endocannabinoid production and its receptor activation may contribute to the development of a spectrum of ALD, ranging from simple alcoholic steatosis to more severe forms such as steatohepatitis and fibrosis. Therefore, understanding the precise physiology of the endocannabinoid system in the liver and unveiling the mechanism underlying the association between ALD progression and hepatic endocannabinoid signaling seem to bear a paramount significance for the advancement of ALD treatment, as well as for the treatment of other chronic liver diseases (e.g., NAFLD, viral hepatitis). Moreover, developing efficacious and highly selective cannabinoid receptor-modulating drugs could be a major breakthrough in the treatment of ALD.

However, efforts to develop second- and third-generation CB1R antagonists must overcome the complications caused by the first generation of CB1R antagonists, which were able to penetrate the blood-brain barrier and produced critical psychiatric side effects. Furthermore, careful implication of the combinatorial effects of CB1R antagonist and CB2R agonist may bring about promising outcomes for the treatment of ALD in the future.

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NATURAL RECOVERY BY THE LIVER AND OTHER ORGANS AFTER CHRONIC ALCOHOL USE

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Chronic, heavy alcohol consumption disrupts normal organ function and causes structural damage in virtually every tissue of the body. Current diagnostic terminology states that a person who drinks alcohol excessively has alcohol use disorder. The liver is especially susceptible to alcohol-induced damage. This review summarizes and describes the effects of chronic alcohol use not only on the liver, but also on other selected organs and systems affected by continual heavy drinking—including the gastrointestinal tract, pancreas, heart, and bone. Most significantly, the recovery process after cessation of alcohol consumption (abstinence) is explored. Depending on the organ and whether there is relapse, functional recovery is possible. Even after years of heavy alcohol use, the liver has a remarkable regenerative capacity and, following alcohol removal, can recover a significant portion of its original mass and function. Other organs show recovery after abstinence as well. Data on studies of both heavy alcohol use among humans and animal models of chronic ethanol feeding are discussed. This review describes how (or whether) each organ/tissue metabolizes ethanol, as metabolism influences the organ's degree of injury. Damage sustained by the organ/tissue is reviewed, and evidence for recovery during abstinence is presented.

KEY WORDS: alcohol use disorder; alcohol-associated liver disease; alcohol abstinence; alcohol cessation; alcohol; alcoholic pancreatitis; alcoholic cardiomyopathy

INTRODUCTION

A vast body of evidence from human studies and animal research clearly indicates that chronic, heavy alcohol consumption causes structural damage and/or disrupts normal organ function in virtually every tissue of the body. In heavy consumers of alcohol, the liver is especially susceptible to alcohol-induced injury.^{1,2} Additionally, several other organs—including the gastrointestinal (GI) tract, pancreas, heart, and bone—exhibit impaired function after chronic ethanol use.³

As the largest internal organ and the first to see blood-borne nutrients, toxins, and xenobiotics absorbed from the GI tract, the liver is especially vulnerable to alcohol-induced damage. The liver plays a key role in the body's metabolic regulation and is a "frontline" organ that rapidly metabolizes (i.e., chemically converts or oxidizes) alcohol to less harmful substances. However, acetaldehyde, the first metabolite generated by alcohol oxidation is actually more toxic than alcohol, but acetaldehyde is rapidly converted to acetate for use in other biochemical reactions in the body.³ Thus, although the liver has the capacity to eliminate toxic substances, continual excessive alcohol consumption can seriously damage the liver and other organs. Recent studies report that alcohol-associated liver disease (ALD) is one of the leading preventable causes of illness and death from liver disease in the United States and the world.⁴

After drinking stops, damaged organs may regain partial function or even heal completely, depending on the extent of organ damage and whether there is relapse (i.e., resumption of drinking). Organ damage due to heavy drinking is greatest in the liver, in part because the liver has higher levels of enzymes that catalyze the metabolism of acetaldehyde from alcohol. Acetaldehyde is more toxic than ethanol because it is highly reactive and binds to biomolecules (e.g., proteins, lipids, nucleic acids) and disrupts their function.^{3,5} However, even after years of chronic alcohol use, the liver has remarkable regenerative capacity and, after sustained cessation of drinking, can recover a significant amount of its original mass.⁶

This review examines injury to selected organs and tissues from chronic alcohol use and their

"natural recovery" after drinking ceases. Data have been obtained from both human studies and studies with experimental animal models of alcohol administration. The main points of emphasis will be how ethanol, the active ingredient and principal component in alcoholic beverages, affects the liver, GI tract, pancreas, heart, and bone. This review describes how (or whether) each organ/tissue metabolizes ethanol, as this property is closely related to the organ's degree of injury. The damage sustained by the organ/tissue is then described, and the evidence for natural recovery after drinking cessation is reviewed. It is important to emphasize that "natural recovery" is that which is unaided by external agents that directly enhance healing of the damaged organ or tissue. In the case of the liver, such agents include drugs or other compounds that suppress inflammation or dietary or medicinal compounds (e.g., betaine, caffeine, aspirin), which alleviate tissue damage by enhancing protective pathways, thereby preventing further damage. Throughout the article, "alcohol" and "ethanol" are used interchangeably, given that they have the same meaning.

LIVER

Alcohol Metabolism in the Liver

In humans (and other animals, such as rodents), the liver is the primary site of alcohol metabolism. The same two enzymes catalyze ethanol oxidation in both species. The major, most catalytically efficient enzyme is alcohol dehydrogenase (ADH), which catalyzes the formation of acetaldehyde from alcohol. The other enzyme, cytochrome P450 2E1 (CYP2E1), is catalytically less efficient than ADH, but it increases in both content and activity severalfold after continual alcohol exposure.³ This increase, called "induction," further accelerates alcohol conversion to acetaldehyde, which is rapidly detoxified by its conversion to acetate by the enzyme aldehyde dehydrogenase (ALDH).^{3,7} Many laboratories utilize rodent models to examine ALD to elucidate the mechanisms responsible for such injury. As in humans, fatty liver (steatosis) is the earliest pathophysiological change that occurs in rodent livers after chronic alcohol administration.

In rodent models, with continued drinking, hepatic steatosis can worsen to further injury such as alcoholic steatohepatitis (ASH). Fibrosis and cirrhosis occur when nutrients such as choline and/or methionine are deleted from the diet, when an endotoxin is simultaneously administered to increase injury, or after continual intragastric infusion of high levels of alcohol in liquid diets.⁸ Other studies have administered alcohol to nonhuman primates (baboons) to induce liver fibrosis.⁹ However, most laboratories utilize rodent models, which are more manageable and can be used in greater numbers than nonhuman primates.

Liver Injury and Recovery After Chronic Alcohol Use in Humans

Fatty liver (steatosis), characterized by an accumulation of lipids in hepatocytes, is one of the earliest pathological changes in the progression of ALD. More than 90% of people who drink heavily consume up to 60 grams or more of ethyl alcohol per day. Most of these individuals develop fatty liver.² Once the liver becomes steatotic, it is more prone to damage by inflammatory mediators (tumor necrosis factor, endotoxin) and/or toxic agents, leading to progression to ASH, fibrosis, and eventually cirrhosis and, in some cases, hepatocellular carcinoma. Even though virtually all heavy-alcohol consumers develop fatty liver, only about 20% to 40% of such people develop steatohepatitis, and a subset of these latter individuals develop the more advanced stages of ASH, cirrhosis, and hepatocellular carcinoma.¹⁰ Progression to further injury depends on the genetic constitution of individuals, their lifestyle (diet and exercise), and their exposure to viral infections, all of which contribute to disease progression and severity.¹¹ The actual mechanisms involved in ALD development are complex and multifactorial, including gut and other tissue dysfunctions that influence liver pathology. Other parts of this review describe such dysfunctions in greater detail. Abstinence from alcohol is considered the most effective therapeutic strategy to recover from ALD, and there is clear evidence that abstinence can improve outcomes at nearly all stages of this disease.⁶

Diagnosis and recovery from ALD steatosis

Excessive use of alcohol ($\geq 60\text{g/day}$) for more than 2 weeks results in development of fatty liver (steatosis), characterized by deposition of fat in more than 5% of hepatocytes resulting in mostly macrovesicular steatosis (large intrahepatocyte lipid droplets) with or without minimal inflammation. Steatosis is mostly asymptomatic, although some people feel weakness, nausea, and pain in the right upper quadrant. Mild elevations in serum alanine transaminase (ALT), aspartate transaminase (AST), and gamma glutamyl transferase (GGT) are seen in patients with ALD. After abstinence from alcohol for 2 to 3 weeks, hepatic steatosis completely resolves and liver biopsies appear normal when examined by electron microscopy.¹² Similarly, Mehta et al. reported that 1 month of abstinence from alcohol by heavy-alcohol consumers (average consumption $\sim 258\text{ g/week}$) reduced serum ALT, AST, GGT, and carbohydrate-deficient transferrin to baseline (abstinence) levels.¹³ In addition, insulin resistance, systolic and diastolic blood pressure, and serum cholesterol levels were also reduced with abstinence from alcohol. These changes were attained without significant lifestyle adjustments such as changes in diet or increased exercise, indicating that abstinence was the major factor in recovery.¹⁴

Alcoholic steatohepatitis

With continued excessive drinking, about 20% to 40% of heavy-alcohol consumers with steatosis develop alcoholic steatohepatitis (ASH), characterized by fatty liver, inflammation with accumulation of neutrophils, ballooning degeneration of hepatocytes with or without Mallory-Denk bodies, and pericentral and perisinusoidal fibrosis. The severity of ASH can range from mild to severe and is superimposed on chronic liver disease. Severity of ASH can be assessed by the model for end-stage liver disease (MELD). A MELD score greater than 20 has been proposed as defining severe ASH with approximately 20% mortality.¹ Steatohepatitis symptoms include reduced appetite, nausea and vomiting, abdominal pain, fatigue, and weakness. People with severe alcoholic hepatitis exhibit

jaundice (yellowing of the skin), dark urine, kidney failure, and confusion. ASH is diagnosed by a serum AST:ALT ratio greater than 1.5:1 with absolute ALT and AST numbers not exceeding 400 international units per liter, increased GGT, serum bilirubin greater than 3 mg/dl, and documented heavy alcohol use until 8 weeks prior to seeking help.¹⁵ Ultrasound and magnetic resonance analyses are additionally used to confirm ASH. Currently, hepatologists recommend liver biopsies for diagnosis of ASH, as one-third of patients who are asymptomatic can show advanced fibrosis histologically.¹⁰ As for steatosis, the major therapy recommended for mild ASH and severe ASH with systemic inflammatory response syndrome is abstinence from alcohol consumption. This provides the best long-term outcome for survival and recovery. Indeed, Kirpich et al. (2017) reported that after 2 weeks of abstinence, patients who presented with inflammation and increased serum endotoxin showed improvement, as indicated by decreased serum AST, ALT, and cytokeratin 18 (a sensitive marker of liver injury), as well as lower levels of tumor necrosis factor alpha and endotoxin.¹⁶ In other articles in this topic series, information is given on pharmacological therapy, in addition to cognitive behavioral therapy, which is known to be key to preventing relapses during abstinence; both of these therapies show increased recovery from ALD.⁶ In addition, nutritional supplementation is beneficial for recovery from ALD.¹⁰

Fibrosis and cirrhosis

Repeated episodes of ASH are accompanied by hepatic fibrosis and characterized by ballooned and dying hepatocytes and abnormal deposition of extracellular matrix around these cells. The stage/intensity of fibrosis (F0–F4) can be evaluated histologically and, in some cases, on the basis of liver stiffness, which is determined by transient elastography (FibroScan).¹⁷ When overexposed to alcohol, the liver loses its efficiency, and inflammatory damage produces scar tissue and fatty deposits in the organ. Normal liver parenchymal cells are replaced by regenerative nodules surrounded by fibrotic (scar) tissue.

If enough scar tissue develops, the liver loses function in those scarred areas. Decompensated liver cirrhosis occurs when the liver can no longer properly perform its functions because of excessive scar tissue. Symptoms include fatigue, spider angioma (radiating blood vessels beneath the skin), palmar erythema (reddening of the palms), and jaundice (yellowing of the skin). These patients also have an increased risk of developing hepatocellular carcinoma, with a lifetime risk of about 3% to 10% and an annual risk of about 1%. The American College of Gastroenterology recommends that patients with alcohol-associated cirrhosis undergo screening with ultrasound examination every 6 months.¹⁸ At this stage, abstinence from alcohol improves survival rates.^{6,14,19}

Liver Injury and Recovery After Alcohol Administration in Animals

Researchers have studied molecular mechanisms of ALD and recovery from ALD in several animal models, most notably in rats and mice, using a wide variety of experimental conditions and various genetic backgrounds. As noted previously, both rats and mice develop fatty liver after alcohol administration, but progression to fibrosis or cirrhosis occurs only with manipulation of the diet and/or injection of an agent such as endotoxin or low-dose carbon tetrachloride to enhance a fibrotic response. This review summarizes cellular mechanisms that contribute to resolution of liver injury in alcohol-fed rats subjected to alcohol cessation. All studies described here used a similar model to investigate effects of alcohol and its cessation: Rats fed control or alcohol-containing Lieber-DeCarli liquid diets for 1 to 6 weeks showed typical serum alcohol concentrations of 200 to 300 mg/dl.²⁰⁻²² Subsequently, randomly chosen alcohol-fed rats were weaned from the alcohol diet.²¹⁻²³

Receptor-mediated endocytosis

Work from Casey and others has identified alcohol-induced defects in protein trafficking and organelle function, both of which recover upon alcohol cessation.^{21,24} The latter studies focused on the asialoglycoprotein receptor, a hepatocyte-specific

receptor, which exhibits decreased function after even 1 week of alcohol administration.²¹ The authors identified impaired binding, internalization, and degradation of several ligands internalized by receptor-mediated endocytosis. In all cases, recovery to control levels of receptor-mediated endocytosis by the asialoglycoprotein receptor was partially restored after 2 to 3 days of refeeding with the control diet, and function was fully restored after 7 days of refeeding. These findings suggest that the detrimental effects of alcohol on protein trafficking pathways occur rather rapidly (1 to 5 weeks) and that complete recovery is obtained within 7 days after cessation of alcohol consumption.

Golgi apparatus organization

Another study reported that alcohol cessation normalizes alcohol-induced Golgi apparatus disorganization in the liver.²⁵ These findings further support the notion that alcohol cessation reverses alcohol-induced trafficking defects. Here, chronic alcohol administration caused de-dimerization of the large Golgi matrix protein giantin in rat hepatocytes, leading to Golgi apparatus disassembly. Alcohol cessation and refeeding with the control diet for 10 days restored the compact, native structure of the Golgi apparatus.

Mg²⁺ levels

In another study, Torres et al. reported that 3 weeks of alcohol administration to rats impairs hepatocytes' ability to increase the level of magnesium ion (Mg²⁺) in the extracellular compartment. Ten days after alcohol cessation, Mg²⁺ homeostasis was restored.²³

Steatosis

Additionally, resolution of alcohol-induced fatty liver after alcohol cessation has been reported. Here, alcohol feeding increases hepatic triglycerides, confirmed by microscopic analyses of liver sections, which clearly show lipid droplet accumulation associated with elevated levels of ADH, CYP2E1, and lipid peroxides, as well as higher levels of serum AST, ALT, and

nonesterified fatty acids (NEFA, or free fatty acids).²² After alcohol removal and refeeding with the control diet, there was normalization of serum NEFA and ALT levels with a significant (but not complete) reduction of hepatic triglycerides. The latter reduction was associated with normalization of ADH and CYP2E1 to control levels. Additionally, there was concomitant reduction of hepatic lipid peroxides, indicating lower levels of oxidants.²² These findings reveal that alcohol cessation attenuates generation of oxidants to alleviate hepatocyte damage, as confirmed by normalization of ALT levels.

NEFA levels

It is well established that impaired liver function affects other organs, and vice versa. For example, high serum NEFA levels in alcohol-fed rats arise from alcohol-induced lipolysis in adipose tissue, generating serum NEFA levels that exacerbate hepatic fat accumulation. This occurs because hepatocytes rapidly take up circulating NEFA,²² which, upon their entry into hepatocytes, are esterified with glycerol to form triglycerides. Notably, alcohol removal and refeeding with the control diet normalize serum NEFA levels, indicating that alcohol cessation slows the hepatic uptake of circulating fatty acids and attenuates adipose lipolysis to alleviate alcohol-induced steatosis in the liver. Also noteworthy is that alcohol cessation enhances hepatic fatty acid oxidation.

Hepatic autophagy

Alcohol cessation also resolves impaired hepatic autophagy, a key intracellular catabolic pathway that breaks down lipid droplets and other obsolete organelles. Chronic feeding of alcohol reduces the nuclear localization of transcription factor EB,²² which coordinates lysosome biogenesis with autophagy. Additionally, chronic alcohol feeding downregulates the activity of lysosomal acid lipase, causing intrahepatic lipid accumulation. Cessation of alcohol restores nuclear transcription factor EB levels to normal, thereby reactivating hepatic autophagy and the normal turnover of lipid droplets.²²

Alcohol cessation and recovery following intragastric alcohol administration

Yin et al. (1988) examined recovery in rats subjected to intragastric alcohol feeding, during which rodents are given continual intragastric infusion of an alcohol diet through an inserted cannula.²⁶ Liver damage in these animals is typically greater than in animals given oral feeding of alcohol ad lib. Alcohol removal for 2 weeks nearly normalized all liver functions in rats previously subjected to 6 weeks of intragastric alcohol administration.²⁶

The foregoing findings indicate that several cellular mechanisms collectively contribute to resolution of steatosis and liver injury following alcohol cessation. First, since alcohol cessation would terminate ethanol metabolism, oxidant generation would be greatly decreased. Second, cessation normalizes circulating NEFA, their uptake by liver cells, and their reesterification into triglycerides. Third, alcohol cessation reactivates hepatic autophagy by restoring nuclear transcription factor EB levels, allowing resumption of lipid droplet degradation and organelle turnover. Interestingly, although alcohol cessation alleviates fat accumulation, it does not completely reverse fatty liver, probably because the amount of residual fat in livers of alcohol-fed rats overwhelms the degradation/oxidative systems. The latter findings indicate a longer recovery period is necessary to reverse fatty liver completely in alcohol-withdrawn rats.

GI TRACT AND ALCOHOL

Alcohol Metabolism in the GI Tract

As the principal site of alcohol absorption, the GI tract plays a particularly significant role in mediating the toxic effects of alcohol on the liver and other organs. GI metabolism of alcohol is significant as it affects the systemic availability of alcohol while it locally generates acetaldehyde. GI mucosal ADH catalyzes alcohol oxidation, especially in the oropharynx and esophagus where ADH class IV activity is relatively high, and it likely contributes to local toxicity because of the acetaldehyde it produces.²⁷

Before alcohol reaches the liver, the stomach lining is the principal site of “first pass” metabolism of the ingested alcohol.²⁷ Various isoforms of gastric ADH oxidize a significant percentage of ingested alcohol before it enters the portal circulation. The total first-pass metabolism of alcohol was calculated to be in the range of 7% to 9% and is influenced by many factors including gastric emptying.²⁸ Besides ADH, the other major enzymes that catalyze alcohol oxidation, CYP2E1 and catalase, are present in GI mucosal cells. Similar to liver, GI CYP2E1 content also increases after chronic alcohol administration. GI tract microflora, including bacteria and yeast, possess ADH activity and metabolize alcohol to produce acetaldehyde, but they also are capable of generating alcohol during fermentation.²⁷ Other factors such as motility, absorption, dilution by GI secretions, and rediffusion of alcohol all influence alcohol clearance from the GI tract. In addition, gender, age, genetics, and gastric morphology modulate gastric ADH activity. ADH levels are significantly lower in younger women compared with age-matched men. This difference probably accounts for greater alcohol-induced liver toxicity in women.²⁷

GI Injury and Recovery After Alcohol Exposure in Humans

Alcohol consumption interferes with the function of all parts of the GI tract. These malfunctions are due to the local production and systemic levels of acetaldehyde. Chronic alcohol use also damages and erodes the upper GI mucosa, which encounter undiluted alcoholic beverages, causing hemorrhagic lesions and increasing the risk of cancer development. Alcohol also impairs the muscles surrounding the stomach, small intestine, and large intestine. This affects motility, which, while delaying gastric emptying, shortens transit time in the small intestine, causing diarrhea. Essentially, alcohol inhibits absorption of a variety of nutrients by the small intestine and contributes to malnourishment commonly seen in patients with alcohol use disorder (AUD).²⁹

Intestinal barrier disruption

Most relevant, chronic alcohol use disrupts the tightly regulated gut barrier function. This barrier consists of a system of highly specialized, intercellular, multiprotein junctional complexes known as tight junctions. These are located at the apical (luminal) ends of intestinal epithelial cells. Studies reveal that alcohol metabolism in the gut disrupts tight junction structural integrity. The consequent loss of the mucosal barrier allows paracellular translocation of pathogenic molecules—including cell wall components from gram-positive and gram-negative bacteria and fungi—into the general circulation, allowing direct access to the liver via the portal vein. Once inside the liver, these microbial components can activate resident macrophages (Kupffer cells) to initiate a necroinflammatory cascade. Alcohol compromises tight junction integrity by the following molecular mechanisms: generating reactive oxygen species, upregulating production of specific micro-RNAs, and disrupting both the epithelial cell methionine metabolic pathway and the intestinal circadian rhythm.²⁹

In addition to the physical barrier, there are immunological and chemical barriers on the luminal surface of the GI tract. The chemical barriers secreted by the epithelial/immune cells include secretory immunoglobulin A, mucins, and antimicrobial peptides, all of which are altered by alcohol metabolism.

Alterations in the microbiota

A symbiotic balance between proinflammatory and commensal bacteria allows only trace amounts of luminal antigens to penetrate the intestinal barrier and enter the portal vein and systemic circulation. However, chronic alcohol administration alters the balance among intestinal microbiota. This is characterized by both quantitative and qualitative changes, including suppression of many commensal probiotic bacteria, vital for bile acid metabolism and for the generation of short- and long-chain fatty acids necessary for maintaining gut health and liver homeostasis.³⁰

Recovery after abstinence

Recent studies have shown that a 3-week abstinence following the removal of alcohol induces a complete recovery of gut barrier function in subjects with AUD who presented with high intestinal permeability.³¹ Similar results were shown by other laboratories that reported a decrease in endotoxemia following the removal of alcohol.¹⁶ However, 3-week abstinence produces only an incomplete recovery of the gut microbiota,³¹ indicating that alcohol consumption has a more long-lasting effect on gut dysbiosis, even after more than 1 month of abstinence.³² A 3-week abstinence also increases bacterial populations known to be beneficial, which leads to a decrease in potential toxins and an increase in beneficial microbial metabolites.³¹

GI Injury and Recovery After Alcohol Exposure in Animals

Most studies conducted to date using animal models have examined whether external agents—such as antibiotics, probiotics, prebiotics, synbiotics, betaine, zinc, indole-3-acetic acid, and long- and short-chain fatty acids—prevent or reverse alcohol-induced changes in the gut and prevent liver damage. Only one animal study has shown that sobering for 24 hours after 4 weeks of alcohol feeding partially restored intestinal barrier function, but such cessation did not reduce the inflammatory response in the colon.³³

PANCREAS

Alcohol Metabolism in the Pancreas

Although the pancreas expresses both ADH and CYP2E1, its capacity for oxidative alcohol metabolism is significantly lower than that of the liver.³⁴ However, the pancreas has a high capacity for nonoxidative alcohol metabolism, which is catalyzed by fatty acid ethyl ester (FAEE) synthases. These enzymes generate FAEE by condensing alcohol with a fatty acid (e.g., oleic acid). FAEE can bind to and accumulate in mitochondria to impair cell function in the pancreas and the heart,³⁵ which is also rich in FAEE synthases.

Pancreatic Injury and Repair After Chronic Alcohol Use in Humans

The association between alcohol consumption and pancreatic diseases has been recognized for more than 100 years. The pancreas contains two functionally distinct compartments: As an endocrine gland, the pancreas secretes insulin and glucagon, the hormones that govern glycemia. As an exocrine gland, the pancreas produces zymogen precursors of digestive enzymes used for food breakdown in the gut. Both compartments can suffer consequences of chronic alcohol use.

Pancreatitis

Chronic alcohol use is commonly associated with pancreatitis, a necroinflammatory disease of the exocrine pancreas that is classified as either acute or chronic. Although the association between chronic alcohol use and pancreatitis has long been recognized, the mechanism or mechanisms by which chronic alcohol use predisposes the pancreas to disease are not entirely understood. Despite this association, chronic alcohol use alone is not sufficient to trigger a clinical event, such as development of acute pancreatitis.³⁶ Heavy drinking is believed to sensitize the pancreas to injury, whereas other factors trigger necroinflammation.

In developed countries, chronic alcohol use is the second most common factor associated with acute pancreatitis.³⁷ In up to 20% of the cases, there are severe clinical complications of pancreatitis with mortalities of up to 10%.³⁷

In contrast, in the Western world, chronic alcohol use is the major etiological factor in chronic pancreatitis, accounting for approximately 70% of reported cases. Alcohol-induced chronic pancreatitis is thought to have an early stage associated with recurrent attacks of alcohol-induced acute pancreatitis and a late stage characterized by steatorrhea, diabetes, fibrotic scarring, and pancreatic calcification. In many cases, it appears that alcohol-induced acute pancreatitis progresses to chronic pancreatitis. This progression is generally associated with frequent, severe, and acute attacks that are common among chronic alcohol users. Little is known regarding the effects of alcohol

in humans after pancreatic damage. Because chronic pancreatitis is commonly associated with recurrent attacks of acute pancreatitis, it appears that continued alcohol consumption impairs proper pancreatic repair. In support of this, one study investigated pancreatic dysfunction associated with alcohol-induced chronic pancreatitis and demonstrated that pancreatic function deteriorated more slowly in patients who quit drinking compared with those who continued heavy drinking. These findings indicate that the functional deterioration of the pancreas associated with alcohol-induced chronic pancreatitis continues even after drinking ceases, although this occurs to a lesser degree than in those who continue to chronically use alcohol.³⁸ A long-term, population-based study demonstrated that progression from acute to chronic pancreatitis is most common among chronic alcohol users. These findings indicate that alcohol consumption delays the normal repair process following acute pancreatitis and it may enhance the progression from acute to chronic pancreatitis. Although more work must be done to determine how alcohol affects repair of the pancreas, it appears that cessation of chronic alcohol use slows progression of alcohol-induced chronic pancreatitis.

Pancreatic Injury and Repair After Alcohol Exposure in Animals

The structural and functional regeneration of the pancreas after acute injury is supported by studies of experimentally induced pancreatitis in rodents.³⁹ One of the main characteristics of alcohol-induced chronic pancreatitis is the aberrant repair of injury that results in fibrotic scarring. Given the close association between chronic alcohol use and chronic pancreatitis, it is reasonable that chronic alcohol consumption adversely affects pancreatic repair. Using the Lieber-DeCarli pair-feeding model of alcohol administration in rats, one group reported that chronic alcohol feeding for 2 to 8 weeks significantly decreased the amylase content of the pancreas after cerulein-induced pancreatitis, indicating that alcohol consumption impaired functional pancreatic regeneration. This treatment did not affect total protein, DNA, or RNA content

of the pancreas. Although no histological evaluation was performed, and amylase production declined, these authors concluded that alcohol consumption does not affect pancreatic regeneration.⁴⁰ In contrast, Pap et al. reported that intragastric alcohol feeding for 2 months slowed the restoration of pancreatic weight and enzyme content in rats with surgically induced pancreatic injury.⁴¹ During this period, alcohol-fed animals developed chronic calcifying pancreatitis. Cessation of alcohol feeding resulted in structural and functional recovery of the pancreas. These results indicate that inhibition of pancreatic regeneration by alcohol is necessary to maintain the state of chronic pancreatitis. Cholecystokinin is a crucial peptide hormone in pancreatic regeneration. Alcohol feeding reduces cholecystokinin release and prevents pancreas regeneration after partial pancreatectomy.⁴² Additionally, using a model of virally induced pancreatitis, it was demonstrated that alcohol administration to mice delays pancreas repair.⁴³ Together, these studies indicate that alcohol delays the structural repair and functional restitution of pancreatic tissue in animal models of alcoholic pancreatitis. Most studies indicate that cessation of alcohol consumption by rodents restores pancreatic structure and function.

HEART

Alcohol Metabolism in the Heart

Cardiac tissue expresses both major alcohol-metabolizing enzymes: ADH and CYP2E1.⁴⁴ There are reports that both enzymes may influence alcohol-induced myocardial damage by converting alcohol to acetaldehyde. However, the heart also has a rich content of FAEE synthases, suggesting that nonoxidative alcohol metabolism prevails in this organ.

Cardiac Injury and Recovery After Alcohol Exposure in Humans

Alcohol-induced dilated cardiomyopathy is an important manifestation of chronic alcohol use. Chronic AUD is accompanied by a high incidence of cardiac morbidity and mortality due

to development of alcoholic cardiomyopathy. Cardiomyopathy can be seen by ventricle dilation, along with a reduced ventricular wall thickness and some contractile dysfunction. Alcohol/acetaldehyde toxicity along with mitochondrial production of reactive oxygen species is one theory proposed for alcohol-induced cardiac injury. Indeed, acetaldehyde can directly impair cardiac contractile function, disrupt cardiac excitation-contraction coupling, and promote oxidative damage and lipid peroxidation. Some resulting effects are oxidative injury, apoptosis, impaired myofilament Ca²⁺ sensitivity, impaired protein synthesis, and altered fatty acid extraction and deposition, along with changes in protein catabolism.⁴⁵ The removal of alcohol is associated with the reduction or disappearance of myocardial damage and the improvement of function.⁴⁶ A study on cardiovascular changes during different phases following the removal of alcohol found that heart rate, systolic blood pressure, and diastolic blood pressure were higher in the early stage of alcohol cessation. These cardiovascular parameters returned to baseline levels after 1 month of abstinence.⁴⁷ Other cardiac effects of chronic alcohol exposure are cardiac arrhythmias (irregular heartbeat), tachycardia (fast heartbeat), and other cardiovascular disease. These cardiovascular parameters also returned to baseline levels after 1 month of abstinence.⁴⁷ There is no evidence for reversal of cardiac fibrosis in humans with alcoholic cardiomyopathy. However, cessation of alcohol consumption can result in significant improvement in left ventricular function.^{48,49} In a case study, Mahmoud et al. showed that a patient who exhibited signs of alcoholic cardiomyopathy demonstrated severe global left ventricular systolic dysfunction with an ejection fraction of 20%.⁵⁰ Moreover, the end-systolic dimension was 4.1 cm and the end-diastolic dimension was 5.0 cm. However, after 1 month of alcohol abstinence, this patient was asymptomatic, with a higher ejection fraction of 62%. The patient's end-systolic dimension was 3.3 cm, and the end-diastolic dimension was 4.8 cm.⁵⁰ Cardiac arrhythmias may explain cases of sudden death in patients with AUD who are abstinent.

The QTc interval (a measure of heart rate) is frequently prolonged during alcohol cessation syndrome and tends to become normal over time.⁵¹ The frequency and nature of arrhythmias, as well as some irregularities of their time-course due to alcohol cessation terms were studied in subjects with chronic AUD. Sinus tachycardia, abnormal excitation, and conduction were more frequently observed in the acute (early) period of alcohol cessation. In most cases, these symptoms ceased within 2 weeks after cessation.⁵²

Cardiac Injury and Recovery After Alcohol Exposure in Animals

Alcoholic cardiomyopathy is a specific heart muscle disease caused by chronic alcohol intake and has been studied in animal models. Chronic alcohol intake tends to increase left ventricular mass and dilatation that leads to heart failure in a rat model of alcohol administration. In one study, the authors postulated that alcohol intake activates the pro-renin receptor and contributes to cardiac remodeling and damage.⁵³ They examined the relationship between the pro-renin receptor and alcoholic cardiomyopathy and found that alcohol intake increases myocardial fibrosis, myocardial oxidative stress, and inflammation response like that seen in humans. Studies examining recovery of cardiac function in animal models have not been described.

BONE

Alcohol Metabolism in Bone

It is not clear whether bone tissue itself metabolizes alcohol by oxidative metabolism (i.e., ADH and CYP2E1 catalysis) or by esterification with fatty acids. Current evidence supports that alcohol alone is the causative agent that delays bone growth and repair.

Bone Injury and Repair After Alcohol Exposure in Humans

Osteopenia

Continued heavy alcohol use decreases bone density. The pathogenesis of osteopenia in AUD remains unclear, and many alcohol-related

abnormalities have been proposed to explain bone loss.⁵⁴ A direct inhibitory effect of alcohol on osteoblast function was suggested by *in vivo* and *in vitro* studies. The rapid increase in serum bone Gla protein (BGP) concentrations following alcohol cessation suggests that low serum BGP concentrations in heavy-alcohol users may result from a direct toxic effect of alcohol on osteoblast function and/or numbers.⁵⁴ The role of alcohol as a risk factor for osteopenia was studied in subjects with AUD who did not have liver cirrhosis. The data show that chronic alcohol ingestion induces osteopenia regardless of whether liver cirrhosis is present, and that some relationship can be expected between the amount and duration of alcohol consumption and the degree of bone loss. Low serum levels of BGP in drinkers are reversible upon alcohol cessation, suggesting that reduction of osteoblast activity is likely the main factor responsible for alcohol-associated bone disease.⁵⁵ Alcohol not only promotes bone loss but also impairs bone formation. Plasma concentrations of osteocalcin, a marker of bone formation, were measured in human male heavy drinkers before and after 3 weeks of alcohol cessation and compared with nondrinking men. Plasma osteocalcin levels in heavy-alcohol-using subjects were significantly lower than in controls. After 21 days of cessation, plasma osteocalcin levels were significantly higher than on the day of admission and were equal to those of controls, who did not have AUD. The results support the notion that the decrease of plasma osteocalcin with chronic alcohol use is reversible within 3 weeks following alcohol removal.⁵⁶

Bone turnover

The biochemical markers for bone formation (osteocalcin, bone-specific alkaline phosphatase, and procollagen type 1 carboxy-terminal peptide) and resorption (c-terminal telopeptide and urine deoxypyridinoline) were studied in men who were heavy-alcohol users and in abstainers with more than 5 years of abstinence. The results were compared with male controls. The findings

suggest that there is an imbalance between bone formation and bone resorption among heavy-alcohol users that results in rapid bone loss. Although most directions tended to normalize shortly following the removal of alcohol, biochemical data suggest that there may still be persistently high bone turnover after more than 5 years of abstinence.⁵⁷

Although most studies suggest that alcohol induces bone loss, epidemiological studies indicate that higher bone mass is associated with moderate alcohol consumption in postmenopausal women. Therefore, a study investigated the hypothesis that moderate alcohol intake attenuates bone turnover after menopause. This study showed that abstinence from alcohol results in increased markers of bone turnover, whereas resumption of drinking reduces bone turnover markers. These results suggest that the inhibitory effect of alcohol on bone turnover attenuates the detrimental skeletal consequences of excessive bone turnover associated with menopause.⁵⁸ Taken together, these studies indicate that alcohol has a direct effect on bone formation and resorption and that these effects are reduced during abstinence.

Bone Injury and Repair After Alcohol Exposure in Animals

Animal (rodent) studies report that the adverse effects of alcohol on bones are limited not only to bone formation and resorption, but that chronic alcohol administration also impairs the healing capacity of fractured bone in rodents.⁵⁹ In vitro studies report that proliferation of alcohol-exposed osteoblasts (precursor bone cells) is impaired and that such treatment enhances oxidant stress by increasing intracellular superoxide, which inhibits osteoblast proliferation.^{60,61} Recent in vivo studies suggest that oxidant stress inhibits bone repair, as fracture healing is restored in alcohol-fed rats treated with the antioxidant, *N*-acetylcysteine.⁶² Given the latter findings, it is reasonable to postulate that alcohol cessation may fully restore osteogenesis in bone.

SUMMARY

Continual heavy alcohol consumption damages multiple organs/systems. This review focused on damage and recovery in five of those tissues in humans and experimental rodents. The greatest degree of alcohol-induced injury occurs in the liver and GI tract, as both these organs/systems are the first to encounter high concentrations of imbibed alcohol. The liver and GI tract are well equipped to oxidatively metabolize alcohol. However, alcohol oxidation comes at a cost, as it generates acetaldehyde, which is capable of forming toxic acetaldehyde-macromolecular adducts as well as free radicals that oxidize lipids and form reactive lipid peroxides. Thus, the continual metabolic generation of these intermediates eventually disrupts homeostasis, causing cell death, inflammation, and the eventual breakdown of organ integrity.

In the pancreas and heart, alcohol is minimally oxidized. Instead, most of it is esterified with fatty acids, forming FAEE. These molecules bind to mitochondria and disrupt the generation of energy that is normally reserved for pancreatic secretion or myocardial contraction. Although it is not clear whether oxidative or nonoxidative alcohol metabolism actually occurs in bone tissue, it is clear that alcohol exposure to osteoblasts inhibits their proliferation by causing oxidant stress. Also, structural weakening of bone and delays in fracture healing are clearly evident after chronic alcohol consumption by rodents.

Despite alcohol-induced damage to these tissues, abstinence, in its simplest form, brings about either complete or partial recovery, but the extent of such recovery depends on the extent of the damage, as shown in Figure 1. For example, it is unlikely that abstinence would be effective in a case of decompensated cirrhosis, but resolution of cirrhosis which involves a portion of the liver (i.e., compensated cirrhosis) is more likely. Thus, the examples provided in this review highlight the value of intrinsic regenerative processes that maintain organ function.

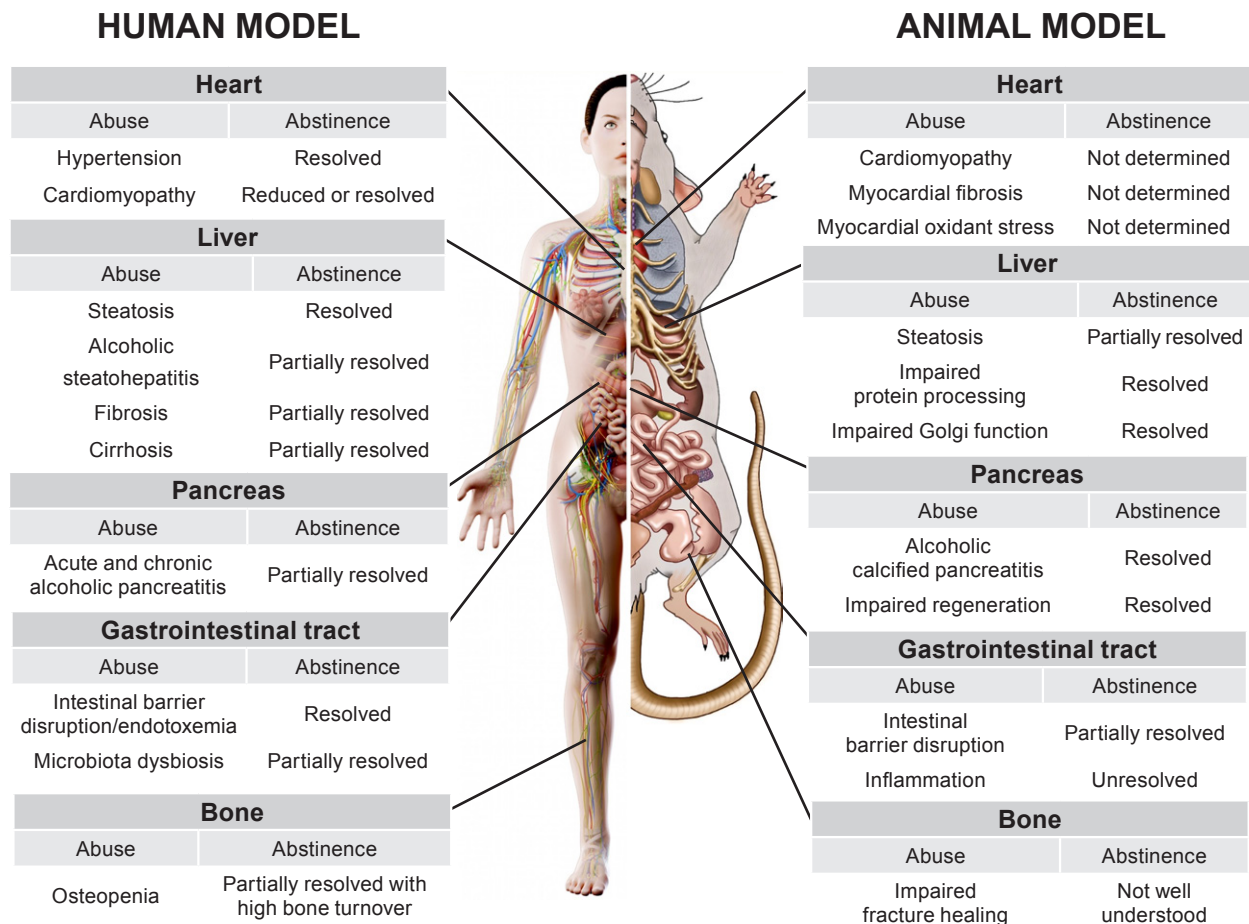


Figure 1 Schematic diagram of the effects of chronic alcohol use and abstinence in humans and rodents on various organs and systems, including the heart, liver, gastrointestinal tract, pancreas, and bone. Each row describes a consequence of chronic alcohol use, whether it is resolved by abstinence, and, if so, to what degree. Adapted with permission from SciePro/stock.adobe.com (human) and Science Photo Library, London (rodent).

Finally, more basic research is needed to clearly evaluate whether abstinence that follows chronic alcohol consumption completely or partially restores the full integrity of the affected organs. To date, the results appear promising that cessation of alcohol consumption indeed allows partial or full recovery, depending on the parameter being measured. It is also worth noting that alcohol-induced pathology in animals (usually rodents) does not fully reflect the extent of injury incurred by human heavy drinkers. However, the use of other feeding models, such as intragastric feeding and the acute-on-chronic feeding model have yielded valuable information on liver damage in animals that consume similar amounts of alcohol and have similar drinking patterns as humans with AUD.

FINAL REMARKS

The focus of this review has been on organ recovery after cessation of chronic alcohol use. Abstaining from alcohol by a person with AUD is not a trivial matter. A recent review by Asrani et al. gives important details on the scope of the global burden of alcohol-associated disease;⁶³ although its principal focus is ALD, it applies to all the alcohol-induced disorders described here. Presently, the problems of alcohol-related morbidity (suffering from AUD) and mortality (death from AUD) are rising worldwide. Their reductions will require multifaceted solutions that focus on early identification of problem drinking and interventions at the population level (e.g., increased taxation of

beverages; youth education) and at the patient level (e.g., early diagnosis of organ injury; counseling by an addiction specialist). Although none of the aforementioned examples, by themselves, are considered innovative, their combined use represents a new approach, especially when they make use of technological advances, including smartphone technology and telehealth. The team approach to treatment is important because, although a physician can diagnose and treat organ injury, an addiction specialist or mental health professional also must be part of the treatment plan to prevent patient relapse. These measures, along with public reeducation about social stigmas related to alcohol addiction, will likely reverse the rising trends toward heavy drinking.

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THE ENDOCRINE SYSTEM AND ALCOHOL DRINKING IN FEMALES

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Sexually dimorphic effects of alcohol exposure throughout life have been documented in clinical and preclinical studies. In the past, rates of alcohol use disorder (AUD) were higher in men than in women, but over the past 10 years, the difference between sexes in prevalence of AUD and binge drinking has narrowed. Recent evidence adds to historical data regarding the influence of sex steroids on alcohol drinking and the interaction with stress-related steroids. This review considers the contribution of the endocrine system to alcohol drinking in females, with a focus on the hypothalamic pituitary gonadal axis and the hypothalamic pituitary adrenal axis and their reciprocal interactions. Emphasis is given to preclinical studies that examined genomic and rapid membrane effects of estrogen, progesterone, glucocorticoids, and GABAergic neurosteroids for their effects on alcohol drinking and models of relapse. Pertinent comparisons to data in males highlight divergent effects of sex and stress steroids on alcohol drinking and emphasize the importance of considering sex in the development of novel pharmacotherapeutic targets for the treatment of AUD. For instance, pharmacological strategies targeting the corticotropin releasing factor and glucocorticoid receptor systems may be differentially effective in males and females, whereas strategies to enhance GABAergic neurosteroids may represent a biomarker of treatment efficacy in both sexes.

KEY WORDS: estrogen; ethanol; glucocorticoid; neurosteroid; progesterone; stress

INTRODUCTION

Alcohol use disorder (AUD), a diagnosis that combines criteria for alcohol abuse and alcohol dependence from the 4th edition of the Diagnostic and Statistical Manual of Mental Disorders into a single disorder in the 5th edition,¹ negatively influences health and is the third-leading preventable cause of death in the United States.²

According to the 2015 National Survey on Drug Use and Health, the prevalence of binge drinking, which is the consumption of an excessive amount of alcohol in a short period of time, and of heavy alcohol use was similar in males and females.² Likewise, a recent meta-analysis confirmed a greater increase in alcohol use and binge drinking in women versus men over the past 16 years,³ representing a narrowing of the historically higher

AUD rate in males. It has been suggested that the increased rate of AUD among women may be due to stress or to drinking to regulate a negative affect.⁴⁻⁶

As elegantly reviewed by Rachdaoui and Sarkar, acute and chronic alcohol administration disrupts functioning of the endocrine system, which is a complex system of glands that work in conjunction with the nervous system to maintain homeostasis.⁷ Glands of the endocrine system produce and secrete hormones into the circulation, which can have long-lasting as well as rapid actions. Hormones affect physiological functions such as metabolism, reproduction, growth, and development, and they facilitate the ability to respond to changes in the environment and to stress.⁷⁻⁸ Additionally, gonadal sex steroid hormones exert organizational (permanent) and activational (transient) effects on the brain to regulate sexual differentiation, secondary sex characteristics, and sex differences in behavior.^{4,9-11} Gonadal steroids also influence the stress response that is mediated by the hypothalamic-pituitary-adrenal (HPA) axis, and elevated stress hormones affect the reproductive or hypothalamic-pituitary-gonadal (HPG) axis.⁸ Finally, sex and stress hormones influence alcohol consumption and behavior in models of addiction.^{4-5,10,12} As a result, it should be considered that alcohol consumption can influence the endocrine system and the reciprocal interaction between the stress and reproductive axes and that gonadal and stress steroid hormones can influence alcohol drinking and addiction-related behaviors.

This review highlights preclinical research on the contribution of gonadal and stress steroids to alcohol drinking in females. It focuses on the HPG and HPA axes and describes how endogenous fluctuations in steroid hormones as well as exogenous administration influence alcohol drinking and other pertinent addiction-related phenotypes. In addition to a discussion of how classical steroid responses are mediated by genomic effects via intracellular receptors, this review considers rapid steroid responses via membrane receptors and the interaction with neurotransmitter systems. Relevant comparisons

to results in males bolster the emerging evidence for sex differences in steroid hormone and stress effects on alcohol drinking behavior and addiction-related phenotypes. These comparisons emphasize the importance of considering sex in the development of novel pharmacotherapies for the treatment of AUD.

OVERVIEW OF THE HPG AND HPA AXES

The HPG axis is the neuroendocrine axis important for reproduction, whereas the HPA axis is the neuroendocrine axis important for the stress response. As depicted in Figure 1, both the HPG and HPA axes are regulated by steroid hormone feedback and reciprocal interactions between steroids in each axis.

The HPG axis comprises the hypothalamus, pituitary, and gonads. Hypothalamic nuclei (e.g., in the preoptic area) release gonadotropin-releasing hormone (GnRH) into the portal vasculature to stimulate the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the anterior pituitary (see Figure 1). Circulating LH and FSH act on the gonads to stimulate the production and release of estrogen and progesterone from the ovary and of testosterone from the testis.^{7,13} In females, FSH stimulates follicle development in the ovary and the secretion of estradiol, which promotes a surge in LH and FSH. LH stimulates ovulation and the subsequent secretion of progesterone. These overall effects of estradiol are similar across species, but phases of the 28- to 30-day menstrual cycle in primates and the 4- to 5-day estrous cycle in rodents are not completely analogous (see the box **Phases of Primate Menstrual and Rodent Estrous Cycles**). Additionally, steroid hormone feedback loops regulate HPG axis function at the level of the hypothalamus and anterior pituitary. Testosterone inhibits GnRH, LH, and FSH through negative feedback, whereas estradiol and progesterone can exert both negative (inhibitory) and positive (stimulatory) feedback actions, depending on the stage of the ovarian cycle (see Figure 1).

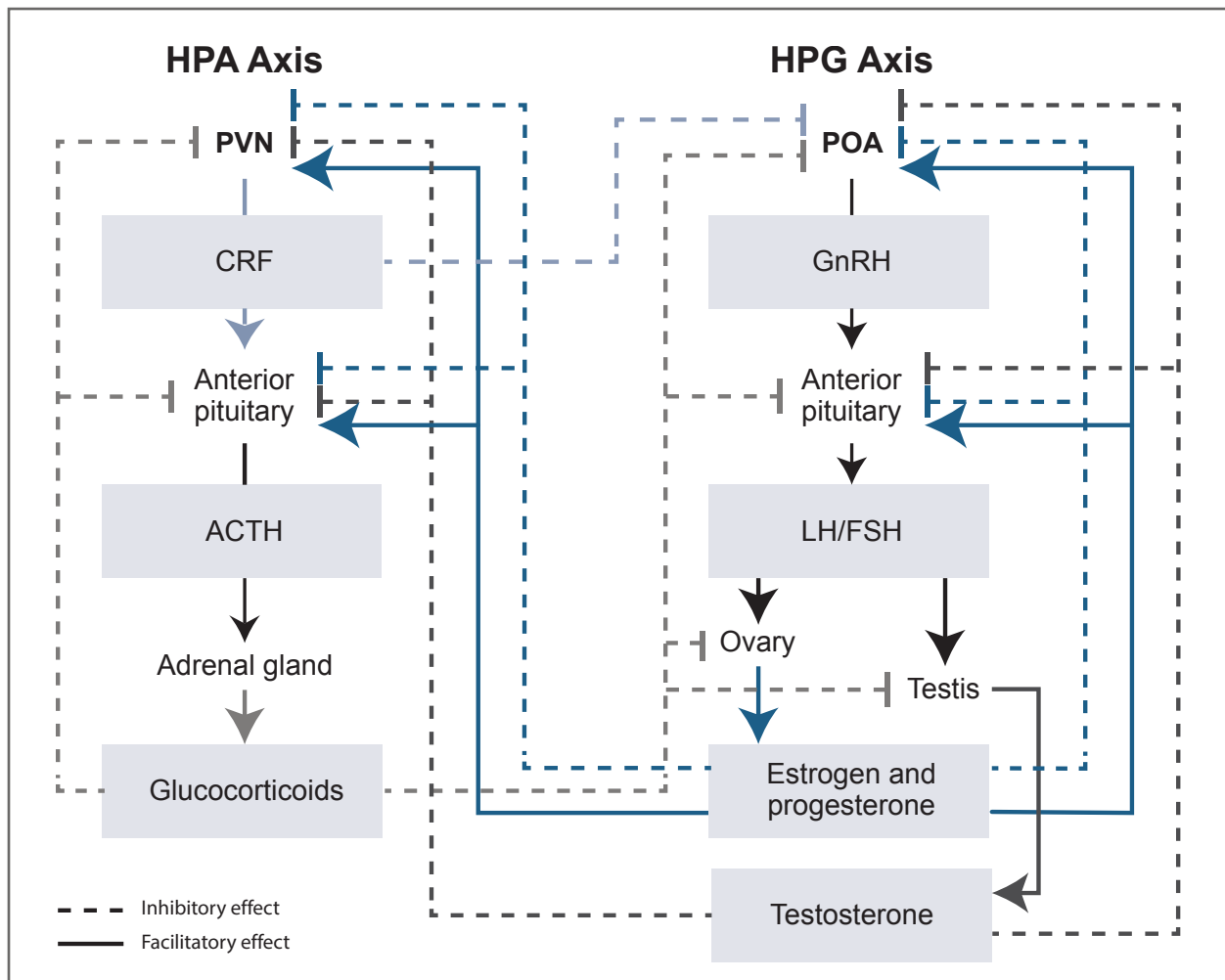


Figure 1 Simplified diagram of the reciprocal interaction between the HPA axis and the HPG axis. Solid lines with arrows depict facilitatory effects. Dashed lines with block symbols depict inhibitory or negative feedback effects. Gonadal steroids are involved in the regulation of the HPA axis at the level of the PVN and the anterior pituitary, and estrogen and progesterone can have either a facilitatory or an inhibitory effect at the PVN and the anterior pituitary. Stress steroids can regulate the HPG axis at the level of the hypothalamic POA, anterior pituitary, and gonads (ovaries or testes). Glucocorticoids (corticosterone in rodents, cortisol in humans and monkeys) exert negative feedback at each level of the HPG axis, and CRF exerts negative feedback at the POA. Upstream regulatory centers for each axis are not shown. Also shown is the negative feedback exhibited by glucocorticoids within the HPA axis, the negative feedback exhibited by testosterone within the HPG axis, and the negative and positive feedback exhibited by estrogen and progesterone within the HPG axis. *Note:* ACTH, adrenocorticotropic hormone; CRF, corticotropin releasing factor; FSH, follicle stimulating hormone; GnRH, gonadotropin releasing hormone; HPA, hypothalamic-pituitary-adrenal; HPG, hypothalamic-pituitary-gonadal; LH, luteinizing hormone; POA, preoptic area; PVN, paraventricular nucleus. *Source:* Modified from a figure by Oyola and Handa.⁸

Responses to stress are mediated by the HPA axis and the sympathetic autonomic response. Short-term activation of the HPA axis produces beneficial effects, whereas chronic activation can result in deleterious effects.¹⁴ Neurons in the paraventricular nucleus (PVN) of the hypothalamus are responsible for the secretion

of corticotropin releasing factor (CRF) and arginine vasopressin into the portal system, and CRF causes the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary. ACTH stimulates the biosynthesis and release of glucocorticoids from the adrenal cortex.¹³ Negative feedback of glucocorticoids at the level of the

Phases of Primate Menstrual and Rodent Estrous Cycles*	
Primate (Human and Monkey)	Rodent (Rat and Mouse)
The average length of the menstrual cycle is 28 to 30 days.	The average length of the estrous cycle is 4 to 5 days.
Follicular phase: As the ovarian follicle develops, estradiol is secreted. Menstruation overlaps with the beginning of the follicular phase.	Metestrus/diestrus phase: As the ovarian follicle develops, estradiol is secreted.
Perioovulatory phase: A rapid estradiol increase triggers an LH surge, which produces ovulation.	Proestrus/estrus phase: A rapid estradiol increase triggers an LH surge, which stimulates progesterone release and produces ovulation.
Luteal phase: The corpus luteum releases high levels of estradiol and progesterone. Menstruation occurs at the end of the luteal phase as hormone levels fall.	No equivalent phase: Female rodents do not have a functional corpus luteum.

*Adapted from a table by Becker and Koob.⁴ Note: LH, luteinizing hormone.

anterior pituitary and PVN inhibits CRF, arginine vasopressin, and ACTH production and helps maintain optimal glucocorticoid levels (Figure 1).

An additional consideration is that the HPA and HPG axes have reciprocal interactions in terms of steroid hormone feedback, as depicted in Figure 1.⁸ For example, glucocorticoids exhibit negative feedback of the HPG axis at the level of the hypothalamus, anterior pituitary, and gonads. As a result, a chronic elevation of glucocorticoids can result in suppressed HPG axis function. Likewise, gonadal steroids may influence HPA axis function, as evidenced by the effects of testosterone, progesterone, and estrogen at the level of the PVN and anterior pituitary.¹³ For example, basal and stress-induced increases in glucocorticoids are greater in female than in male rodents. Evidence from studies that used gonadectomy and hormone replacement suggests that testosterone exerts an inhibitory influence on HPA axis activity in male rodents, whereas estrogen primarily produces a facilitatory effect on HPA axis activity in female rodents. Some of the differing results for estrogen on HPA axis function may be due in part to the opposing actions of two types of estrogen receptors.¹³

STEROID HORMONE RECEPTORS AND CIRCUITRY IMPORTANT FOR STRESS AND DRINKING

Steroid hormones produce effects through several mechanisms. First, steroid hormones bind to their classical intracellular receptors, which act as ligand-activated transcription factors to alter gene expression and produce long-lasting actions.¹³ Progestins, such as progesterone and dihydroprogesterone, bind to two progesterone receptor isoforms: A and B.¹⁵ Estrogens, such as 17beta-estradiol, bind to two distinct receptor subtypes: estrogen receptor-alpha and estrogen receptor-beta.^{13,16} Androgens, such as testosterone and dihydrotestosterone, bind to androgen receptors.¹³ Glucocorticoids, such as corticosterone in rodents and cortisol in humans and monkeys, bind to mineralocorticoid receptors (type I) and glucocorticoid receptors (type II).¹³ Endogenous glucocorticoids have higher affinity for mineralocorticoid receptors than for glucocorticoid receptors.¹³

Second, through classical and nonclassical receptors located in the cell membrane, steroids have rapid effects that influence second-messenger

pathways and ion channel function.¹⁶⁻²² Finally, steroid hormone derivatives can rapidly alter ion channel function via allosteric interactions with ligand-gated ion channels.²³⁻²⁶ For example, the progesterone derivative allopregnanolone and the deoxycorticosterone derivative tetrahydrodeoxycorticosterone (THDOC) are very potent positive allosteric modulators of gamma-aminobutyric acid_A (GABA_A) receptors and can rapidly alter neuronal inhibition. Rapid actions at the cell membrane gave rise to the terms “neuroactive steroids” and “neurosteroids” (Refer to the Finn and Jimenez article on neurosteroid networks for more information about neurosteroid synthesis and pathways.)²⁴ Thus, steroid hormones

and their derivatives can influence brain function and behavior through classic genomic actions and rapid membrane effects.

Neuroanatomical overlap occurs between gonadal and adrenal steroid hormone receptors within the hypothalamic (the PVN) and extrahypothalamic (e.g., in the amygdala and the bed nucleus of the stria terminalis) stress circuitry (see Figure 2). Overlap also occurs within components of the mesocorticolimbic circuitry (e.g., in the medial prefrontal cortex, nucleus accumbens, ventral tegmental area, and hippocampus). Ultimately, this overlap can affect output of the PVN (i.e., the stress response) and alcohol drinking. Figure 2 shows simplified circuitry of

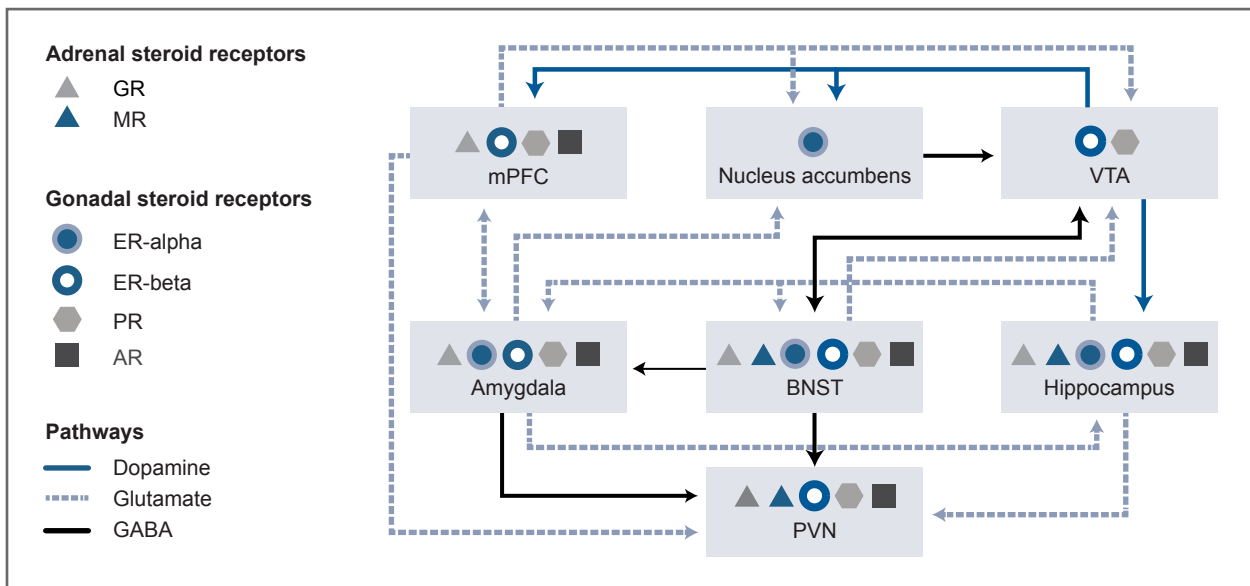


Figure 2 Simplified stress and mesocorticolimbic circuitry, including inputs to the HPA axis and the distribution of gonadal and adrenal steroid receptors. Rapid steroid actions at associated receptors and neurosteroid actions at GABA_A receptors represent additional mechanisms for fine-tuning central nervous system excitability. Gonadal and adrenal steroid receptors have considerable overlap in expression within the hypothalamic (PVN) and extrahypothalamic (e.g., amygdala, BNST) stress circuitry, as well as among components of the mesocorticolimbic (e.g., mPFC, nucleus accumbens, VTA, and hippocampus) circuitry, which ultimately can affect output of the PVN (i.e., the stress response) and alcohol drinking. This simplified circuitry shows GABAergic (red), glutamatergic (green), and dopaminergic (blue) projections within the brain regions that input to the PVN, either directly or indirectly through an inhibitory projection from the peri-PVN (which contains ER-alpha and GR, not shown). The brain regions involved and the overall influence on the output of the PVN (and HPA axis activity) depend on the stressor modality, the level of acute or chronic alcohol consumption, and the various steroid and neurosteroid levels and actions at their associated receptors. *Note:* AR, androgen receptor; BNST, bed nucleus of the stria terminalis; ER-alpha, estrogen receptor-alpha; ER-beta, estrogen receptor-beta; GABA, gamma-aminobutyric acid; GR, glucocorticoid receptor; HPA, hypothalamic-pituitary-adrenal; mPFC, medial prefrontal cortex; MR, mineralocorticoid receptor; PR, progesterone receptor (both isoforms); PVN, paraventricular nucleus; VTA, ventral tegmental area. *Source:* Circuitry^{13,24} and steroid receptor distribution^{13,15,21,33-36} are modified from other sources.

glutamatergic, GABAergic, and dopaminergic projections in brain regions important for responses to stress and alcohol drinking behavior. These responses to stress and alcohol drinking behavior may be modulated by steroid actions at receptors localized within the brain regions.

For example, the brain regions involved and the overall influence on PVN output depends on the stress, on various steroid hormone levels and actions at associated receptors,^{8,13} and on GABA_A receptor–active neurosteroid levels and actions at GABA_A receptors.²⁴ Alcohol's ability to activate the HPA axis relies on activation of the PVN.²⁷ Synaptic connections within the PVN are primarily GABAergic and glutamatergic.^{28,29} As a result, glutamatergic afferents in the forebrain that increase GABA release in the PVN, and upstream GABAergic projection neurons that activate the PVN, produce tonic inhibition of the PVN.³⁰

Additionally, stress-induced elevations in GABA_A receptor–active neurosteroids can modulate PVN activity, given that physiological concentrations of allopregnanolone (i.e., 10 nM to 100 nM) inhibit output of PVN neurons (i.e., CRF release) via a potentiation of GABA_A receptors.^{31,32} A neurosteroid-induced inhibition of CRF release likely represents another mechanism for terminating the stress response.

Another consideration is that alcohol-induced alterations to neurotransmission within the circuitry depicted in Figure 2 can be modulated by steroid hormone and neurosteroid levels. For instance, estradiol and progesterone can rapidly affect dopamine signaling via actions at their respective steroid receptors, functional coupling between estrogen receptors (both alpha and beta) and metabotropic glutamate receptors (Group I or Group II) can activate distinct signaling pathways, and neurosteroids can rapidly increase GABA_A receptor–mediated signaling.^{21,23,24,33-36} Thus, rapid steroid actions at associated receptors and neurosteroid actions at GABA_A receptors are other mechanisms for fine-tuning central nervous system excitability.

STEROID HORMONE EFFECTS ON DRINKING AND OTHER ADDICTION-RELATED BEHAVIORS

Investigations of sex differences in drug misuse and self-administration behavior have gained momentum, particularly after 2015, when the National Institutes of Health announced a policy of including sex as a biological variable. Clinical and preclinical alcohol research offers many examples of sex differences, given that alcohol exposure can produce sexually dimorphic effects throughout life. Discussion of all these studies is beyond the focus of this review, but several excellent reviews describe sex differences in the effects of alcohol exposure across development. Reviews have summarized findings from prenatal³⁷ and adolescent³⁸⁻⁴¹ alcohol exposure, as well as from exposure during adulthood.^{4,7} Marked sex differences in self-administration patterns have been well-documented and observed at every stage of the course of drug exposure, from acquisition to maintenance to relapse, although more evidence has been reported for psychostimulants than for alcohol.^{42,43}

In general, results from preclinical alcohol models indicate that females acquire self-administration of alcohol more rapidly and consume larger alcohol doses during maintenance phases than males, but females exhibit a reduced severity in somatic and negative affective symptoms of alcohol withdrawal than males.⁴ Although the potential role of organizational steroid effects in controlling sex differences in alcohol responses cannot be ruled out, this review focuses primarily on the effects, during adulthood, of estrogen, progesterone, and neuroactive metabolites on alcohol drinking and pertinent addiction-related phenotypes in females.

Gonadal Steroids

In a variety of models of alcohol access, preclinical research in rodents documents that females consume larger doses of alcohol than males. This sex difference appears to be partly due to

a facilitatory effect of estrogen in females and an inhibitory effect of testosterone in males.^{4,44} In female rodents, the estrous cycle phase had minimal effects on alcohol drinking or operant self-administration.⁴⁵ Reduced self-administration of alcohol was observed in females during proestrus and estrus only when their cycles had been experimentally synchronized (the effect was not observed in randomly cycling females that were not synchronized). Likewise, microanalysis of alcohol drinking patterns revealed increased frequency of bouts but less alcohol consumed within each bout during proestrus,⁴⁶ suggesting subtle differences in the pattern of alcohol drinking across the estrous cycle. In several models, more recent evidence confirmed that the phase of estrous cycle did not significantly influence alcohol drinking, including binge drinking,⁴⁷ escalated drinking among dependent animals,⁴⁸ self-administration of alcohol,⁴⁹ or cue plus yohimbine-induced reinstatement of alcohol-seeking.⁴⁹

In contrast to studies of rodents, a recent, longitudinal study of female rhesus monkeys with systematic and extensive hormonal monitoring of menstrual cycle phase across 15 months of active alcohol drinking determined that the monkeys drank more alcohol during the luteal versus the follicular phase and drank the most alcohol during the late luteal phase, when progesterone declines rapidly.⁵⁰ These results from a nonhuman, primate model of self-administration of alcohol were the first to show that typical menstrual cycle-related fluctuations in progesterone, especially during the late luteal phase, modulated alcohol drinking. Previous studies that used less accurate characterization of menstrual cycles and differing histories of alcohol intake revealed inconsistent effects of the menstrual cycle on alcohol drinking. Therefore, Dozier and colleagues' method of extensive menstrual cycle characterization during periods of active drinking⁵⁰ likely was necessary to show the significant menstrual cycle-related fluctuation in alcohol drinking.

The results by Dozier and colleagues are consistent with clinical studies in which increases in premenstrual distress and negative affective

states in women were positively correlated with greater alcohol drinking during the late luteal phase.^{4,51} Thus, existing data support the conclusion that typical hormonal fluctuations during the menstrual cycle, but not during the estrous cycle, can influence alcohol drinking. These differences may reflect hormonal changes during the menstrual cycle that are distinct from those in the estrous cycle,⁵¹ because rodents have no equivalent luteal phase (see the box **Phases of Primate Menstrual and Rodent Estrous Cycles**).

Despite minimal effects of the estrous cycle phase on alcohol drinking, several lines of evidence in studies of rodents indicate that the hormonal milieu contributes to sex differences in models of alcohol drinking behavior and alcohol reward. First, development of the four core genotype (FCG) mouse model has enabled researchers to examine the sex chromosome complement (XX versus XY) and the gonadal phenotype (testes versus ovaries) and their independent contributions to sex differences.⁵² This model produces four different progeny, each with a different combination of sex chromosomes and gonadal sex: XXF (XX gonadal females), XXM (XX gonadal males), XYF (XY gonadal females), and XYM (XY gonadal males). Use of the FCG model determined that gonadal phenotype predicted self-administration of alcohol, independent of the sex chromosome complement.⁵³ That is, gonadal females consumed more alcohol than gonadal males.

Second, several studies that used gonadectomy and hormone replacement found that when compared with intact female rats, female rats with gonadectomy drank significantly less alcohol.^{54,55} After the gonadectomized rats received estradiol replacement, the low levels of alcohol drinking increased significantly to baseline levels. Also, in female mice, gonadectomy significantly reduced binge drinking from the high levels of consumption among intact females to levels of consumption equivalent to that of intact males.⁴⁷ The lower levels of binge drinking among female mice with gonadectomy increased significantly following replacement with 17beta-estradiol.⁴⁷

Similarly, gonadectomy in male and female rats produced shifts in operant alcohol self-administration toward the pattern of the opposite sex (i.e., reduced for females and increased for males).⁴⁹ In these rats, estradiol replacement in females with gonadectomy significantly increased self-administration of alcohol, and testosterone replacement in males with gonadectomy significantly decreased self-administration of alcohol. However, in rodent males, the suppressive effect of testosterone on alcohol drinking contrasts with fairly consistent clinical reports that found positive associations between blood or salivary testosterone levels and alcohol drinking among human adolescent and adult males.¹⁰

Third, in studies that used conditioned place preference as a measure of alcohol reward, only intact female rats exhibited conditioned place preference to an intermediate alcohol dose.⁵⁶ Intact male rats and female rats with gonadectomy (males with gonadectomy were not tested) did not exhibit the preference for the drug paired side of the testing chamber. Subsequent studies in female mice determined that in females with gonadectomy, 17beta-estradiol facilitated alcohol-induced conditioned place preference due to activation of both estrogen receptor-alpha and estrogen receptor-beta.⁵⁷

The facilitatory effects of estradiol on alcohol drinking and a measure of alcohol reward may be due, in part, to estradiol's rapid enhancement of dopaminergic signaling.³⁶ In the prefrontal cortex, the ability of a low dose of alcohol (0.5 g/kg) to enhance extracellular dopamine levels in female rats during estrus was eliminated by gonadectomy and restored by estradiol treatment.⁵⁸ In the striatum, the well-documented ability of estradiol to enhance dopaminergic signaling in females was hypothesized to be associated with effects of estradiol on membrane-localized estrogen receptor-alpha and estrogen receptor-beta that were functionally coupled to metabotropic glutamate receptors.^{34,36} Collectively, research confirms that within each sex, activational effects of gonadal steroids can modulate alcohol drinking behavior.

The organizational effect of testosterone-derived estrogen, which causes sex-specific differentiation of the mammalian brain,^{9,52,59} during a critical period of brain development, also influences alcohol drinking. Early work found that neonatal exposure to estrogen among female rats, which conferred a male phenotype on a genetically female brain, produced levels of alcohol drinking that were lower than levels in intact females but similar to levels in intact males.⁶⁰

More recent work has determined that gonadectomy alone in male and female rats shifted self-administration of alcohol toward the pattern of the opposite sex, but it did not eliminate the sex difference.⁴⁹ Females with gonadectomy still self-administered more alcohol than males with gonadectomy. Likewise, during tests of alcohol-seeking (cue plus yohimbine-induced reinstatement), intact females engaged in active lever presses more than intact males. Females with gonadectomy still had more lever presses than males with gonadectomy, and lever presses were not altered by steroid replacement (i.e., estradiol in females and testosterone in males). These results suggest that in addition to the contribution of the activational effects of gonadal steroids on alcohol drinking in males and females, permanent factors, such as sex chromosomes and the organizational effects of gonadal steroids, contribute to sex differences in alcohol-drinking and alcohol-seeking behaviors.

Use of the FCG model also determined that independent of gonadal phenotype, the sex chromosome complement mediates habitual responding for alcohol reinforcement after moderate instrumental training.⁵³ Specifically, XY mice (XYM and XYF) were insensitive to alcohol devaluation, a procedure that established conditioned taste aversion by pairing alcohol consumption with lithium chloride injections. Both valued (no conditioned taste aversion) and devalued (with conditioned taste aversion) XY mice responded similarly, indicating that XY mice were responding in a habitual manner. XX mice (XXM and XXF) were sensitive to alcohol devaluation (devalued XX mice responded less

than valued XX mice), indicating that XX mice retained goal-directed responding.⁵³

Given that AUD involves a transition from casual to habitual use, as well as a transition from ventral striatal circuitry including the prefrontal cortex to a more dorsal circuit involving the dorsolateral striatum,⁶¹ the results from Barker and colleagues⁵³ suggest that sex chromosomes mediate sex differences in habit formation for alcohol, and they may underlie sex differences in alcohol-induced neuroadaptation. Additional studies are necessary to disentangle the contribution of sex chromosomes and the organizational effects of gonadal steroids on alcohol-motivated behavior.

Neurosteroids

Studies have examined whether manipulation in levels of the progesterone derivative allopregnanolone, which is a potent, positive allosteric modulator of GABA_A receptors,²³⁻²⁶ alters alcohol drinking and alcohol's subjective effects. In general, females have higher endogenous allopregnanolone levels than males. Allopregnanolone levels in females fluctuate across the estrous and menstrual cycles and increase during pregnancy in a time-dependent manner that is related to fluctuations in endogenous progesterone.^{25,62,63} The majority of studies, which were conducted in male rodents, consistently have shown that allopregnanolone, after systemic and intracerebroventricular administration, exerts a biphasic effect (i.e., increases with low physiological doses and decreases with supraphysiological doses) on alcohol drinking and operant self-administration.⁶⁴

In contrast, research has shown that allopregnanolone does not alter alcohol drinking in female mice (see Figure 3).⁶⁵ Administration of the 5 α -reductase inhibitor finasteride to mice, which decreased endogenous GABA_A receptor-active neurosteroids such as allopregnanolone,⁶⁵ produced a decrease in the acquisition and maintenance phases of self-administration of alcohol in males, with females, again, being less sensitive to these modulatory effects.⁶⁶⁻⁶⁸

A priming dose of allopregnanolone promoted reinstatement of alcohol-seeking behavior in male mice and rats,^{69,70} but similar studies in females have not been conducted.

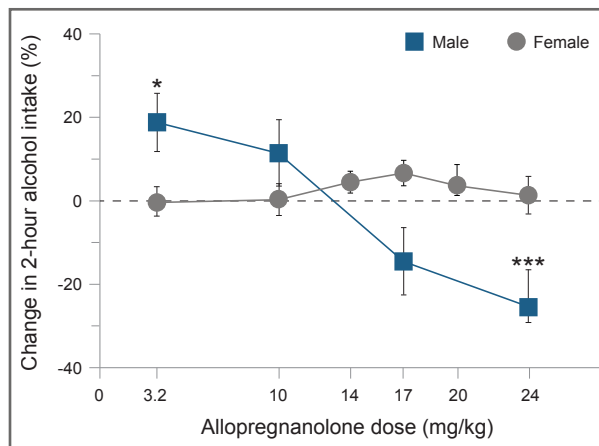


Figure 3 Sex differences in the modulatory effect of allopregnanolone on limited-access alcohol drinking in mice. Dose response is shown as a percentage of change from baseline values (vehicle treatments). The graph depicts the means and standard errors for 18 male and 24 female C57BL/6J mice. The dashed line represents the baseline values. Note: * $p \leq 0.05$; *** $p \leq 0.001$ versus respective vehicle treatment (20% beta-cyclodextrin). Source: Adapted from Finn DA, Beckley EH, Kaufman KR, et al.⁶⁴

Finally, evidence also suggests that allopregnanolone and its 5 β -isomer, pregnanolone, like alcohol, possess positive motivational effects, as demonstrated by conditioned place preference among male mice,⁷¹ preference for drinking steroids versus water in male mice and rats,^{72,73} and intravenous self-administration in four rhesus monkeys, with the highest self-administration of pregnanolone in the one female versus the three male monkeys.⁷⁴ Both allopregnanolone and pregnanolone produced potent, alcohol-like, discriminative stimulus effects in male and female cynomolgus monkeys.⁷⁵ Also, during the luteal phase of the menstrual cycle, when endogenous allopregnanolone levels were highest, female cynomolgus monkeys were more sensitive to the discriminative stimulus effects of alcohol and

to the alcohol-like effects of allopregnanolone.⁷⁶ Collectively, these results suggest that GABAergic neurosteroid levels may enhance the reinforcing effects of alcohol, and that in rodents, sensitivity to neurosteroid effects differs by sex.

A comparison of results in female mice and monkeys suggests that female monkeys are more sensitive to allopregnanolone's modulatory effects on alcohol drinking behavior. However, the relative insensitivity in female mice contrasts with the enhanced sensitivity to the anticonvulsant effect of allopregnanolone and THDOC during alcohol withdrawal in female rats and in female mice that have a low withdrawal phenotype.⁷⁷⁻⁷⁹

Based on evidence that local allopregnanolone metabolism in hippocampal subregions significantly altered GABA_A receptor-mediated inhibition,⁸⁰ a sex difference in allopregnanolone

metabolism in discrete brain regions in mice possibly contributes to low sensitivity to allopregnanolone's modulatory effects on alcohol drinking. Belelli and Herd used the 3alpha-hydroxysteroid dehydrogenase (3alpha-HSD) inhibitor indomethacin to inhibit oxidation of allopregnanolone to dihydroprogesterone, which increased local allopregnanolone levels and enhanced GABA_A receptor-mediated inhibition.⁸⁰ Early work indicated that female rats, when compared with males, had about twice the activity of 3alpha-HSD from rat-liver cytosol, and that this sex difference was induced by ovarian estrogen.⁸¹ So, in female rodents, more 3alpha-HSD activity within neurocircuitry fundamental to the regulatory processes underlying alcohol intake possibly contributes to insensitivity to the effects of allopregnanolone on alcohol drinking. Consistent with this idea, administration of allopregnanolone and indomethacin in female mice did not alter alcohol drinking when administered separately but produced a significant decrease in alcohol drinking when administered in combination (see Figure 4, DA Finn and MM Ford, unpublished data, May 2013).

Another strategy for avoiding potential confounds of rapid allopregnanolone metabolism is use of a synthetic allopregnanolone analog, such as ganaxolone.⁸² Ganaxolone has a similar pharmacological profile to allopregnanolone, but it has an additional 3beta-methyl group that protects the steroid from metabolic attack at the 3alpha-position and extends the half-life about three to four times longer than that of allopregnanolone. In male rodents, ganaxolone produced a biphasic effect on alcohol drinking and self-administration when administered systemically⁸³⁻⁸⁵ or bilaterally into the nucleus accumbens shell.⁸⁶ Systemic ganaxolone also promoted reinstatement of alcohol-seeking.⁸⁷ These effects of ganaxolone on alcohol drinking and seeking were similar to those observed following allopregnanolone administration. Preliminary results suggest that ganaxolone also

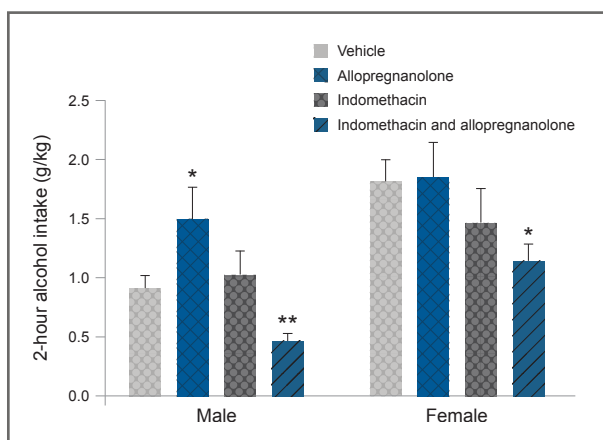


Figure 4 Modulatory effect of a combination of allopregnanolone and indomethacin in male and female mice. Female mouse insensitivity to allopregnanolone's modulatory effect on limited-access alcohol drinking was overcome by administering 0.1 mg/kg indomethacin along with 10 mg/kg allopregnanolone. Indomethacin blocks the oxidation of allopregnanolone and thereby enhances allopregnanolone's effect on GABA_A receptor-mediated inhibition. The graph depicts the means and standard errors for 10 male and 10 to 11 female C57BL/6J mice. Note: * $p \leq 0.05$; ** $p \leq 0.01$ versus respective vehicle treatment (20% beta-cyclodextrin). Source: DA Finn and MM Ford, unpublished data, May 2013.

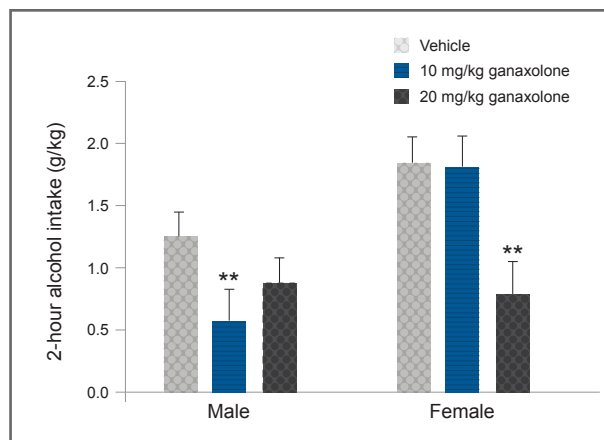


Figure 5 Sex differences in the modulatory effect of the synthetic neurosteroid ganaxolone in mice. Ganaxolone significantly decreased limited-access alcohol drinking in males and females. To significantly suppress alcohol drinking, female mice required a higher dose (20 mg/kg) than male mice (10 mg/kg). The graph depicts the means and standard errors for 10 male and 10 to 11 female C57BL/6J mice. *Note:* ** $p \leq 0.01$ versus respective vehicle treatment (20% beta-cyclodextrin). *Source:* DA Finn and MM Ford, unpublished data, April 2013.

significantly reduces alcohol drinking in female mice, although a higher dose was required to produce a comparable reduction to that observed in male mice (see Figure 5, DA Finn and MM Ford, unpublished data, April 2013).

The U.S. Food and Drug Administration recently approved the allopregnanolone analog brexanolone for treatment of postpartum depression. In addition, ganaxolone is in phase 2 clinical trials for treatment of various disorders, such as postpartum depression, treatment-resistant depression, post-traumatic stress disorder (PTSD), and epilepsy. Allopregnanolone analogs and strategies to stabilize allopregnanolone levels also are being examined in clinical trials for the treatment of various central nervous system disorders.⁸⁸ Collectively, evidence suggests that targeting neurosteroid synthesis or use of neurosteroid analogs such as ganaxolone may represent innovative therapies for the treatment of AUD in males and females.²⁶

EFFECTS OF CHRONIC ALCOHOL USE ON GONADAL STEROID LEVELS

Alcohol misuse and AUD produce significant hormonal disruptions in the endocrine system.⁷ For sex steroids, the majority of evidence in rodents and humans suggests that chronic alcohol exposure significantly increases estradiol levels in both males and females, produces a slight or significant decrease in progesterone levels in both males and females, decreases testosterone levels in males, and produces a transient increase in testosterone levels in females. Additional work found that chronic exposure to alcohol vapor to induce dependence significantly increased testosterone levels in female mice and suggested that the increased testosterone levels in dependent female mice contributed to an observed estrous cycle disruption (i.e., prolonged diestrus).⁸⁹

Thus, the HPG dysfunction that occurs in people with AUD can be associated with deleterious effects on reproduction in both males and females. However, some preclinical studies suggest that 6 weeks of binge drinking by female rodents⁴⁷ or 15 months of active drinking by female monkeys⁵⁰ did not significantly alter the estrous or menstrual cycles, respectively, in terms of overall cycle length or the length of specific cycle phases. Fifteen months of active drinking also did not alter progesterone or estradiol levels in the female monkeys.⁵⁰ The method of chronic alcohol exposure and resulting blood alcohol concentrations, which are considerably higher for vapor exposure (e.g., 200 mg%) than for drinking models (e.g., 80 mg% to 100 mg%), may contribute to the differences among studies with regard to whether chronic alcohol exposure disrupted the estrous or menstrual cycle.

EFFECTS OF CHRONIC ALCOHOL USE ON NEUROSTEROID LEVELS

Preclinical models of chronic alcohol drinking and vapor exposure both produce significant alterations in neurosteroid levels. Most of the evidence supports changes to allopregnanolone levels in plasma and in discrete brain regions.²⁴ The majority of available data are from studies in male rodents and monkeys. The results consistently show that chronic alcohol drinking and vapor exposure significantly decrease plasma allopregnanolone levels during acute withdrawal, a finding in harmony with the limited results reported for males and females with AUD.

In a small cohort of females with AUD, a significant reduction in allopregnanolone, progesterone, and estradiol levels was detected upon detoxification, and levels recovered to baseline values after 4 months of abstinence.⁹⁰ In contrast, chronic alcohol drinking did not significantly alter serum allopregnanolone levels in female monkeys,⁵⁰ nor did withdrawal from chronic alcohol vapor exposure alter plasma allopregnanolone levels in female mice (DA Finn and JP Jensen, unpublished data, Feb 2019 and Nov 2019).

Regarding brain regional changes, chronic alcohol exposure and withdrawal significantly decreased allopregnanolone levels in the amygdala of male monkeys and in the nucleus accumbens, ventral tegmental area, and medial prefrontal cortex of male rodents, with divergent changes reported in hippocampal subregions in male rodents.²⁴ However, preliminary results in female mice suggest that withdrawal from chronic alcohol exposure did not significantly alter cortical or hippocampal allopregnanolone levels (DA Finn and JP Jensen, unpublished data, Feb 2020 and Mar 2020).

Collectively, preclinical results in male rodents and monkeys suggest that independent adrenal and brain region regulation of neurosteroid synthesis occurs after chronic alcohol exposure and withdrawal. More preclinical research in females is necessary, but the available preclinical results suggest that females may be protected

from chronic alcohol–induced suppression of allopregnanolone synthesis. Given the preclinical evidence that severity of alcohol withdrawal is reduced in females versus males,⁴ and that allopregnanolone has anticonvulsant, anxiolytic, and antidepressant properties,²⁴ females may have the ability to maintain endogenous allopregnanolone levels after chronic alcohol exposure. This maintenance, versus the suppression seen in males, may contribute to the female phenotype for reduced severity and duration of alcohol withdrawal.

STRESS STEROIDS AND ALCOHOL-RELATED BEHAVIOR

Clinical studies provide evidence for a positive association between stress and alcohol drinking and other phases of AUD, including evidence of stress as a trigger of alcohol relapse.⁹¹ Additionally, males and females have different sensitivities to alcohol and stress.⁴⁻⁶ Acute stress exposure and alcohol intoxication both activate the HPA axis, and the HPA and HPG interact reciprocally (Figure 1).⁸ Therefore, sex differences in HPA axis responsivity following acute stress or acute alcohol intoxication (i.e., enhanced elevation in glucocorticoids in females versus males) are not surprising. Discussion of all studies on this topic is beyond the scope of this review, but other reviews provide more detail.^{5,8,13,92}

Preclinical studies demonstrate conflicting evidence regarding the influence of various stressors on alcohol drinking in rodents, and sex- and stress-related alterations in drinking vary with the stress model used.^{5,93} However, a few examples of results show a sex difference in the relationship between corticosterone levels and alcohol drinking or alcohol-seeking.

First, studies have shown that exposure to predator odor stress (PS), which is considered a traumatic stress and used as a model of PTSD, significantly increases alcohol drinking and self-administration in rodents.⁹⁴ Evidence supports greater PS-enhanced drinking among female

versus male mice.^{93,95} Plasma corticosterone levels following PS exposure have been shown to be significantly higher in female versus male mice when mice were naïve and also when the mice had a history of alcohol drinking.^{93,95} Also, investigators have reported a significant positive correlation between plasma corticosterone levels and alcohol intake on the first day after PS exposure. When all mice were considered, the goodness of fit of the regression line ($R^2 = 0.26$, $p < 0.05$) indicated that the variation in PS-induced corticosterone levels accounted for 26% of the variance in alcohol drinking on the day after PS exposure. The relationship was stronger in females ($R^2 = 0.42$, $p < 0.05$), confirming that the amount of HPA axis activation after PS exposure significantly influenced alcohol drinking the following day.⁹³

Second, studies examining cue plus yohimbine-induced reinstatement of alcohol-seeking in male and female rats determined that active lever presses during the reinstatement tests were significantly higher in females versus males.⁹⁶ During the reinstatement testing for female rats only, corticosterone and estradiol levels were significantly, positively correlated with active lever presses.⁹⁶

Third, in mice deficient in beta-endorphin (knockout mice), a peptide that regulates HPA axis activity via mu opioid receptor-mediated inhibition, the females had elevated basal levels of anxiety, plasma corticosterone, and CRF in the extended amygdala when they were compared with female wild-type mice.⁹⁷ High binge alcohol intake in the female beta-endorphin knockout mice normalized their high levels of basal anxiety, corticosterone, and CRF. This relationship was not observed for the male beta-endorphin knockout mice when they were compared with wild-type mice.

Fourth, in mice with a history of alcohol drinking and exposure to PS, the PS-induced increase in plasma corticosterone was significantly lower in male mice, and tended to be lower in female mice, versus respective naïve mice.⁹⁵ This result is consistent with evidence that AUD in humans and alcohol dependence in rodents can lead to a dampened neuroendocrine state in

terms of HPA axis responsivity.⁷ Collectively, the results suggest that overlapping stress and gonadal steroids, as well as sex differences in HPA axis responsivity, contribute to sex differences in alcohol drinking, alcohol-seeking, and interaction with stress.

Preclinical studies also demonstrate cellular and molecular sex differences in stress response systems.^{5,8,13,92} Both glucocorticoid receptors and CRF₁ receptors are being pursued as potential targets for AUD pharmacotherapies, but preclinical data in support of these targets have been generated primarily in males.⁹⁸ Recent work in male and female mice found that a history of alcohol drinking and intermittent PS exposure produced sexually divergent and brain region differences in protein levels for glucocorticoid receptors and CRF₁ receptors.⁹⁵ Increased cortical glucocorticoid receptor levels and hippocampal CRF₁ receptor levels were only found in female mice. These findings are consistent with evidence for impaired glucocorticoid negative feedback resulting from inhibition of glucocorticoid receptor translocation and evidence for increased CRF₁ receptor signaling and decreased CRF₁ receptor internalization in female versus male rodents.⁹²

Collectively, an increased endocrine response to stress and alcohol consumption in females may result from sex differences that occur at the molecular and systems level. The sex differences in CRF₁ receptor and glucocorticoid receptor protein levels described above suggest that sexually divergent mechanisms may contribute to HPA axis dysregulation following a history of alcohol drinking and repeated stress exposure. As a result, pharmacological strategies targeting the CRF₁ receptor and glucocorticoid receptor systems may be differentially effective in males versus females.

EFFECTS OF STRESS ON NEUROSTEROID LEVELS

Exposure to stress³¹ and models of acute alcohol intoxication^{24,99} also significantly increase levels of GABA_A receptor-active neurosteroids, although some species differences in the effects of alcohol

administration on neurosteroid levels have been reported.¹⁰⁰ In addition, most of these studies were conducted in males. In male rats, alcohol's steroidogenic effect was shown to be regulated by an alcohol-induced increase in ACTH release and by de novo synthesis of adrenal steroidogenic acute regulatory protein.¹⁰¹ Chronic alcohol exposure blunts alcohol's steroidogenic effect on neurosteroid levels, but administration of ACTH restores the steroidogenic effect.¹⁰² Although comparable studies have not been conducted in females, limited data have indicated that CRF and ACTH tests in women significantly increase serum allopregnanolone, progesterone, and dehydroepiandrosterone levels.⁶³ Studies also have reported that binge alcohol intoxication in male and female adolescent humans significantly increased serum allopregnanolone levels.^{103,104}

Preclinical studies found that exposure to various stressors significantly increased plasma allopregnanolone levels in male and female mice that had been consuming alcohol for weeks,⁹³ whereas weeks of alcohol consumption alone (i.e., without stress exposure) significantly increased brain allopregnanolone levels in male mice but not in female mice.⁶² Thus, data available for females suggest that stress and activation of the HPA axis increases neurosteroid levels, whereas acute alcohol administration produces inconsistent effects. Additional studies in females are necessary to determine whether an alcohol-induced steroidogenic effect can exert a protective effect against further alcohol drinking, as has been proposed for males.⁹⁹

Two studies with small cohorts of male and female patients with co-occurring AUD and cocaine use disorder found that progesterone administration decreased cue-induced craving and cortisol responses.¹⁰⁵ The male and female subjects with the highest allopregnanolone levels after progesterone administration showed the greatest reductions in craving,¹⁰⁶ with no sex differences in these relationships. Consequently, despite no direct data on neurosteroid treatment in patients with AUD, strategies to enhance levels of GABA_A receptor-active neurosteroids, such as

allopregnanolone, may represent a biomarker of treatment efficacy among men and women.^{5,91}

CONCLUSION

The current review considered the contribution of the endocrine system to alcohol drinking and addiction-related behaviors in females, with a focus on the HPG and HPA axes and their reciprocal interactions. The majority of results from preclinical models indicate that females acquire self-administration of alcohol more rapidly and consume higher alcohol doses during maintenance phases than males. However, aspects of alcohol withdrawal, especially somatic and some negative affective symptoms, are less severe in females than in males. Some of these behavioral differences are due to the organizational and activational effects of gonadal steroids.

Numerous studies that used gonadectomy and steroid replacement documented that gonadal steroids have activational effects and that these activational effects contribute to the higher alcohol drinking, self-administration, and responding during reinstatement tests of alcohol-seeking in females versus males. However, additional studies determined that permanent factors, such as sex chromosomes and the organizational effects of gonadal steroids, also can contribute to sex differences in alcohol drinking and alcohol-seeking. For example, elegant studies that used the FCG mouse model determined that the sex chromosome complement mediated habitual responding for alcohol reinforcement. Additional studies are necessary to distinguish how sex chromosomes and the organizational effects of gonadal steroids contribute to alcohol-motivated behavior.

Sex steroids also influence the stress response, and elevated glucocorticoids can suppress HPG axis function (Figure 1). In addition to the facilitatory and inhibitory feedback mechanisms within and between the HPA and HPG axes, steroid hormones and their derivatives (e.g., neurosteroids) can influence brain function and behavior through classic genomic actions and rapid membrane effects at receptors localized

within brain regions important for stress responses and for alcohol-related behaviors (Figure 2). For example, ovarian steroids can modulate dopamine signaling and distinct signaling pathways through actions at their membrane receptors, and neurosteroids can rapidly increase GABA_A receptor-mediated signaling. These effects represent another way that steroids and neurosteroids modulate alcohol-drinking and -seeking behaviors.

Likewise, sex steroids modulate PVN output (e.g., the stress response). Estrogen has a facilitatory effect, and testosterone has an inhibitory effect. These effects are consistent with enhanced HPA axis responsivity and elevated glucocorticoids in females versus males. In both sexes, a neurosteroid-induced inhibition of CRF release via enhancement of GABAergic inhibition likely is a mechanism for terminating the stress response.

Another consideration is that the well-documented effects of chronic alcohol use and exposure on steroid levels provides another level of complexity toward understanding the influence of gonadal and stress steroids on alcohol-related behaviors.

Evidence for a positive association between stress and alcohol drinking is strong in clinical studies and mixed in preclinical studies. However, stress is a potent trigger of alcohol relapse in clinical studies and of alcohol-seeking in preclinical studies. HPA axis responsivity is enhanced in females versus males. So, it is interesting that only female rodents exhibited positive correlations between corticosterone levels following stress and stress-enhanced drinking as well as between corticosterone and estradiol levels and lever presses during cue- and stress-induced reinstatement tests of alcohol-seeking. In addition to the facilitatory effect of estrogen on the HPA axis, these sex differences could be due, in part, to impaired glucocorticoid receptor negative feedback and increased CRF₁ receptor signaling in female rodents.

Both glucocorticoid receptors and CRF₁ receptors are being pursued as potential targets for treatment of AUD, but most preclinical and

clinical data examining medications that target these receptor systems have used male subjects. The few clinical studies that included female subjects were underpowered to examine for sex effects. In the single study conducted with females—who had anxiety and AUD—the CRF₁ receptor antagonist verucerfont reduced HPA responsivity without altering measures of alcohol craving.⁹¹ Considering the preclinical data indicating that CRF₁ receptor antagonists effectively reduce escalation in alcohol drinking in dependent male rodents, it is not known whether verucerfont would reduce measures of alcohol drinking in females with AUD.

Regarding glucocorticoid receptor antagonists, the mixed glucocorticoid receptor and progesterone receptor antagonist mifepristone (also known as RU-486) significantly reduced measures of alcohol craving and alcohol consumption in participants with AUD.⁵ These participants were predominantly male (the mifepristone treatment group was 82% male). Because of its progesterone receptor antagonism, mifepristone is used in females to terminate pregnancy. Thus, use of mifepristone in females may be confounded by its mixed pharmacological properties, with the progesterone receptor antagonism producing more serious side effects in females versus males.

More selective glucocorticoid receptor antagonists, such as CORT113176, are being pursued, but data for females are not available. Preliminary data in mice selectively bred for a high binge drinking phenotype determined that CORT113176 significantly decreased binge drinking in both male and female mice, and that female mice were more sensitive to the effect.¹⁰⁷

Pharmacological strategies targeting the CRF₁ receptor and glucocorticoid receptor systems may be differentially effective in males versus females, and new strategies targeting these systems could have greater specificity for females.⁹² For example, inhibiting molecules that facilitate the transport of glucocorticoid receptors to their classical intracellular receptor might normalize high glucocorticoid levels in females. Likewise,

compounds that target the CRF₁ receptor and shift signaling away from pathways that enhance CRF₁ receptor signaling might make females more resilient to stress-induced hyperarousal.⁹²

Strategies targeting GABA_A receptor–active neurosteroids or their biosynthesis may represent an approach to effectively treat AUD in males and females. Results from preclinical models suggest that chronic alcohol drinking or the induction of dependence in females does not significantly alter allopregnanolone levels, as is seen in males. These results are consistent with the idea that the ability of females to maintain endogenous levels of a GABAergic neurosteroid following chronic alcohol exposure may contribute to the reduced severity of their alcohol withdrawal phenotype. Alternately, strategies to enhance neurosteroid synthesis may exert a protective effect against further alcohol drinking in females, as has been proposed for males.⁹⁹

Neurosteroid analogs with a longer half-life than allopregnanolone show promise as another effective strategy. For instance, brexanolone was recently approved for the treatment of postpartum depression. Currently, ganaxolone also is in clinical trials for treatment of postpartum depression, as well as for treatment-resistant depression, PTSD, and epilepsy. Preclinical results indicate that ganaxolone significantly reduces alcohol drinking in male and female mice (Figure 5, DA Finn and MM Ford, unpublished data, April 2013). Thus, neurosteroid analogs may be effective at reducing alcohol drinking in individuals with co-occurring AUD and depression or co-occurring AUD and PTSD, or in individuals with AUD who drink to alleviate stress and negative affect.

Finally, use of progesterone as a “prodrug” to increase allopregnanolone levels has been an effective strategy to decrease cue-induced craving and cortisol responses in small cohorts of male and female patients with co-occurring AUD and cocaine use disorder.^{105,106} The greatest reduction in craving was observed in male and female participants who had the highest allopregnanolone levels after progesterone administration.^{105,106}

Thus, strategies to use allopregnanolone analogs with longer half-lives, or to stabilize or enhance levels of GABA_A receptor–active neurosteroids such as allopregnanolone, may represent new efficacious treatments for both males and females with AUD.

Collectively, the importance of arriving at a more complete understanding of the neuroendocrine mechanisms underlying sex differences is clear, as treatment strategies and their effectiveness may revolve around sex differences in the endogenous steroid and neurosteroid environments and in sexually divergent downstream signaling mechanisms. In addition, variations in neurosteroid physiology also may help explain individual differences in susceptibility to AUD, vulnerability to relapse, and the negative health consequences of alcohol intake.

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ALCOHOL AND LIVER FUNCTION IN WOMEN

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Alcohol-related liver disease generally has been ascribed to men because men reportedly consume alcohol at an increased rate and quantity as compared to women. Recent literature has reported, however, that rates of liver disease attributed to alcohol use by women have increased, largely due, in part, to the increased number of women who consume alcohol regularly. This increase is a paramount concern, as women are more susceptible than men to the effects of alcohol-related liver injury. Health care providers should make efforts to counsel women on the risks of excess alcohol consumption to prevent further increase in alcohol-related liver disease and its associated complications.

KEY WORDS: alcohol; estrogen; liver disease; women

EPIDEMIOLOGY

The prevalence of alcohol use disorder is increasing, and one of the most devastating complications is end-stage liver disease. Interestingly, the consequences of alcohol use do not affect all heavy-drinking individuals with the same frequency. Only 15% of people who drink heavily develop cirrhosis from heavy alcohol consumption.¹ Certain populations, including those with genetic predispositions (e.g., presence of the *PNPLA3* genotype) and women, are more susceptible to end-stage effects of alcohol-related liver injury.

Historically, alcohol-associated liver injury has been reported to be more prevalent in men, despite women's increased susceptibility to the detrimental

effects of alcohol.² This difference in prevalence largely is due to the fact that men generally consume more alcohol than women. However, a recent study that examined the presence of alcohol-related liver disease from 2009 to 2015 demonstrated increased incidence (50%) of alcohol-related liver injury in women, as compared to a 30% increase among men during the same time period.³ The increase in alcohol-related liver injury among women appears to parallel the increase in alcohol consumption observed in women.

A study examining alcohol use patterns in the United States from 2001 to 2002, as compared with 2012 to 2013, reported an 80% increase in

heavy alcohol consumption among women and a 30% increase among men.⁴ Similar patterns have been seen globally, with a Japanese study noting a twofold to fourfold increase in alcohol consumption among women from 1968 to 1987.⁵ In this study, the rates of alcohol consumption in men remained static. A meta-analysis examining the effects of alcohol use and cirrhosis reported that cirrhosis was more frequent in women versus men, despite similar amounts of alcohol consumption.⁶

MECHANISTIC FACTORS

Previous studies have shown that, when controlling for the amount of alcohol consumed and for body weight, women had increased levels of blood alcohol when compared with men.⁷ This increase likely is due to decreased body water content in women, thus leading to a smaller volume of distribution. Moreover, women have reduced gastric alcohol dehydrogenase compared with men and therefore impaired first-pass metabolism, resulting in increased susceptibility to injury.⁷ Additional studies also have shown gender differences in alcohol metabolism by hepatic enzymes such as cytochrome P450 2E1, with lower levels in women due to regulation of growth hormone.⁸ The role of estrogen is also a culprit.

Kupffer cells reside within hepatic sinusoids and play a role in clearance of foreign compounds within the liver. Activation of Kupffer cells leads to cytokine release and subsequent hepatic inflammation.⁹ Rat models have shown that estrogen exposure increases Kupffer cell susceptibility to endotoxin. When animals that received exogenous estrogen were studied, increased Kupffer cell sensitization to lipopolysaccharide was observed.¹⁰ Additional animal models have demonstrated that increased endotoxin release related to Kupffer cell activation resulted in more severe hepatic injury and necrosis.¹¹ In fact, estrogen blockade in mouse models has been shown to attenuate alcohol-related injury in females.¹²

IMPLICATIONS

These factors likely account for studies showing that women, compared to men, are more susceptible to liver disease with less alcohol consumption, and that women have a faster progression to cirrhosis over a shorter time period. In a study conducted in Australia, the rate of progression to cirrhosis for women was 13.5 years, as compared to 20 years for men, when controlling for less alcohol consumption among the women.¹³ More vexing is that although alcohol abstinence has been linked to fibrosis regression, reports show that among people who had cirrhosis and then abstained from alcohol, women had lower 5-year survival rates than men.¹⁴

Current recommendations from the “Dietary Guidelines for Americans 2015–2020” advise that women should not consume more than 14 grams of alcohol daily, and men should not consume more than 28 grams of alcohol daily.¹⁵ The relative risk of alcohol-related liver disease increases in women who drink any more than one drink per day. Recently, the Million Women Study in the United Kingdom published prospective data and reported observed liver disease patterns among women from 1996 to 2001.¹⁶

An interesting observation from the Million Women Study is that people who reported drinking daily were more susceptible to liver injury than those who reported binge drinking.¹⁶ Thus, recommendations from this study advise that women abstain from drinking daily. This study also noted that women who drank alcohol with meals were less susceptible to alcohol-related injury than those who drank without eating. A possible explanation for this finding is the increased metabolism of alcohol for those who drank with meals as compared to the metabolism of those who did not drink with meals.

The effects of alcohol consumption outside of meals appear to coincide with the observation that women with eating disorders (e.g., bulimia, anorexia) are more susceptible to alcohol-related liver injury than women with no eating disorder.^{17,18} These findings may be explained by the nutritional deficiencies associated with eating

disorders, which are hepatotoxic independent of the effects of alcohol. Other studies have shown that increases in alcohol-related liver disease coincide with obesity.¹ Thus, the presence of eating disorders is not the only risk factor that implicates accelerated progression of alcohol-related liver disease. In a study examining risk factors for liver disease in both men and women, an increased waist-to-hip ratio (a measure of fat distribution) portended a worse prognosis for development of severe liver disease.¹

OBESITY AND ALCOHOL USE

A possible explanation for the paradoxical discrepancy between alcohol-related liver injury in people with eating disorders and the recent observed increase in those with obesity may be due to the overlap of non-alcoholic fatty liver disease co-existing with alcohol-related liver disease, thus explaining the latter.

In a non-gender focused study, researchers replaced alcoholic beverages with non-alcoholic beverages to examine the effects on hepatic triglyceride fat content.¹⁹ Individuals who received a sugary beverage as a substitute for alcohol, as compared with those who received a non-sugary beverage, had increased hepatic triglyceride fat content. Even more intriguing was that the hepatic triglyceride levels for those who consumed the sugary beverage were comparable to the levels observed for those who consumed the alcoholic beverage. The effects of non-alcoholic beverages on the liver warrant further study, but these results may explain the increase of cirrhosis in patients with concomitant alcohol use and obesity.

MANAGEMENT

Abstinence for individuals with alcohol-related liver injury is paramount to preventing liver-related complications. Although liver disease progression may persist even with abstinence, prevention of further hepatic damage is crucial. After enrolling in alcohol treatment programs, women had higher

rates of abstinence than men.²⁰ However, women are less likely to use face-to-face counseling and pharmacologic therapy to prevent relapse because of family/childcare barriers and a perceived stigma associated with attending programs.²¹

Moreover, if a woman experiences complications of liver disease and needs a transplant, she is often disadvantaged. A recent study that examined early liver transplantation across multiple centers within the United States reported that few women undergo early liver transplantation for alcoholic hepatitis.²² In addition, few women with any type of alcohol-related liver disease receive transplants. In a retrospective study of individuals evaluated for transplantation for alcohol-related liver disease, men were more likely than women to be listed for transplantation.²³ Also, of all the participants listed, men were more likely than women to receive a transplant.

The lack of proper counseling for alcohol use disorder must be addressed, as studies have demonstrated increased risk of relapse of harmful drinking among women with alcohol-related liver disease who received transplants.²⁴ This increased relapse for women is problematic, as it has been associated with a higher incidence of recurrent disease for women than for men.

Determining why women are drinking more and exceeding the drinking observed among men is imperative. Several hypotheses include the paradigm shift of women assuming male gender roles, for example, more women are working outside the home and fewer women are having children.²⁵ Another hypothesis is that the increasing stress of family and work balance for women leads to the use of alcohol to manage stress.²⁶ In addition, alcohol advertisements targeted toward females have increased, beginning with advertisements for wine coolers in the early 2000s²⁷ to the advertisements for “female-friendly” drinks such as wine in the current decade, and have made alcohol use more socially acceptable. Increased alcohol use may inadvertently be used to manage stress.

Research shows that the association between problematic drinking and post-traumatic stress

disorder, anxiety, and depression is stronger for women than for men.²⁸ Moreover, women are more likely to use alcohol to regulate negative reinforcement, whereas for men, investigators have speculated that drinking results in positive reinforcement.

FUTURE AREAS OF RESEARCH

It is quite evident from currently available literature that women, compared to men, have an increased risk of end-stage liver disease from alcohol use. Although it has been established that women should consume less alcohol than men, observations vary as to whether binge drinking or moderate daily drinking (i.e., not exceeding 14 grams per day) is more likely to lead to end-stage liver disease. Future studies should be conducted to provide more detailed recommendations, although in the interim, health care practitioners should advise women to consume no more than one drink per day.

In addition, the Million Women Study's observation that women who did not eat meals while consuming alcohol had increased alcohol-related liver injury needs further corroborative evidence. Currently available literature also indicates that women with obesity should be advised to avoid drinking heavily and to avoid substituting alcohol with beverages that have high sugar content, as these beverages may lead to further hepatic fibrosis despite alcohol abstinence.

Moreover and more significantly, public awareness of current hazardous drinking is needed, as many women are unaware they are increasing their risk of liver disease. Public policies need to minimize alcohol advertising targeted toward women.

CONCLUSION

Although alcohol-related liver injury previously has not been linked to women, it is paramount to educate women about the dangers of consuming alcohol given that women are more susceptible

than men to injury after consuming less alcohol. Globally, alcohol consumption has increased, particularly among women. Safe drinking habits, including not exceeding 14 grams of alcohol consumption in a day, not drinking without eating meals, and avoiding daily drinking, should be recommended. If alcohol use disorder is identified, adequate and appropriate counseling and pharmacologic therapy should be provided. Additionally, further study into the neurobiologic basis leading to alcohol use disorder should be made by clinicians and researchers.

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ALCOHOL'S EFFECTS ON BREAST CANCER IN WOMEN

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Globally, more than 2 million new cases of breast cancer are reported annually. The United States alone has more than 496,000 new cases every year. The worldwide prevalence is approximately 6.8 million cases. Although many risk factors for breast cancer are not modifiable, understanding the role of the factors that can be altered is critical. Alcohol consumption is a modifiable factor. Studies of alcohol in relation to breast cancer incidence have included hundreds of thousands of women. Evidence is consistent that intake, even intake of less than 10-15 grams per day, is associated with increased risk of this disease. In addition, evidence, although less extensive, shows that possible early indicators of risk, such as benign breast disease and increased breast density, are associated with alcohol consumption. Evidence is less strong for differences based on geographic region, beverage type, drinking pattern, or breast cancer subtype. Some studies have examined the association between alcohol and recurrence or survival after a breast cancer diagnosis. These findings are less consistent. Public awareness of alcohol as a risk factor for breast cancer is low, and public health measures to increase that awareness are warranted.

KEY WORDS: alcohol drinking; breast cancer incidence; breast cancer survival; drinking pattern; women

INTRODUCTION

In 1987, the *New England Journal of Medicine* published two reports about alcohol consumption and breast cancer risk.^{1,2} In the two reports, both prospective cohorts, alcohol consumption, even at modest levels of intake, was associated with risk of breast cancer. An accompanying editorial indicated that based on the existing epidemiologic studies, approximately 17 at the time, one could conclude “despite variations in

study design, population, culture and language of the country of origin, and methods of determining the amount of alcohol ingested, most investigations have found at least a small increase in risk with increases in intake, particularly among premenopausal women.”³ Since those landmark papers were published, studies have been conducted among hundreds of thousands of women. Findings of an association between

alcohol consumption and an increase in breast cancer risk for women have persisted.

SCOPE OF THE PROBLEM

Breast cancer affects more than 2 million women each year around the world.⁴ The age-adjusted rate is 46.3 new cases of this disease per year for every 100,000 women. In the United States, more than 496,000 new cases are diagnosed every year, and the age-adjusted incidence is 84.8 per 100,000 women. Globally, 626,679 deaths from breast cancer occur annually, and in the United States, close to 89,000 deaths were reported. The age-adjusted breast cancer mortality rates are 13.0 deaths per 100,000 women globally, and 12.6 deaths per 100,000 women in the United States. It is estimated that the prevalence of breast cancer around the world is 6.8 million cases.

ALCOHOL AND BREAST CANCER INCIDENCE

A large body of research provides evidence that alcohol is a risk factor for incidence of breast cancer. The World Cancer Research Fund and the American Institute for Cancer Research (WCRF-AICR) collaborated to organize a continuous systematic review of dietary factors in relation to cancer.⁵ The WCRF-AICR reports include examinations of alcohol and breast cancer. In a 2018 update, they concluded that, based on the existing literature (16 prospective studies of premenopausal breast cancer and 34 of postmenopausal disease), alcohol consumption is a “probable cause” and a “convincing cause” for premenopausal and postmenopausal breast cancer, respectively. The meta-analysis showed that for a 10-gram increase in alcohol consumed per day on average, risk increased 5% among premenopausal women and 9% among postmenopausal women. A standard drink contains approximately 14 grams of alcohol.⁶

As noted in the 1987 editorial in the *New England Journal of Medicine*, an association between alcohol and breast cancer was found

across geographic locations for a range of beverage types consumed and for a variety of drinking patterns.³ Most of the studies on alcohol and breast cancer have been conducted in North America and Europe, but there are some from other locations.

The WCRF-AICR meta-analysis reported some differences by location.⁵ For premenopausal breast cancer, the summary meta-analysis was significant only for North America. Results were similar in magnitude but not statistically significant for analyses of findings from Europe and Asia. For postmenopausal cancer, in the meta-analysis of dose-response, the association was statistically significant only for studies of Europe and North America.

In a study that pooled data from 20 cohorts in the United States, Canada, Europe, Australia, and Japan, no significant heterogeneity was found among studies, although the association between alcohol and breast cancer was stronger for the North American cohorts than for the others.⁷ Even within regions, there can be considerable differences in quantities of alcohol consumption, types of beverages consumed, and intensities of drinking (e.g., frequency of binge drinking, drinking with meals or not). For example, within Europe, drinking patterns vary considerably. In a study of 335,000 women in Europe, of whom 11,600 had invasive breast cancer, a significant, 4% increase in risk was shown for each additional 10 grams of alcohol consumed per day.⁸

Studies of individual European countries, including Italy,⁹ France (among postmenopausal but not premenopausal women),¹⁰ and the United Kingdom,¹¹ but not Greece,¹² also reported evidence of increased risk. In a case-control study of more than 2,000 cases and 2,000 controls from 3 countries in sub-Saharan Africa, an association between alcohol consumption and risk was reported, despite considerable differences in the prevalence of alcohol consumption in those countries.¹³ In South America, studies in Brazil reported some evidence of an association.^{14,15} For studies in Asia, where women’s alcohol consumption generally is lower, results have been inconsistent.¹⁶⁻²⁰

Few studies have examined the association between alcohol and breast cancer by race/ethnicity. The African American Breast Cancer Epidemiology and Risk (AMBER) Consortium, a pooled analysis of studies of African American women, found a J-shaped association between alcohol consumption and breast cancer risk.²¹ The magnitude of the association for higher intakes of alcohol was similar to results reported in other studies of women of European descent.

Overall, there is strong evidence that alcohol increases breast cancer risk. Evidence is strongest for North America and Europe, where more studies have been conducted, but other regions also show some evidence of a similar association. Much additional research has been done regarding the details of the alcohol consumption (e.g., beverage type, drinking pattern, the participant's age at the time of consumption) and the details of the breast cancer (e.g., tumor subtype). These findings are less consistent.

Variability in findings may be a function of the small sample size of some studies, for instance, in those studies that examined associations between alcohol consumption for breast cancer by subtype (e.g., estrogen receptor–positive or –negative). In addition, alcohol consumption can be difficult to assess for a variety of reasons, including difficulty recalling usual intake, change in consumption over the lifetime, and response bias. In this context, the consistency of the findings regarding overall risk of breast cancer associated with alcohol consumption is noteworthy.

Beverage Type

Several studies of alcohol and risk examined whether there are differences depending on the beverage consumed: wine, beer, or spirits. The pooled analysis of 20 cohorts reported no difference in risk based on the beverage type.⁷ The Million Women Study in the United Kingdom reported similar associations for those who drank wine only and for those who consumed other drinks.¹¹ In the WCRF-AICR meta-analysis, only beer was associated with a statistically significant increase in risk among premenopausal women,

and only wine was associated with risk among postmenopausal women.⁵ However, in all of the studies, there was an indication of increased risk with each of the beverages, even if not statistically significant. In addition, the evidence was that there was not a statistical difference of the association with each of the three types of beverage for both premenopausal and postmenopausal analyses. Some studies provided evidence of a stronger effect for a particular beverage, but most of the evidence pointed to effects from any alcoholic beverage.

Drinking Pattern

When examining the effects of alcohol consumption on health and disease, how participants consumed the alcohol must be considered. Not only the absolute quantity consumed, but also the intensity of consumption may have biological effects. For example, the effects of an average consumption of seven drinks per week may differ for consumption of one drink daily and for seven drinks on one day once per week.

Just a few studies have examined drinking intensity. In the Nurses' Health Study I (NHS), binge drinking (defined as six or more drinks in one day) was associated with increased risk, even after adjusting for total consumption.²² The frequency of alcohol consumption was not associated with risk in that cohort after adjusting for total consumption. In the Sister Study, a cohort of women with a family history of breast cancer, self-report of ever binge drinking (defined as four or more drinks in one sitting) or ever having blacked out while drinking were associated with increased breast cancer risk.²³ These associations were not adjusted for overall alcohol intake.

Even among people who drink lightly, evidence of increased risk has been reported. In a systematic review of light drinking, which used the World Health Organization definition of less than 21 grams of alcohol consumed per day, Shield and colleagues found consistent evidence of increased risk.²⁴ In a meta-analysis, Choi and colleagues found statistically significant increases in risk of 4%, 9%, and 13% for individuals who drank less than 0.5 drinks per day, less than or

equal to 1 drink per day, and 1 to 2 drinks per day, respectively; in this analysis, one drink was defined as 12.5 grams of alcohol.²⁵ There is no evidence of a lower threshold for an effect of alcohol consumption on risk of breast cancer. Collectively, results from these studies on intake indicate that drinking pattern may affect risk, as drinks per drinking day are associated with increased risk even after adjusting for total intake.

Breast Cancer Subtype

Breast cancer can be classified into subtypes by tumor markers. The subtypes may have different risk factors, and they are different in terms of aggressiveness, treatment, and prognosis. A number of studies have examined the association between alcohol consumption and invasive breast cancer by subtype.

In the European Prospective Investigation into Cancer and Nutrition (EPIC) study, which examined a cohort of more than 360,000 women from 23 centers in 10 countries in Europe, the association between alcohol consumption and risk was stronger for women with estrogen receptor–positive tumors than for those with estrogen receptor–negative tumors.²⁶ In a report on postmenopausal breast cancer from the Million Women Study in the United Kingdom, no heterogeneity by estrogen receptor status was found for the association between alcohol consumption and risk.²⁷ A pooled analysis of 20 cohort studies, which comprised more than 1 million women, reported no difference in the associations of alcohol and estrogen receptor–positive tumors or of alcohol and estrogen receptor–negative tumors.⁷ Finally, in the systematic review by the WCRF-AICR, the findings for postmenopausal cancer indicated an increase in risk for estrogen receptor–positive tumors but not for estrogen receptor–negative tumors.⁵

In one study, alcohol consumption and risk of human epidermal growth factor receptor 2 (HER2)–positive and triple-negative breast cancers were compared to risk of estrogen receptor–positive tumors.²⁸ Alcohol consumption was associated with a lower risk of HER2-positive tumors and no difference in the risk of triple-

negative tumors, as compared to its association with risk for estrogen receptor–positive tumors. In an analysis of data from the AMBER Consortium of African American women, the association between alcohol consumption and risk was stronger for estrogen receptor–negative, progesterone receptor–negative, and HER2-negative tumors than for tumors with positive receptor status.²¹ Overall, findings from studies of associations between alcohol consumption and breast cancer subtypes have been inconsistent.

Period of Exposure

Alcohol consumption patterns generally vary during the life span, and effects of exposures may differ depending on the stage of breast development when the drinking occurred. A number of studies have examined risk associated with alcohol consumption at particular time periods, especially during adolescence and early adulthood.

The NHS II, a prospective study of women ages 24 to 44 at baseline, reported an 11% increase in breast cancer risk associated with consumption of 10 grams of alcohol per day between menarche and first pregnancy, adjusting for subsequent intake.²⁹ A similar increase in risk was observed for consumption of alcohol after the first pregnancy, adjusting for intake before that time. In NHS I, a cohort of women ages 30 to 55 at baseline, there was an 8% increase in risk associated with 10 grams of alcohol consumed per day between ages 18 and 40, even after adjusting for consumption after age 40.²² For consumption after age 40, there was a 7% increase in risk, after adjusting for earlier intake.

Benign breast disease is associated with increased breast cancer risk and may be an early indicator of risk. In the NHS II, evidence indicated a 15% increase in risk of benign breast disease for each additional 10 grams per day of alcohol consumed during adolescence.³⁰ Another study of young women reported a 50% increase in risk of benign breast disease for each additional drink per day during the period of ages 9 to 15.³¹ In one study, associations for alcohol with risk

were similar for pre-cancerous conditions as for invasive breast cancer.³²

The EPIC cohort study examined the association between risk and alcohol consumption for parous women before their first, full-term pregnancy compared with women who did not begin drinking until after their first pregnancy.⁸ Point estimates were similar but there was a significant association only for those who started drinking before their first pregnancy. In addition to intake during adolescence and young adulthood, even exposure to alcohol *in utero* may predispose to increased risk. Evidence from animal models indicates that ethanol exposure *in utero* can lead to increased breast tumorigenesis in the adult offspring when exposed to carcinogens.³³

These studies indicate that the association of lifetime alcohol consumption with breast cancer risk may be different depending on when the alcohol was consumed. Evidence shows, with some inconsistency among studies, that consumption in adolescence and before a first pregnancy may particularly affect risk.

Breast Density

Breast density is a measure of breast tissue from radiography. It is associated with subsequent breast cancer and is one of the strongest breast cancer risk factors.^{34,35} Understanding factors related to increased density may provide insight into early stages of carcinogenesis. A number of cross-sectional analyses have shown that alcohol consumption is associated with increased breast density. In a study in Germany, consumption of more than 10 grams of alcohol per day was associated with increased risk of high mammographic density.³⁶ Similarly, increases in risk of increased breast density were associated with alcohol drinking in Japan,³⁷ Sweden,³⁸ and the United States in Hawaii³⁹ and New York City.⁴⁰ There was a nonsignificant association in a study in China.⁴¹

In some studies, the association between alcohol consumption and risk varied depending on other breast cancer risk factors. In the Swedish study, the association was strongest for the group that also had other factors that predicted

increased risk of breast cancer.³⁸ In a multicultural population in New York City, the association was strongest among individuals who had lower body mass index.⁴⁰ In a study of Mexican women, alcohol use was associated with increased breast density.⁴² In a study of NHS II participants, no association was found between breast density and alcohol consumption.⁴³ A meta-analysis of studies reported an association between increased breast density and higher levels of alcohol consumption.³⁵ Although these reported findings are not consistent, effects of alcohol consumption on breast density may be one mechanism for the associations with risk for breast cancer.

Diet

A number of studies have examined alcohol consumption in concert with other known breast cancer risk factors. In particular, there has been study of interactions of alcohol with other dietary factors such as folate and other B vitamins, which play a role in alcohol metabolism. Alcohol negatively affects folate status, impacting folate absorption and metabolism and increasing folate excretion.⁴⁴ A systematic review reported evidence of interaction between alcohol and folate in relation to breast cancer risk.⁴⁵ Breast cancer risk decreased with increased folate consumption among individuals who drank heavily but not lighter drinkers.

Several recent studies examined plasma folate as a measure of vitamin status. In the NHS II, there was an interaction between alcohol and plasma vitamin concentrations, with a trend toward plasma folate being protective for breast cancer risk among individuals who consumed greater amounts, but not among those consuming lesser amounts of alcohol.⁴⁶ However, in the NHS I, plasma folate was not associated with breast cancer risk and did not vary by alcohol consumption.⁴⁷

Further, in the EPIC cohort study in Europe, no interaction was found for alcohol and plasma folate consumption in relation to breast cancer risk.⁴⁸ This study found some evidence of an interaction of alcohol and plasma vitamin B₁₂ consumption in

relation to breast cancer risk; vitamin B₁₂ also is a cofactor in one-carbon metabolism. A study that examined the Women's Health Study cohort found no interaction between plasma concentrations of B vitamins and alcohol consumption in relation to risk.⁴⁹ A systematic review found evidence for an association between higher levels of folate consumption and decreased risk of breast cancer among participants with moderate or high alcohol intake.⁵⁰ Collectively, these results show that diet, particularly vitamins related to one-carbon metabolism, may modify the association between alcohol and the risk for breast cancer.

Genetic Factors

Several studies have examined genetic variation in the association between alcohol consumption and breast cancer risk. There have been several studies of the genes that code for the alcohol dehydrogenases (ADH), which are critical enzymes for alcohol metabolism. In a cohort in the Netherlands, variants in the genes for ADH were not associated with breast cancer risk nor did they modify the risk associated with alcohol consumption.⁵¹ The NHS I reported similar findings; the association between alcohol consumption and risk for breast cancer was not modified by genetic variation in ADH.⁵² There was, however, evidence that an association between alcohol and steroid hormone levels differed depending on ADH genotype.

A Danish cohort study examined variation in the *CYP19A1* gene, which codes for aromatase, an enzyme important to estrogen metabolism.⁵³ Although these researchers found an interaction of genetic variation with blood steroid hormones with acute alcohol consumption, they found no evidence of an association of the genetic variant with breast cancer risk. Among women who have the *BRCA1* or *BRCA2* genes, mutations that confer a particularly elevated risk of breast cancer, alcohol was not associated with breast cancer risk.⁵⁴ Overall, the evidence for genetic factors modifying the association between alcohol consumption and the risk for breast cancer is not strong.

Other Potential Modifying Factors

Understanding of whether other factors modify the observed association between alcohol consumption and breast cancer is another area of active research. In a pooled analysis, alcohol was positively associated with risk among both nulliparous and parous women.⁵⁵ Point estimates of risk were similar and not significantly different for the two groups. There is some evidence of a stronger association between alcohol and breast cancer risk among women receiving hormone therapy as compared to those not receiving hormone therapy, particularly the risk for estrogen receptor–positive breast cancer.⁵⁶ Further examination of modifying factors such as other dietary factors, body mass index, level of physical activity, and smoking is warranted.

ALCOHOL AND SURVIVAL AFTER DIAGNOSIS

Although most of the research regarding the association between consuming alcohol and the risk for breast cancer has focused on incidence, some studies have examined the effects of alcohol on survival after a breast cancer diagnosis. Studies used different time frames (before or after diagnosis) for the alcohol consumption and different outcome measures, such as breast cancer recurrence, breast cancer–specific survival, and all-cause mortality. Most studies did not distinguish by breast cancer subtype, which can affect prognosis.

A meta-analysis of 11 studies found evidence of improved survival after breast cancer diagnosis among individuals who reported any prediagnostic alcohol consumption, when compared with those who reported none.⁵⁷ The association differed somewhat by the estrogen receptor status of the tumor, with some evidence of reduced all-cause mortality for women with estrogen receptor–negative disease and no association with mortality in those with estrogen receptor–positive disease. Studies of lifetime alcohol intake found no association with all-cause mortality or

death from breast cancer (breast cancer–specific mortality).^{58,59}

In the National Institutes of Health (NIH)-AARP Diet and Health Study cohort, alcohol consumption at the study baseline was not statistically significantly associated with breast cancer–specific survival.⁶⁰ In the Women’s Health Initiative, there was no association between prediagnostic alcohol consumption and breast cancer–specific or all-cause mortality.⁶¹ There was some evidence of decreased breast cancer–specific mortality for estrogen receptor–negative tumors. Among breast cancer patients from the Moffitt Cancer Center, self-reported alcohol consumption one year before diagnosis was associated with improved breast cancer–free survival.⁶² Another study of women in the United States reported that prediagnostic alcohol intake was associated with an increased risk of breast cancer–specific mortality.⁶³

Alcohol consumption pattern may affect mortality as well as incidence. In a study in western New York among women who had postmenopausal breast cancer, drinking intensity before diagnosis was associated with prognosis.⁵⁹ Participants who drank four or more drinks per drinking occasion had increased mortality from breast cancer and from all causes, and participants who drank fewer drinks per drinking occasion had decreased mortality from both breast cancer and all causes.

Few studies have examined alcohol consumption following a breast cancer diagnosis. One study reported an increased risk of breast cancer recurrence with alcohol consumption after diagnosis among premenopausal but not postmenopausal women.⁶⁴ In another study, investigators found no association between postdiagnostic intake and breast cancer–specific mortality.⁶³ There was better overall survival for those with greater postdiagnostic alcohol consumption. Findings regarding alcohol consumption and prognosis after a breast cancer diagnosis are not consistent. More research is needed to examine alcohol consumption, including patterns of consumption, following diagnosis.

More analyses regarding breast cancer subtype and treatment are required to better understand a possible role of alcohol consumption following diagnosis. Recent studies examining alcohol consumption and the efficacy of breast cancer treatments have not found any effect of alcohol consumption on radiotherapy⁶⁵ or on adjuvant hormone therapy.⁶² More data regarding in-depth analysis of alcohol consumption both before and after diagnosis are needed, along with more research examining the total amount of alcohol consumed, drinking patterns in relation to outcomes, and the effects of drinking alcohol during treatment.

MECHANISMS FOR ALCOHOL EFFECTS

The role of alcohol consumption in breast carcinogenesis is a complex process likely acting through a number of mechanisms. Although alcoholic beverages contain a variety of compounds, for breast carcinogenesis, alcohol itself appears to be the more important carcinogen,⁶⁶ consistent with the finding that overall, risk does not differ based on the type of beverage consumed. However, much is not understood regarding the underlying mechanisms for alcohol and breast carcinogenesis. Potential mechanisms include oxidative stress, cell proliferation, effects on hormones, particularly steroid hormones, and effects on one-carbon metabolism.

Alcohol likely contributes to carcinogenesis partly through oxidation from alcohol metabolism and through oxidative stress from production of the alpha-hydroxyethyl radical, a reactive oxygen species.⁶⁷ Alcohol is metabolized to acetaldehyde, classified as a carcinogen by the International Agency for Research on Cancer (IARC), part of the World Health Organization, in 2010.⁶⁷ Although production of acetaldehyde from alcohol primarily occurs in the liver, it also occurs in breast tissues.

There is *in vivo* evidence that acetaldehyde can concentrate in mammary cells following a single exposure. In an animal model, acetaldehyde accumulated and persisted in higher concentrations

in breast tissue than in blood.⁶⁸ Adverse effects of acetaldehyde include DNA adduct formation, oxidation, and altered DNA methylation.⁶⁷ Further, in vitro, at low concentrations, alcohol can increase cell proliferation, including proliferation of breast cells.⁶⁹ Higher concentrations of alcohol and red wine exposure may reduce cell proliferation.

In addition to the carcinogenic effects of alcohol consumption and acetaldehyde on breast tissue, alcohol consumption's effects on hormones also may contribute to cancer in the breast. There are both acute and chronic effects of alcohol on steroid hormone level. At doses of even 15 to 30 grams of alcohol per day, serum estrogens increase.²⁴ In one study of premenopausal women, alcohol consumption was associated with plasma estrogens, but not androgens, when measured during the luteal phase. Neither hormone was associated with alcohol during the follicular phase.⁷⁰ In that same cohort, urinary estradiol measured at the mid-luteal phase was more than 20% higher in women who drank more than 15 grams per day, when compared with those who did not drink.⁷¹ Further, a mediation analysis provided evidence that changes in hormones associated with alcohol consumption may explain part of the relationship between alcohol and breast cancer.⁷²

Altered DNA methylation also contributes to carcinogenesis. Alcohol significantly affects one-carbon metabolism, including DNA methylation, in part by effects on folate status, as discussed previously. Studies that examined DNA methylation in breast tumors made comparisons based on drinking history and found differences by the amount of alcohol consumption.^{73,74} Another study found some evidence of these differences in normal, noncancerous breast tissues.⁷⁵ Alcohol's effects on estrogen also may play a role in altered DNA methylation. There is evidence that higher concentrations of the steroid hormone affect DNA methylation.²⁴

Other possible mechanisms for an effect of alcohol on carcinogenesis in general and breast cancer in particular are still emerging. For

example, the microbiome in the mouth and gut may affect breast cancer risk,^{76,77} and alcohol can affect the microbiome.^{78,79} Alcohol likely has other effects on breast carcinogenesis, including effects on metastasis, angiogenesis, and cancer stem cells, affecting both cancer initiation and tumor aggressiveness.⁸⁰

Alcohol's effects on oxidative stress, cell proliferation, steroid hormones, and one-carbon metabolism may explain, in part, the observed associations with breast cancer risk. Additional research is needed regarding these and other mechanisms, including research on those specific to tumor subtypes and mechanisms for exposures following a breast cancer diagnosis.

PUBLIC AWARENESS OF RISK

A limited number of studies have examined public understanding of alcohol and breast cancer. In a study of women attending a breast screening clinic in the United Kingdom, only 19% were aware that alcohol consumption is a breast cancer risk factor.⁸¹ Among university students in a survey conducted in 23 countries around the world, overall, 3.3% were aware of alcohol consumption as a breast cancer risk factor.⁸² Although awareness was highest in the United States, only 10% of students correctly identified alcohol consumption as a risk factor.

Awareness tends to be greater among women who have been diagnosed with breast cancer, with resulting lower alcohol intake in that group. In a systematic review, 62% to 97% of participants adhered to recommendations to limit alcohol consumption in a study of women completing initial treatment for breast cancer.⁸³ These studies were conducted primarily in the United States; a small number of participants were in Europe. In spite of the strength of the overall evidence connecting alcohol consumption to breast cancer,^{5,67} there is little public awareness of alcohol consumption as a breast cancer risk factor.

RECOMMENDATIONS

Reduction of alcohol consumption could measurably affect the burden of disease related to breast cancer. Based on global data of the prevalence of alcohol consumption and of the incidence rate of breast cancer, an estimated 144,000 new cases of breast cancer and 38,000 breast cancer deaths annually are accounted for by alcohol consumption, which is 8.6% of all incidence and 7.3% of mortality.²⁴ The magnitude of effect of a decrease in consumption in a particular region depends on the prevalence of alcohol consumption in that region. For example, in Australia, it has been estimated that any regular consumption of alcohol accounts for 12.6% and 6.6% of premenopausal and postmenopausal breast cancer, respectively.⁸⁴ Alcohol consumption accounts for 12% of breast cancer in the United Kingdom.¹¹ In the United Kingdom, regular consumption of each additional drink per day accounts for 11 additional breast cancers per 1,000 women in their lifetime, up to age 75.¹¹ As further indication of the effect, one estimate is that the increase in cancer risk for drinking one bottle of wine per week is approximately equivalent to smoking 10 cigarettes per week, with breast cancer accounting for most of that increase.⁸⁵

Although the evidence is strong for an increase in breast cancer with alcohol consumption, some areas of research still require further attention. A better understanding of the roles of drinking pattern, or drinking intensity, in relation to total consumption is needed. More studies of alcohol consumption and breast cancer subtypes would help increase insight into the relationship. A clearer understanding of the effects of exposures in early life, including *in utero* exposure, is warranted. Examination of how other breast cancer risk factors (e.g., physical activity, body mass index, smoking, reproductive history) interact with alcohol consumption in relation to both breast cancer risk and prognosis is needed. More studies of the association by race/ethnicity, by age at diagnosis, and conducted in regions outside of Europe and North America would contribute to

our understanding. Additional research linking epidemiological information with biological information regarding the role of alcohol in carcinogenesis could enhance the ability to leverage this important relationship toward prevention efforts.⁴⁴ Further, additional study is needed of the effects of alcohol consumption, both before and after diagnosis, on breast cancer recurrence, breast cancer–specific mortality, and overall mortality.

Given the strength of the evidence linking alcohol to breast cancer, increasing awareness of risk is critical. It is time for a clear public health message identifying the role of alcohol in breast carcinogenesis and indicating that there is no apparent lower threshold of effect. Consumption levels of less than one drink per day are associated with increased risk. Further, drinking alcohol affects risk at all phases of life, including early and late life. The science is consistent and clear, but awareness is low. It is time for a focus on developing public understanding of alcohol, which is a very common exposure, and its connection with increased risk of breast cancer.

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Binge Drinking's Effects on the Body

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Studies have focused on the effects of chronic alcohol consumption and the mechanisms of tissue injury underlying alcoholic hepatitis and cirrhosis, with less focus on the pathophysiological consequences of binge alcohol consumption. Alcohol binge drinking prevalence continues to rise, particularly among individuals ages 18 to 24. However, it is also frequent in individuals ages 65 and older. High blood alcohol levels achieved with this pattern of alcohol consumption are of particular concern, as alcohol can permeate to virtually all tissues in the body, resulting in significant alterations in organ function, which leads to multisystemic pathophysiological consequences. In addition to the pattern, amount, and frequency of alcohol consumption, additional factors, including the type of alcoholic beverage, may contribute differentially to the risk for alcohol-induced tissue injury. Preclinical and translational research strategies are needed to enhance our understanding of the effects of binge alcohol drinking, particularly for individuals with a history of chronic alcohol consumption. Identification of underlying pathophysiological processes responsible for tissue and organ injury can lead to development of preventive or therapeutic interventions to reduce the health care burden associated with binge alcohol drinking.

Key words: Alcohol and other drug (AOD) intoxication; alcoholic hepatitis; alcoholic liver cirrhosis; alcohol-induced disorders; binge drinking; blood alcohol content

Introduction

Alcohol misuse is the fifth-leading risk factor for premature death and disability worldwide,¹ and, adjusting for age, alcohol is the leading risk factor for mortality and the overall burden of disease in the 15 to 59 age group.² According to the World Health Organization, in 2004, 4.5% of the global burden of disease and injury was attributable to alcohol: 7.4% for men and 1.4% for women.²

Alcohol can permeate to virtually all tissues in the body, resulting in significant alterations in organ function, which leads to multisystemic pathophysiological consequences. The effect of alcohol misuse on multiple organ systems outside the liver, mediated through direct and indirect effects beyond those associated with alterations in the nutritional state of

the individual, has been well-established.^{3,4} The resulting tissue injury has increasingly been recognized and examined as a contributing factor to alcohol-related comorbidities and mortality. Several pathophysiological mechanisms have been identified as causative factors of tissue and organ injuries that resulted from excessive alcohol consumption, including acetaldehyde generation, adduct formation, mitochondrial injury, cell membrane perturbations, immune modulation, and oxidative stress (Figure 1). Some of these mechanisms are the result of direct alcohol-induced cell perturbations, whereas others are the consequence of tissue alcohol metabolism (Figure 2). The oxidative stress caused by excess production of reactive oxygen species (ROS) or a reduction in reducing antioxidant

equivalents in tissue has been consistently demonstrated to be an overall mechanism of the tissue injury that results from chronic alcohol misuse. Dose-dependent relationships between alcohol consumption and incidence of diabetes mellitus, hypertension, ischemic heart disease, dysrhythmias, stroke, pneumonia, and fetal alcohol syndrome have been reported.⁴ However, recognition of alcohol as an underlying causal factor in comorbid conditions remains a challenge in the clinical setting.

Several factors associated with alcohol consumption, including pattern, amount, and frequency, and the type of alcoholic beverage, may contribute differentially to the risk for alcohol-induced tissue injury. The question of whether all types of alcohol produce similar pathophysiological consequences remains to be answered.

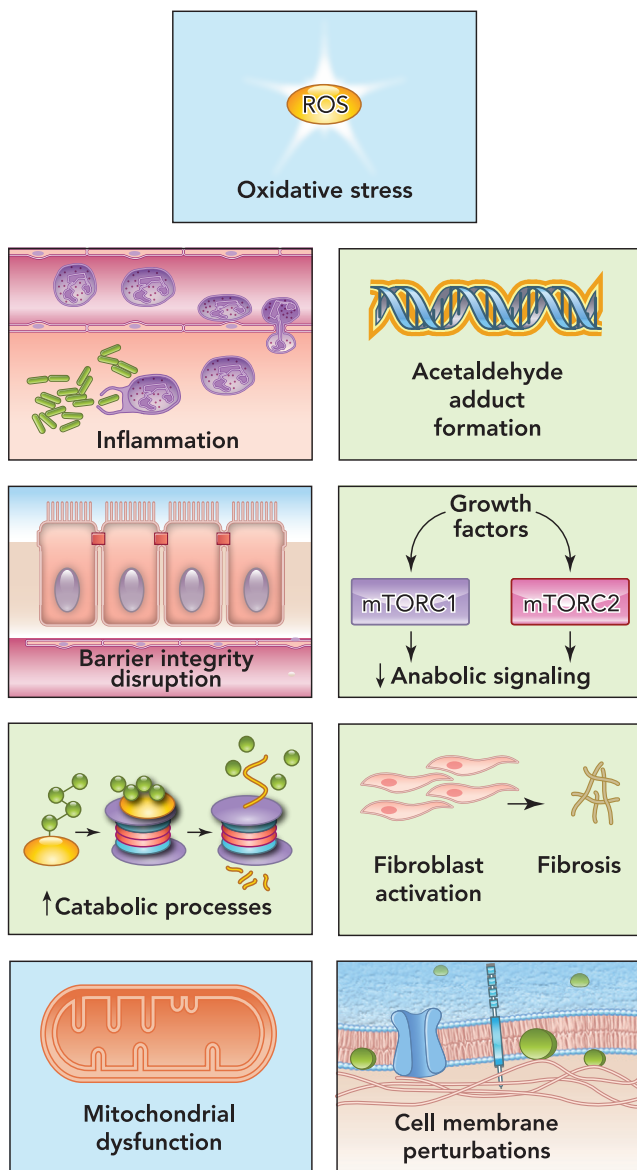


Figure 1 Mechanisms of alcohol-induced tissue injury. Alcohol contributes to tissue injury directly and indirectly through mechanisms including oxidative stress, inflammation, acetaldehyde adduct formation, barrier integrity disruption, decreased anabolic signaling, enhanced catabolic processes (particularly through the ubiquitin proteasome pathway), profibrotic changes, mitochondrial dysfunction and injury, and cell membrane perturbations. *Note:* mTORC1, mammalian target of rapamycin complex 1; mTORC2, mammalian target of rapamycin complex 2; ROS, reactive oxygen species. *Source:* Molina PE, Gardner JD, Souza-Smith FM, et al. Alcohol abuse: Critical pathophysiological processes and contribution to disease burden. *Physiology*. 2014;29(3):203-215.

However, the particularly detrimental effects of binge drinking have increasingly gained attention. Binge drinking, as defined by the National Institute on Alcohol Abuse and Alcoholism (NIAAA), is a pattern of alcohol consumption that brings blood alcohol concentration to .08 g/dL, which typically occurs following the intake of five or more standard alcohol drinks by men and four or more by women over a period of approximately 2 hours.⁵ Results from the 2015 National Survey on Drug Use and Health show overall prevalence of binge drinking (during the past 30 days) of 26.9% among U.S. adults ages 18 and older.⁶ Those data show that binge drinking prevalence and intensity are highest among those ages 18 to 24 but also occur in high frequency among older individuals (ages 65 and older). Thus, binge drinking prevails in two vulnerable segments of the population, raising their risks for greater severity of injury and frequency of comorbidities.

Understanding the Biomedical Consequences of Binge Drinking

A limitation to our understanding of the consequences of binge alcohol consumption on organ injury is the lack of information on the time period, duration, and number of binge occurrences that describe the long-term practice of binge drinking. Preclinical studies conducted under controlled conditions provide opportunities to examine quantity and frequency variables in the investigation of the effects of alcohol consumption on organ injuries. However, interpreting, comparing, and integrating the patterns of alcohol consumption described in clinical reports is difficult because of the different types of data collected across studies. This difficulty underscores the need for researchers to perform more rigorous comprehensive and systematic data collection on alcohol use patterns. The Timeline Followback (TLFB) tool, for example, uses a calendar and

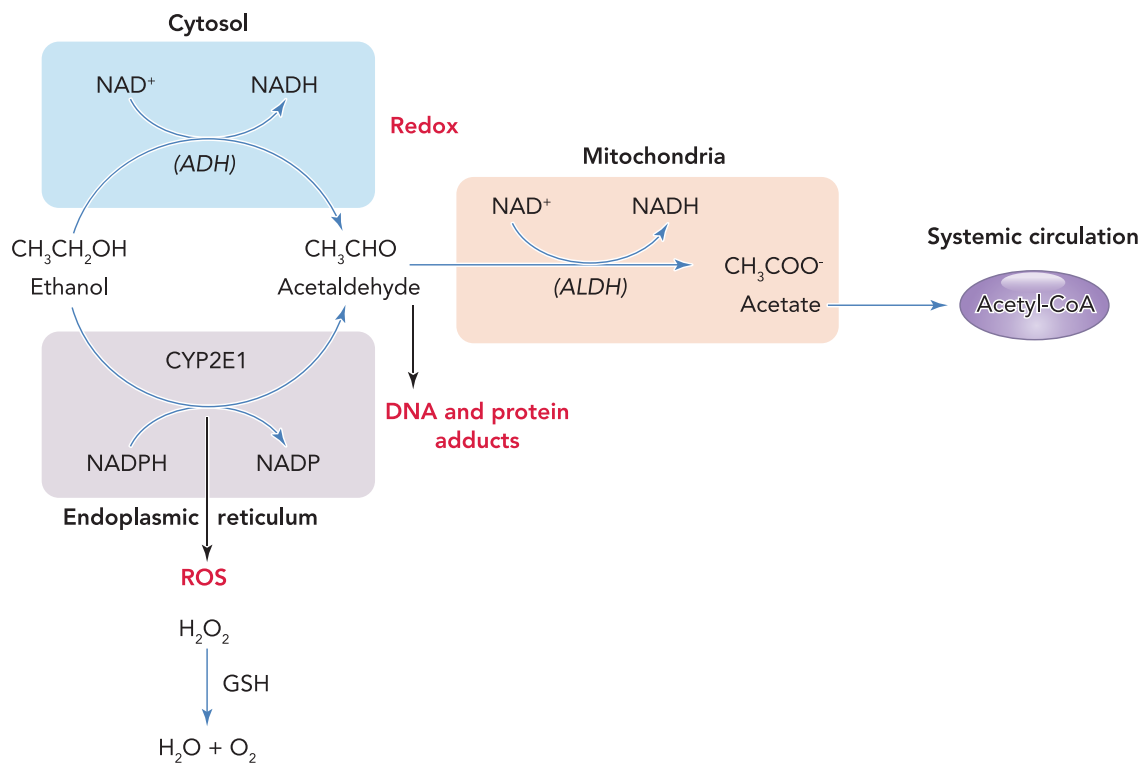


Figure 2 Tissue alcohol metabolism contributes to tissue and organ injury through altered redox potential, generation of ROS, and generation of metabolites, such as acetaldehyde, that form DNA and protein adducts. Alcohol (ethanol) is metabolized to acetaldehyde primarily by ADH in the cytosol and CYP2E1 in the endoplasmic reticulum. Acetaldehyde is converted to acetate in the mitochondria by the enzyme ALDH. Acetaldehyde can form adducts with DNA and proteins that can produce injury through activation of immune responses. During the oxidative process, both ADH and ALDH reactions reduce NAD^+ to NADH , shifting the cellular redox ratio. In addition, the cytochrome P450 enzymes, particularly CYP2E1, contribute to the oxidation of alcohol to acetaldehyde, particularly at increasing alcohol concentrations, as well as following their induction by chronic alcohol misuse. The pathway of alcohol oxidation results in the production of large amounts of ROS, including H_2O_2 , and is thought to be an important mechanism contributing to alcoholic liver injury. ROS are eliminated by antioxidants like GSH under normal conditions. Alcohol depletes cellular GSH stores, thereby exacerbating ROS-mediated injury. ROS can interact with lipids, producing lipid peroxidation, which leads to formation of reactive molecules such as MDA and HNE, which can then form protein adducts. *Note:* Acetyl-CoA, acetyl coenzyme A; ADH, alcohol dehydrogenase; ALDH, acetaldehyde dehydrogenase type 2; CYP2E1, cytochrome P450 2E1; GSH, glutathione; H_2O , water; H_2O_2 , hydrogen peroxide; HNE, 4-hydroxy-2-nonenal; MDA, malondialdehyde; NAD^+ , nicotinamide adenine dinucleotide (oxidized); NADH , nicotinamide adenine dinucleotide (reduced); NADP , nicotinamide adenine dinucleotide phosphate (oxidized); NADPH , nicotinamide adenine dinucleotide phosphate (reduced); O_2 , oxygen; ROS, reactive oxygen species. *Source:* Molina PE, Gardner JD, Souza-Smith FM, et al. Alcohol abuse: Critical pathophysiological processes and contribution to disease burden. *Physiology*. 2014;29(3):203-215.

a structured interview to collect retrospective information on the types and frequency of alcohol use over a given time period.^{7,8} Nevertheless, accounting for a lifetime pattern of binge alcohol consumption remains challenging when conducting clinical studies. Alcohol consumption patterns

should be taken into consideration for future development of alcohol use screening tools, because binge drinking has been suggested to result in greater alcohol-related harm.⁹

Different types of alcoholic beverages consumed in binge drinking episodes could also differentially affect

the health consequences associated with binge drinking. Epidemiological studies that compared the prevalence of coronary heart disease in “wine-drinking countries” and beer- or liquor-drinking countries have proposed that red wine, but not beer or spirits, consumed with a meal may

confer cardiovascular protection.¹⁰ The proposed protective effects of red wine include decreased blood clot formation, vascular relaxation, and attenuation of low-density lipoprotein (LDL, or bad cholesterol) oxidation, an early event preceding formation of cholesterol-filled plaque. These effects are attributed to polyphenols, especially resveratrol, and their antioxidant properties.

However, not all reports support the link between consuming a specific beverage type (i.e., wine vs. beer or spirits) and health benefits. Some reports suggest that beverage amount is more directly linked to health outcomes.^{11,12} The differential contribution of alcoholic beverages to beneficial or detrimental health outcomes remains to be examined in both preclinical and clinical studies. In binge drinking episodes, the form of alcohol consumed most frequently is beer (67.1%), followed by liquor (21.9%) and wine (10.9%).¹³ Moreover, beer accounts for most of the alcohol consumed by drinkers who are at the highest risk of causing or incurring alcohol-related harm, including drinkers ages 18 to 20, those with more frequent binge episodes per month, and those drinking 8 or more drinks per binge episode. Therefore, dissecting how pattern of drinking and type of alcoholic beverage contribute to overall outcomes is challenging.

The Gastrointestinal Tract, Liver, and Pancreas

Of all tissues affected by binge-like alcohol consumption, the gastrointestinal tract bears the greatest burden due to its direct exposure to high tissue concentrations of alcohol following ingestion (Figure 3). Binge drinking often occurs apart from meals, which may also contribute to its deleterious effects on organs. Food consumed at the time of alcohol consumption influences not only the alcohol absorption rate and blood alcohol concentration, but also the direct effect of alcohol on the gastrointestinal mucosa. Hence,

binge drinking is more likely to contribute to organ injury than paced, moderate alcohol drinking that is associated with a meal.

The gut mucosa is particularly susceptible to alcohol-induced injury, and alcohol consumption can result in a loss of intestinal barrier integrity. Several direct and indirect mechanisms have been identified that disrupt the structural and functional components involved in maintaining the integrity of the gut mucosal barrier. Alcohol and its breakdown products directly damage epithelial cells through generation of ROS and through disruption of tight junction protein expression and signaling.¹⁴ This process disrupts the integrity of the intestinal barrier, allowing bacteria and toxins to reach the bloodstream. Acute alcohol binge drinking in healthy human volunteers can produce a significant increase in serum endotoxin levels and bacterial 16S ribosomal DNA, suggesting the gastrointestinal microbial origin of endotoxin.¹⁵⁻¹⁷

More recently, attention has focused on the changes in intestinal microbiome that contribute to alcohol-associated intestinal inflammation and permeability. Alcohol promotes both dysbiosis (decreased diversity or an imbalance in the types of microbes) and bacterial overgrowth in the gastrointestinal system.¹⁸⁻²¹ Alcohol alters the balance between bacterial strains, decreasing the presence of beneficial bacteria, such as *Lactobacillus* and *Bifidobacterium*, and increasing that of Proteobacteria and Bacilli.¹⁹ This imbalance adds to the possibility that bacterial overgrowth may contribute to local mucosal inflammation through bacterial metabolism of alcohol and enhanced local production of metabolites such as acetaldehyde.²² Moreover, increased bacterial load, together with shifts in intestinal bacterial strains, brings about diverse profiles of bacterial-derived metabolites.

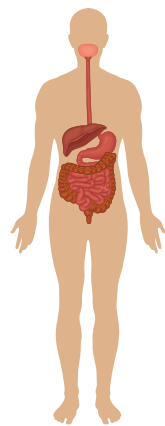
How these shifts in bacterial strains, load, and metabolites contribute to organ injury remains to be fully elu-

cidated. However, it is reasonable to speculate that greater bacterial burden and altered bacterial profiles, together with increased permeability of the gut mucosa, would lead to continuous entry of bacterial toxins into the systemic circulation. These changes could produce chronic and sustained activation of immune responses that, in turn, could lead to immune exhaustion and dysfunction. Preclinical studies show that binge-on-chronic alcohol feeding alters the gut microflora at multiple taxonomic levels, influencing hepatic inflammation, neutrophil infiltration, and liver steatosis,²³ which highlights the need for clinical investigation into the relationship between gut microflora and hepatic liver disease.

Local and Systemic Consequences of Gut Injury

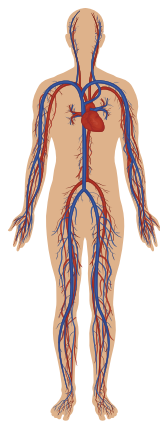
Toxins and bacterial products leaked from the gastrointestinal tract can be transported through the lymphatic system. This route of dissemination, which escapes hepatic clearance, may prove critical in the enhanced systemic delivery of toxins. Preclinical studies have shown that repeated binge-like alcohol intoxication increases lymphatic permeability and inflammation in the adipose tissue that immediately surrounds the mesenteric lymphatics. Inflammatory response in mesenteric perilymphatic adipose tissue is associated with altered adipose tissue insulin signaling and circulating adipokine profiles, which suggests a link between lymphatic leak, adipose tissue inflammation, and metabolic dysregulation.²⁴

Whether chronic alcohol consumption not in a binge pattern produces similar alterations in lymphatic permeability and mesenteric adipose inflammation remains to be determined. However, localized alterations in mesenteric adipose tissue metabolic regulation, including insulin signaling, may prove to be relevant to the enhanced risk for metabolic syndrome that is associated with binge alcohol consumption.²⁵ After burn injury



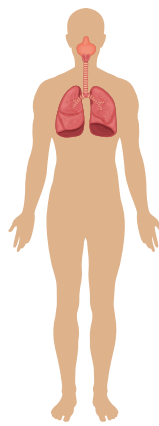
Gastrointestinal tract, liver, and pancreas

- Esophageal and gastric dysmotility
- Liver oxidative stress, steatosis, hepatitis, and fibrosis
- Gastritis and mucosal atrophy
- Impaired intestinal nutrient absorption, disruption of intestinal barrier and lymphatic function, and increased bacterial toxin translocation
- Increased pancreas inflammation



Cardiovascular system

- Cardiomyocyte mitochondrial and sarcoplasmic reticulum damage, altered calcium dynamics, and cardiac fibrosis
- Myocardial oxidative stress, impaired cardiomyocyte contraction, hypertension, and potentiation of the renin-angiotensin-aldosterone system



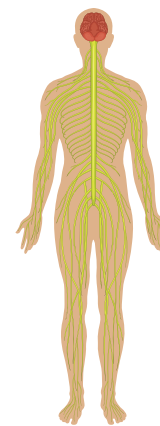
Pulmonary system

- Oxidative stress and diminished lung host defense mechanisms



Musculoskeletal system

- Decreased growth factor signaling and responsiveness, increased ubiquitin proteasome pathway activation, upregulation of negative regulators of skeletal muscle growth, and disruption of bone remodeling



Nervous system

- Impaired behavioral and cognitive function, impaired impulse control and motor skills, and blackouts
- Structural changes in prefrontal and parietal regions, and gender-specific differences in frontal, temporal, and cerebellar brain activation during working memory tasks
- Enlargement of lateral ventricles and cisterns, and degradations in neural white matter
- Reduced neurogenesis
- Increased neuroimmune gene expression

Figure 3 The systemic effects of chronic binge alcohol consumption and the principal organ systems affected.

and a binge-like pattern of alcohol intoxication, rodents showed similar exacerbation of adipose tissue inflammation.²⁶ This suggests that a possible synergism between binge-like alcohol intoxication and injury promotes a dysregulated adipose environment conducive to insulin resistance, and potentially metabolic syndrome, if these alterations are sustained beyond the immediate period following binge drinking or burn injury.³

Second to the gastrointestinal tract, the liver has the most exposure to high alcohol concentrations during periods of binge drinking. Hepatocellular

metabolism of alcohol and the resulting ROS generation; acetaldehyde formation and the resulting adducts; immune response activation, particularly in Kupffer and stellate cells; and alterations in cell signaling are all proposed as mechanisms that underlie liver injury associated with binge-like alcohol consumption. For people with chronic alcoholism, binge drinking augments liver injury^{27,28} and is a major trigger for the progression from steatosis to steatohepatitis.²⁹⁻³¹ In one study, rodents that received binge-on-chronic alcohol exposure had accentuated elevation in liver enzymes (alanine

aminotransferase), hepatic steatosis, and inflammatory cytokine expression compared to rodents subjected only to chronic or to acute alcohol exposure.³² These results demonstrate that binge-on-chronic alcohol exposure results in greater insult than either chronic or acute alcohol exposure alone. Clinical studies have provided evidence of associations among alcohol binge drinking patterns, immune activation (high CD69 and low TLR4, CXCR4, and CCR2 expression), and decreased chemotactic responses to SDF-1 and MCP-1.³³ These associations reflect an altered immune profile that may be as-

sociated with liver injury and increased susceptibility to infection. More recently, attention has been drawn to the potential greater liver injury in individuals with metabolic syndrome. A population-based study showed a direct association between binge drinking frequency and liver disease risk, after adjusting for average daily alcohol intake and age.³⁴ In this study, binge drinking and metabolic syndrome produced supra-additive increases in the risk of decompensated liver disease. Because of increasing rates of obesity and metabolic syndrome, research on the effects of alcohol misuse and the biomedical consequences is needed for this particular segment of the population.

Located strategically between the liver and the gastrointestinal tract, the pancreas also has high susceptibility to alcohol-induced tissue injury. Heavy, chronic alcohol consumption is a recognized contributing factor in the development of pancreatitis. However, how dose and pattern of alcohol consumption affect pancreatic function and structure is not known. Studies show that alcohol consumption of more than 40 g per day is increasingly detrimental for any type of pancreatitis.³⁵ Retrospective clinical studies have shown that binge alcohol drinking is associated with aggravation of first-attack severe acute pancreatitis, which is reflected in higher admission levels of serum triglycerides, Balthazar computed tomographic score, and Acute Physiology and Chronic Health Evaluation II score, as well as higher mortality and incidence of complications.³⁶

Insight into the mechanisms involved in pancreatic injury is derived from preclinical studies that show detrimental effects of binge alcohol exposure on the pancreas. These effects include tissue edema, inflammation, acinar atrophy and moderate fibrosis, endoplasmic reticulum stress, oxidative stress, and apoptotic and necrotic cell death. These structural changes are associated with pancreatic dysfunctional changes, which are reflected by altered

levels of alpha-amylase, glucose, and insulin, strongly suggesting a detrimental effect of acute binge alcohol exposure on the pancreas. Specifically, preclinical studies have proposed that, alone, chronic and binge alcohol exposure caused minimal pancreatic injury, but chronic plus binge alcohol exposure resulted in significant apoptotic cell death; alterations in alpha-amylase, glucose, and insulin; pancreatic inflammation; and protein oxidation and lipid peroxidation, which are indicative of oxidative stress.³⁷ The pathogenesis of alcoholic pancreatitis involves acinar cell alcohol metabolism. The direct toxic effects of alcohol and its metabolites on acinar cells, in the presence of an appropriate trigger factor, may predispose the gland to injury. In addition, pancreatic stellate cells are implicated in alcoholic pancreatic fibrosis.³⁸ Thus, experimental and clinical data suggest that alcohol consumption alone does not initiate pancreatitis, but it sensitizes the pancreas to disease from other insults, including smoking, exposure to bacterial toxins, viral infections, and binge alcohol consumption.³⁹

Cardiovascular Consequences

The effect of alcohol consumption on cardiovascular function has been the subject of much debate. The relationship between alcohol consumption and cardiovascular health is not linear and is thought to follow a J-shaped curve, with low amounts of alcohol consumption frequently reported as cardioprotective.⁴⁰ However, data suggest that binge drinking is associated with transient increases in systolic and diastolic blood pressure (Figure 3).⁴¹⁻⁴³ The prevalence of hypertension has been reported to be higher in individuals who consume more than six drinks per day. However, the pattern of alcohol consumption was not considered in these studies.⁴⁴ The effect of even a modest rise in blood pressure is considerable, as it is a recognized risk factor for cardiovascular mortality.^{45,46}

Binge drinking has been associated with increased risk of cardiovascular comorbidities, including hypertension, stroke, myocardial infarction, and sudden death, and this risk may extend to the younger population as well.⁴⁷⁻⁵¹ Acute elevations in blood alcohol levels resulting from binge alcohol consumption are associated with an increased risk of new-onset atrial fibrillation, a most common arrhythmia strongly associated with adverse cardiovascular events and sudden death.⁵² A higher risk for myocardial infarction has been reported after 1 day of heavy alcohol consumption (which could reflect a binge-like pattern of alcohol consumption).⁵³

Few preclinical studies have examined the effect of binge drinking on cardiac function. In one study, over a 5-week period, rodents received repeated episodes of alcohol administration that modeled a binge drinking pattern.⁵⁴ These rodents did not show changes in cardiac structure, but this drinking pattern resulted in increased phosphorylation of myocardial p38 mitogen-activated protein kinase and transient increases in blood pressure, which became progressively higher with repeated episodes of binge drinking. These effects were partly mediated by adrenergic mechanisms. More recently, the combined binge-on-chronic pattern of alcohol feeding to rodents has been shown to result in alcohol-induced cardiomyopathy, characterized by increased myocardial oxidative/nitrative stress, impaired mitochondrial function and biogenesis, and enhanced cardiac steatosis.^{55,56} The role of oxidative stress has been confirmed by other preclinical studies.⁵⁷

Pulmonary Consequences

Preclinical studies have identified impairments in multiple aspects of lung function after chronic and binge-like alcohol administration, including altered epithelial barrier function, suppressed immunity, impaired bacterial clearance, depleted glutathione (GSH),

and impaired pulmonary epithelial ciliary function (Figure 3).^{58,59} Moreover, alcohol binge drinking increases the risk for sustaining traumatic injuries and aggravates outcomes from traumatic injuries,⁶⁰ such as burns,^{26,58,61-63} bone fractures,⁶⁴ and hemorrhagic shock.⁶⁵ For alcohol-intoxicated hosts, similar detrimental effects have been reported on bacterial pneumonia outcomes, a frequent comorbid condition associated with traumatic injury.⁶⁶ Binge-like alcohol administration impairs innate and adaptive immune responses in the lungs, thereby increasing infection susceptibility, morbidity, and mortality.^{61,62} It is possible that, in hosts previously exposed to chronic alcohol consumption, binge drinking detrimentally affects pulmonary outcomes from traumatic injury by priming host defense mechanisms. This combined effect may prevent clear isolation of binge alcohol consumption effects from chronic alcohol consumption effects.

Musculoskeletal Consequences

The incidence of skeletal muscle dysfunction (i.e., myopathy) resulting from chronic alcohol misuse surpasses that of cirrhosis.⁶⁷ This progressive loss of lean mass is multifactorial and involves metabolic, inflammatory, and extracellular matrix alterations, which promote muscle proteolysis and decreased protein synthesis (Figure 3).⁶⁸ An additional severe complication of binge drinking is the development of acute muscle injury, rhabdomyolysis. Binge drinking that precedes coma or immobility can lead to rhabdomyolysis and, consequently, to renal injury, as documented in case reports in the literature.⁶⁹⁻⁷¹ The mechanisms are not well-understood, but they may involve acute hypokalemia.⁷² This phenomenon may warrant further study, as environmental factors such as high ambient temperature and individual drug-drug interactions can obscure presentation and hinder management of alcohol-induced rhabdomyolysis.

Preclinical studies suggest that, after binge-like alcohol administration, physical exercise may ameliorate cognitive impairment and suppressed neurogenesis.⁷³ The effect of binge alcohol consumption on exercise performance and recovery remains to be systematically investigated. One clinical study reported no change in isokinetic and isometric muscle performance, central activation, or creatine kinase release during or after acute moderate alcohol intoxication.⁷⁴ Short-term reductions in lower-extremity performance were reported in a study that investigated athletes after an alcohol drinking episode and the associated reduced sleep hours.⁷⁵ Another study found that alcohol consumption following a simulated rugby game decreased lower-body power output but did not affect performance of tasks requiring repeated maximal muscular effort.⁷⁶ However, the same researchers found that alcohol consumption following eccentric exercise accentuated the losses in dynamic and static strength in males.⁷⁷

In contrast, alcohol consumption following muscle-damaging resistance exercise did not alter inflammatory capacity or muscular performance recovery in resistance-trained women,⁷⁸ suggesting possible gender differences in alcohol's modulation of exercise performance and recovery. These studies were conducted using healthy volunteers and athletes. Other studies that investigated patients with alcoholic liver disease showed lower muscular endurance, maximal voluntary isometric muscle strength, and total work of knee extensors.⁷⁹ Controlled studies are needed, particularly in light of the popularity of binge drinking events frequently associated with collegiate and professional sports.

Neuropathological Consequences

The behavioral and cognitive effects of binge drinking include difficulties in decision-making and impulse con-

trol, impairments in motor skills (e.g., balance and hand-eye coordination), blackouts, and loss of consciousness (Figure 3).⁸⁰ All of these effects have serious health consequences ranging from falls and injuries to death.⁸¹ In particular, adolescents are vulnerable to the cognitive manifestations and memory loss associated with binge drinking. National estimates suggest that significant numbers of people who binge drink report at least one incident of blacking out in the previous year.^{82,83} Blackouts, defined as short periods of amnesia during which a person actively engages in behaviors (e.g., walking or talking) without creating memories for them, often occur at blood alcohol concentrations exceeding .25 g/dL.^{84,85} Blackouts are common among college students who drink alcohol. Estimates suggest that up to 50% of students that engaged in drinking reported a blackout episode during the past year.^{86,87} The pattern of rapid consumption of large doses of alcohol, frequently on an empty stomach, is characteristic of the adolescent period.⁸⁸

The consequences of binge drinking are not short-lived or limited to the period of intoxication. Imaging studies of binge drinking adolescents document long-lasting changes. Reports indicate structural changes in the prefrontal and parietal regions, as well as in regions known to mediate reward, and these changes are thought to reflect long-lasting effects of alcohol bingeing on critical neurodevelopmental processes.⁸⁹ Functional imaging studies of the brains of binge drinking and nondrinking adolescents found that binge drinking adolescents showed greater responses in frontal and parietal regions, no hippocampal activation to novel word pairs, and modest decreases in word-pair recall, which could indicate disadvantaged processing of novel verbal information and a slower learning slope.⁹⁰ In another study, adolescent binge drinking resulted in gender-specific differences in frontal, temporal, and cerebellar brain activation during a special working memory

task, reflecting differential effects of binge drinking on neuropsychological performance and possibly greater vulnerability in female adolescents.⁹¹ Other researchers have reported that degradations in neural white matter were linked with impaired cognitive functioning in adolescents who binge drank.⁹²

Adolescent rodent intermittent ethanol exposure that modeled human adolescent binge drinking produced a range of pathophysiological and neurobehavioral sequelae, including altered adult synapses, cognition, and sleep; reduced adult neurogenesis; increased neuroimmune gene expression; and increased adult alcohol drinking associated with disinhibition and social anxiety.⁹³ Preclinical studies indicated that binge drinking could produce brain structural abnormalities. Binge alcohol

administration to rodents produced increases in cerebrospinal fluid volume in the lateral ventricles and cisterns, decreased levels of *N*-acetylaspartate and total creatine, and increased choline-containing compounds, glutamate, and glutamine, all of which recovered during abstinence.⁹⁴ Moreover, preclinical data suggested that adolescent binge drinking sensitized the neurocircuitry of addiction, possibly inducing abnormal plasticity in reward-related learning processes, which could contribute to adolescent vulnerability to addiction.⁹⁵

Summary

Although the effects of chronic alcohol consumption and the mechanisms of tissue injury underlying alcoholic

hepatitis and cirrhosis have received much attention, less attention has been focused on the pathophysiological consequences of binge alcohol consumption. The differential duration of the intoxication period, excessive concentrations of alcohol at the tissue level, accelerated alcohol metabolism and generation of ROS and alcohol metabolites, and acute disruption of antioxidant mechanisms are some of the salient differences between chronic and binge-like alcohol-mediated tissue injury. Because of the differences in male and female alcohol metabolism rates, it is possible that greater tissue injury is produced in females who consume alcohol in binge-like patterns. Furthermore, in an aging population already riddled with polypharmacy, there is heightened potential for toxicity during an alcohol binge (Figure 4). Also, pre-existing comorbid conditions such as cardiovascular disease, renal failure, or steatohepatitis may predispose binge drinkers to accelerated tissue injury.

Additional research is needed to better recognize the differential effects of binge, chronic, and binge-on-chronic patterns of alcohol consumption. Animal models that reflect these patterns of alcohol exposure are needed. In addition, greater effort toward documenting a history of alcohol consumption, including the frequency, quantity, and quality of alcoholic beverages consumed, should help us better understand the effects of binge drinking on biological systems.

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Figure 4 Factors that contribute to disease processes associated with binge alcohol drinking. For individuals who drink alcohol, factors such as type of alcohol, pattern of consumption, duration of alcohol misuse, and the age and diet of the drinker contribute to the incidence and severity of tissue injury. Another factor, polypharmacy, particularly affects the older adult population, as multiple medications increase the potential for toxicity during an alcohol binge. Similarly, pre-existing comorbid conditions may predispose binge drinkers to accelerated tissue injury. Finally, genetic predisposition and environmental toxins are likely to be determining factors that affect the incidence and severity of tissue and organ injury.

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The authors declare that they have no competing financial interests.

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Development, Prevention, and Treatment of Alcohol-Induced Organ Injury

The Role of Nutrition

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Alcohol and nutrition have the potential to interact at multiple levels. For example, heavy alcohol consumption can interfere with normal nutrition, resulting in overall malnutrition or in deficiencies of important micronutrients, such as zinc, by reducing their absorption or increasing their loss. Interactions between alcohol consumption and nutrition also can affect epigenetic regulation of gene expression by influencing multiple regulatory mechanisms, including methylation and acetylation of histone proteins and DNA. These effects may contribute to alcohol-related organ or tissue injury. The impact of alcohol–nutrition interactions has been assessed for several organs and tissues, including the intestine, where heavy alcohol use can increase intestinal permeability, and the liver, where the degree of malnutrition can be associated with the severity of liver injury and liver disease. Alcohol–nutrition interactions also play a role in alcohol-related lung injury, brain injury, and immune dysfunction. Therefore, treatment involving nutrient supplementation (e.g., with zinc or S-adenosylmethionine) may help prevent or attenuate some types of alcohol-induced organ damage.

Key words: Alcohol consumption; alcohol use, abuse, and disorder; heavy alcohol consumption; alcohol–nutrition interactions; organ injury; tissue injury; intestine; nutrition; nutrients

The effect of alcohol on organ health and injury is complex and influenced by a host of different factors, such as dose of alcohol consumed; duration and pattern of drinking (e.g., binge drinking); and, as reviewed in this article, potential interactions with nutrition. The *2015–2020 Dietary Guidelines for Americans* (U.S. Department of Health and Human Services and U.S. Department of Agriculture 2015) highlight the concept of the standard drink and the fact that if alcohol is consumed, it should be in moderation (i.e., up to 1 drink per day for women and 2 drinks per day for men in adults of legal drinking age). It is becoming increasingly accepted that this moderate form of drinking may have health benefits that seem to lessen many types of organ injury. This concept

was popularized in 1991, when Morley Safer presented information on the television show *60 Minutes* related to the “French paradox”—that is, the observation that the French seemed to have lower rates of heart attacks despite higher fat consumption. This outcome was postulated as possibly resulting from the beneficial effects of wine consumption by the French. Subsequent studies have shown that all forms of alcohol, when consumed in moderation, seem to lower the risk of coronary artery disease (Yang et al. 2016). The beneficial effect can be represented by a J-shaped curve, in which low alcohol consumption has protective effects compared with abstention, whereas excessive alcohol consumption is harmful. Moderate drinking also may have

beneficial effects on several other organs and organ systems, including the following:

- Decreased risk of ischemic stroke (Sacco et al. 1999);
- Protection against type 2 diabetes (Conigrave et al. 2001);
- Decrease in rheumatoid arthritis (Di Giuseppe et al. 2012);
- Improved cognition (Anstey et al. 2009);
- Decreased progression of liver disease to fibrosis in obese individuals (Thomson et al. 2012); and
- Improved renal function (Koning et al. 2015).

Indeed, moderate alcohol consumption may be associated with an overall modest survival benefit (Ford et al. 2011).

Moderate alcohol consumption also has been shown to decrease biomarkers of inflammation, such as C-reactive protein, and reduced inflammation could be one unifying mechanism underlying alcohol's protective effects (Imhof et al. 2004). On the other hand, long-term heavy alcohol abuse can cause organ injury, which may, at least in part, result from alcohol–nutrient interactions and alcohol-related nutrient deficiencies. As described in this article, people who abuse alcohol frequently consume large amounts of alcohol, which may contribute to the displacement of needed nutrients (see figure 1). Indeed, recent analyses of nutritional status and alcohol consumption in people with alcohol use disorder (AUD) who were admitted to a rehabilitation program demonstrated that the participants generally had a normal body mass index, were not overtly malnourished, and did not have clinical evidence of alcohol-induced organ injury. However, these people were consuming, on average, 14 drinks per day, which would amount to about 2,000 calories

per day or more consumed as alcohol (Vatsalya et al. 2016). Considering that the participants had a normal body mass index, this suggests that they replaced normal nutrients with alcoholic beverages, resulting in potential nutrient deficiencies. Nutritional supplementation may either help ameliorate such deficiencies or have pharmacologic effects.

Alcohol and nutrition can interact at multiple levels. For example, alcohol metabolism can result in the generation of reactive oxygen species, which can deplete endogenous nutritional antioxidant stores and contribute to oxidative stress. Heavy alcohol consumption also can cause poor intestinal absorption of certain nutrients (e.g., zinc) or increase nutrient losses (e.g., by

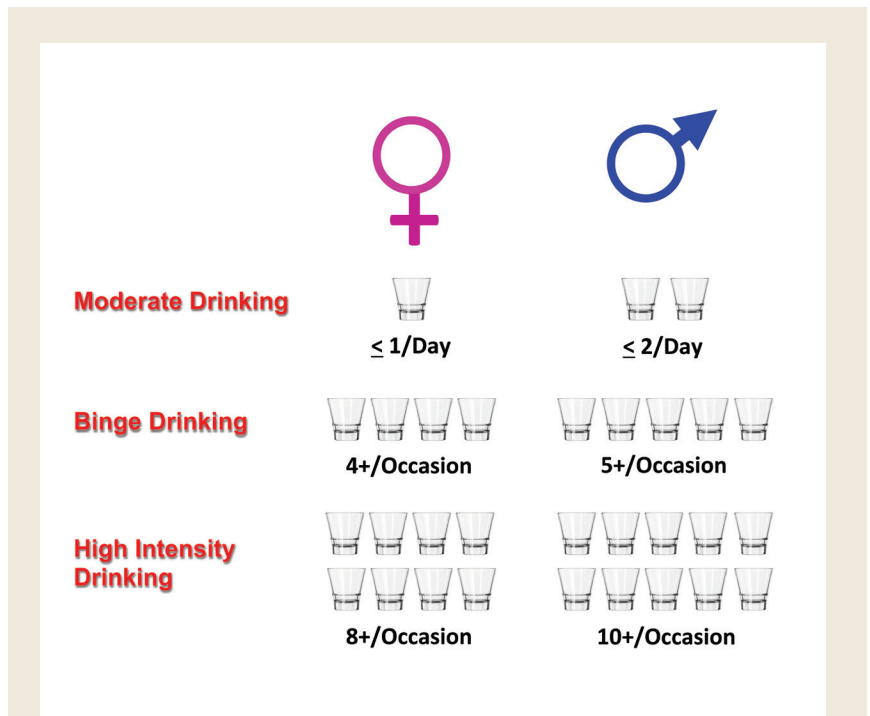


Figure 1 Drinking levels and their consequences. In the United States, drinking levels are expressed in terms of standard drinks consumed—that is, the number of alcoholic beverages drunk, each containing about 0.6 fluid ounce or 14 grams of pure alcohol. The *Dietary Guidelines for Americans 2015–2020* defines moderate drinking as consuming up to 2 drinks/day for men and up to 1 drink/day for women. The Substance Abuse and Mental Health Services Administration defines binge drinking as consuming 5 or more (for men) or 4 or more (for women) alcoholic drinks on the same occasion on at least 1 day in the past 30 days (National Institute on Alcohol Abuse and Alcoholism 2016). High-intensity drinking refers to drinking at levels far beyond the binge threshold, resulting in high peak blood alcohol concentrations. Some studies define high-intensity drinking as two or more times the gender-specific binge drinking thresholds (Patrick et al. 2016); others use a higher threshold (Johnston et al. 2016). Some individuals drink considerably more than this. For example, one study found that patients admitted to a National Institutes of Health treatment facility with a diagnosis of alcohol use disorder consumed the equivalent of 13 drinks per day (Vatsalya et al. 2016). In these drinkers, the metabolic effects of alcohol and altered nutrient intake may set the stage for alcohol–nutrient interactions and organ injury.

increasing zinc and magnesium excretion in the urine). Moreover, nutrition can have a far-reaching impact through altering epigenetic mechanisms, such as methylation and acetylation of DNA and associated proteins. Finally, the degree of alcohol-related malnutrition can be associated with the severity of organ injury (e.g., alcoholic hepatitis). This article reviews how nutritional alterations may predispose to alcohol-induced organ injury and how nutritional supplementation may prevent and/or treat alcohol-induced organ injury. The article specifically highlights the effects of certain alcohol–nutrient interactions, with a focus on zinc and linoleic acid, and their impact on epigenetics and selected organ injury.

Nutrition and Nutritional Alterations Following Alcohol Use/Abuse

Alcohol: Nutrition Overview

From a nutrition perspective, alcohol is a significant source of calories, but these can be considered “empty” calories—that is, they contain few micronutrients, such as vitamins and minerals, normally found in most food sources (Antonow and McClain 1985). The main site of beverage alcohol (i.e., ethanol) metabolism is the liver, where ethanol is converted to carbon dioxide and water, with an energy yield of 7 kcal/g of alcohol. Regular alcohol intake can be a major source of calories, because beer has approximately 150 kcal per 12-ounce can and bourbon or scotch with a mixer has approximately 125 kcal per drink. Thus, a person can easily consume 200 to 500 calories or more per day by consuming 2 to 3 drinks. For people attempting weight reduction, alcohol consumption therefore can be considered a source of unwanted and empty calories. Moreover, when alcohol intake is combined with fructose-containing sugared drinks, the

intake of empty calories increases even further, enhancing the opportunity for alcohol-induced organ injury. Finally, alcohol can be an expensive source of calories compared with traditional foods, and this may become a major problem for people with limited incomes.

The issue of alcohol as a nutrient becomes more prominent when dealing with people with AUD and those with alcohol-induced organ injury. Analyses of the nutritional status of people with AUD admitted to treatment programs found that these individuals often consumed 35 to 50 percent of their total calories as alcohol, and some exhibited inadequate micronutrient intake and micronutrient serum levels (Antonow and McClain 1985). However, most had little or no evidence of protein-calorie malnutrition and loss of muscle mass. In contrast, patients admitted to hospitals for severe alcoholic hepatitis who also consumed 50 percent of their total calories as alcohol not only regularly showed depletion of certain micronutrients but also loss of muscle mass (Mendenhall et al. 1995a). The following sections focus on the micronutrient zinc, which may be deficient or have altered metabolism with heavy alcohol consumption, and a macronutrient (i.e., dietary fat) that may play a role in alcohol-induced organ injury. Some of the other micronutrients for which heavy alcohol intake may cause deficiency states or altered metabolism are listed in the table.

Zinc

Zinc is an essential trace element required for normal cell growth, development, and differentiation, including such processes as DNA synthesis, RNA transcription, and cell division and activation. It is a critical component of many proteins/enzymes, including zinc-dependent transcription factors. Zinc deficiency or altered zinc metabolism is frequently observed in heavy alcohol drinkers and may result from decreased dietary intake, increased urinary excretion, abnormal activation of



Figure 2 Chronic alcohol user who had been consuming large amounts of beer before admission. Note classical skin lesions of zinc deficiency around the eyes, nose, and mouth.

certain zinc transporters, and induction of hepatic metallothionein (Mohammad et al. 2012). Zinc deficiency may manifest itself in many ways in alcoholics, ranging from raised, crusting skin lesions around the eyes, nose, and mouth (figure 2) to impaired wound healing or liver regeneration, altered mental status, or altered immune function (Mohammad et al. 2012). Importantly, oxidative stress (e.g., resulting from ethanol metabolism) may cause release of zinc from critical zinc-finger proteins and cause loss of DNA-binding activity. Specifically, oxidative stress causes modification of certain amino acids (i.e., cysteine residues) that hold the zinc in place in zinc-finger proteins such as hepatocyte nuclear factor 4 (HNF4), a transcription factor that is essential for liver development.

Zinc supplementation has been documented to block or attenuate experimental organ injury and dysfunction in the gut, liver, lung, and brain through multiple pathways. Thus, zinc may

strengthen the integrity of the intestinal wall by stabilizing tight junctions, reduce transfer of toxic bacterial molecules (e.g., endotoxin) into the blood, lower the levels of metabolic toxins such as ammonia in the blood, decrease production of inflammation-promoting (i.e., proinflammatory) cytokines, reduce oxidative stress, and attenuate apoptotic cell death (Zhong et al. 2010, 2015) (figure 3). The dose of zinc used for treatment of alcohol-induced organ injury such as liver disease usually is 50 mg of elemental zinc taken with a meal to decrease the potential side effect of nausea. Intake of greater than 50 mg of elemental zinc per day can cause dose-related side effects, such as copper deficiency resulting from reduced copper absorption.

Dietary Fats

The critical role for specific types of dietary fat (i.e., saturated versus unsaturated fats) in intestinal and liver injury has been demonstrated and extensively studied in preclinical animal models of alcohol feeding using various sources of dietary lipids. Experimental evidence has shown that dietary saturated fats (SFs) attenuated, and unsaturated fats (USFs) enhanced, alcohol-induced liver damage (Nanji and French 1989). Thus, in contrast to the general assumption that SFs are less healthy than USFs, in this situation SFs had a protective effect and USFs had a harmful effect.

Further analyses focused on the role of different types of dietary polyunsaturated fatty acids (PUFAs) in alcohol-induced gut and liver injury. There are two major families of dietary PUFAs—omega-6 [ω -6] and omega-3 [ω -3] PUFAs—each of which includes numerous related metabolites. It has been demonstrated that linoleic acid, an ω -6 PUFA [18:2 ω -6], is required for the development of experimental alcohol-induced intestinal and liver injury and that the severity of alcoholic liver disease (ALD) is correlated with the amount of linoleic acid in the diet (Nanji and French 1989; Ronis et al.

Table Types of Nutrient Deficiency Caused by Heavy Drinking and the Associated Signs and Symptoms

Selected Nutrient Deficiency	Signs/Symptoms
Magnesium	Insulin resistance, muscle cramps
Selenium	Myopathy, cardiomyopathy
Vitamin B1/Thiamine	Wernicke-Korsakoff syndrome, neurologic symptoms
Vitamin B2/Riboflavin	Glossitis, cheilitis, and lingual papillae atrophy
Vitamin A/Retinol	Abnormal dark adaptation, rough skin
Vitamin C	Scurvy with purpura and petechiae
Vitamin D	Altered bone metabolism, altered gut barrier/immune function
Vitamin E	Oxidative stress
Niacin	Skin photosensitivity, confusion, pellagra
Folate, S-Adenosylmethionine	Anemia, altered methylation, epigenetic effects

2004). Conversely, fish oil (a rich source for ω -3 PUFAs) or purified ω -3 PUFAs (e.g., eicosapentaenoic acid [EPA] and docosahexaenoic acid [DHA], which are known to be important in brain development) may be beneficial in ALD. For example, in mice, prior ingestion of fish oil, specifically tuna fish oil, in amounts that provided 30 percent of the total caloric intake, resulted in reduced hepatic fat accumulation caused by a single dose of ethanol administration. This effect was mediated, at least in part, through marked reductions in the expression of the hepatic enzyme stearoyl-CoA desaturase-1 and in the activity of the transcription factor sterol regulatory element-binding protein (Wada et al. 2008). Mice supplemented with highly purified DHA also had significantly decreased alcohol-induced liver steatosis, inflammation, and injury (Huang et al. 2013). The beneficial role of ω -3 PUFAs in experimental ALD also has been supported by the observation that when rhesus monkeys who had free access to an ethanol solution were fed a diet that was generally nutritionally adequate (including the linoleic acid amount), but with a low ω -3 PUFA content (i.e., a very low

concentration of α -linolenic acid), the animals developed hepatic steatosis and fibrosis (Pawlosky and Salem 2004). The ω -3 PUFAs also are precursors to factors that resolve injury and inflammation, such as resolvins (e.g., E- and D-series resolvins generated from EPA and DHA, respectively), and a high dietary ω -6/ ω -3 PUFA ratio may be disadvantageous to resolving inflammation (Serhan and Petasis 2011). Thus, emerging evidence suggests that dietary fats can play a role in both initiation and treatment of alcohol-induced organ injury in the gut and liver as well as in the brain (which will be discussed later in this article).

Nutrition–Alcohol Interactions and Epigenetics

In virtually every cell type, epigenetic mechanisms—that is, modifications to the genetic material that do not alter the DNA sequence—play a critical role in both the physiologic and pathologic regulation of gene expression. These mechanisms, which involve chromatin remodeling initiated by posttranslational modifications of

histones and changes in DNA methylation status, can activate or deactivate gene transcription. The proteins that are involved in posttranslational histone modifications and DNA methylation changes require a variety of cofactors, including acetyl coenzyme A, S-adenosylmethionine (SAM), nicotinamide adenine dinucleotide, and zinc (Moghe et al. 2011). A person's nutritional status can significantly influence the availability of these cofactors and, consequently, epigenetic mechanisms, gene expression, and disease pathogenesis. Chronic alcohol consumption is known to affect nutritional status at many levels, including nutrient intake, absorption, utilization, and excretion, causing nutritional disturbances and deficiencies in these cofactors. Research has determined that alcohol-induced nutrient fluctuations can impact transcriptional activity and expression of genes by modulating epigenetic parameters, including histone modifications and DNA methylation (Moghe et al. 2011; Zakhari 2013). Hence, in people with AUD, the combined effects of alcohol metabolism and compromised nutrition are likely to influence epigenetic mechanisms, gene expression, and disease pathogenesis involving intestinal barrier dysfunction, immune suppression, and organ injury.

Alcohol's Effects on Histone Acetylation and Methylation

It is becoming increasingly evident that histone-associated epigenetic modifications, such as histone acetylation and methylation, play a significant role in the regulation of gene expression and development of alcohol-induced organ pathology, such as liver disease and immune dysfunction (Moghe et al. 2011). In particular, histone acetylation in promoter regions is a key regulator of gene expression and is associated with enhanced transcriptional activity, whereas deacetylation typically is associated with transcriptional repression. Steady-state levels of acetylation result from the balance

between the opposing activities of two groups of enzymes—histone acetyltransferases and histone deacetylases. The expression and activities of both types of enzymes can be influenced by alcohol and cofactors, such as nicotinamide adenine dinucleotide and zinc (Ghare et al. 2014; Moghe et al. 2011). Taken together, epigenetic histone modifications provide a likely link between alcohol-mediated nutrient alterations in gene expression and disease pathogenesis.

Alcohol's Effects on DNA Methylation

Investigation of the dietary influences on epigenetic processes has revealed a direct link between SAM, which serves as the primary biological methyl donor, and DNA methylation changes that

epigenetically influence gene expression (McCabe and Caudill 2005). In general, DNA hypermethylation at DNA sequences called CpG islands in gene promoters leads to transcriptional silencing, whereas DNA hypomethylation allows for transcription to occur.

Excessive alcohol consumption can decrease SAM levels via multiple mechanisms, such as reduced folate levels and inhibition of key enzymes in one-carbon metabolism. The reduced SAM levels lead to aberrant DNA methylation patterns and pathogenic alterations in gene expression (Varela-Rey et al. 2013). Importantly, alcohol-induced perturbations in global and regional DNA methylation have been linked with diverse pathological conditions, including ALD, carcinogenesis in various organs, alcohol dependence, and fetal alcohol spectrum disorders

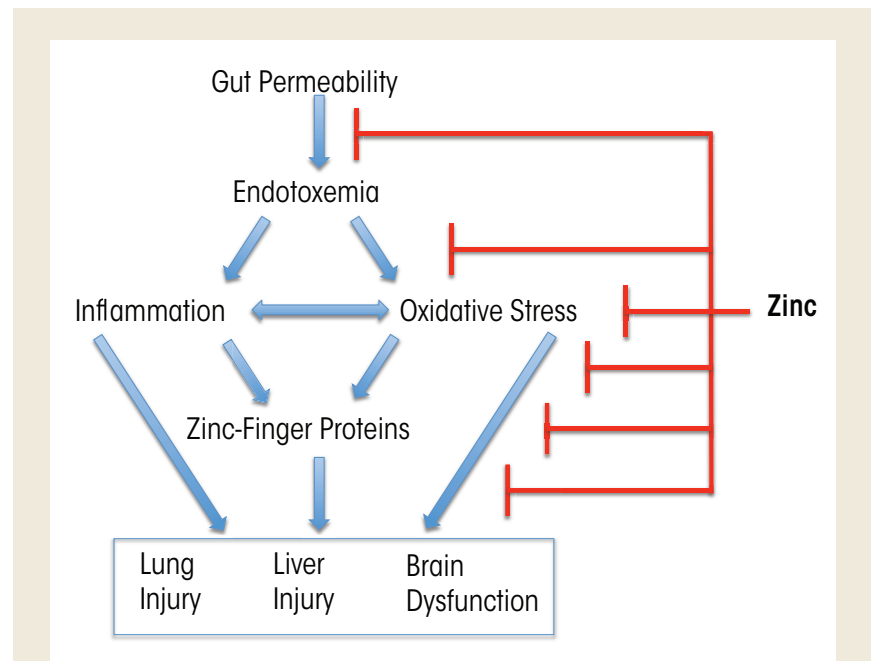


Figure 3 Zinc therapy positively affects multiple mechanisms of alcohol-induced organ injury. Thus, zinc enhances the gut barrier and tight junctions, thereby reducing gut permeability and the risk of transfer of bacterial endotoxin into the blood (i.e., endotoxemia). In addition, zinc decreases proinflammatory cytokine production and oxidative stress and ensures proper functioning of important zinc-dependent regulatory proteins (e.g., zinc-finger proteins). Through these and other mechanisms, zinc supplementation can improve liver injury and may attenuate lung and brain dysfunction.

(FASD), to name only a few. Clearly, further research is needed to detail the alcohol–nutrient interactions that influence epigenetic mechanisms underlying pathogenic changes in gene expression and disease progression, with the goal of developing nutrient-based therapies.

Examples of Nutrition–Alcohol Interactions in Alcohol-Induced Organ/Tissue Injury

Intestine

The intestinal mucosa plays a critical role in preventing passage of toxins from the intestine into the blood-

stream, as well as in immune function, detoxification, and metabolism. The importance of the gut in alcohol-mediated multiorgan pathology is becoming increasingly recognized. Clinical and experimental data have demonstrated that the gut-derived bacterial product, lipopolysaccharide, also referred to as endotoxin, plays a crucial role in the development and progression of alcohol-induced organ injuries, including ALD. Significantly increased endotoxin levels in the blood (i.e., endotoxemia) have been found in patients with different stages of ALD, including fatty liver, hepatitis, and cirrhosis (Parlesak et al. 2000).

Multiple mechanisms contribute to alcohol-associated endotoxemia, including alcohol-mediated alterations

in the composition of the bacterial population of the gut (i.e., gut microbiome) (Mutlu et al. 2009) and increased lipopolysaccharide translocation as a result of disruption of intestinal barrier integrity. Recent studies in mice have demonstrated that the type of dietary fat consumed can influence alcohol-induced changes in the gut microbiome composition (and, therefore, function), intestinal injury/inflammation, and intestinal barrier function (figures 4 and 5). Specifically, when comparing animals that were fed either dietary USFs or SFs plus ethanol (EtOH),¹ the studies found the following:

¹ The diet containing USFs was rich in corn oil, whereas the diet containing SFs was rich in medium-chain triglycerides.

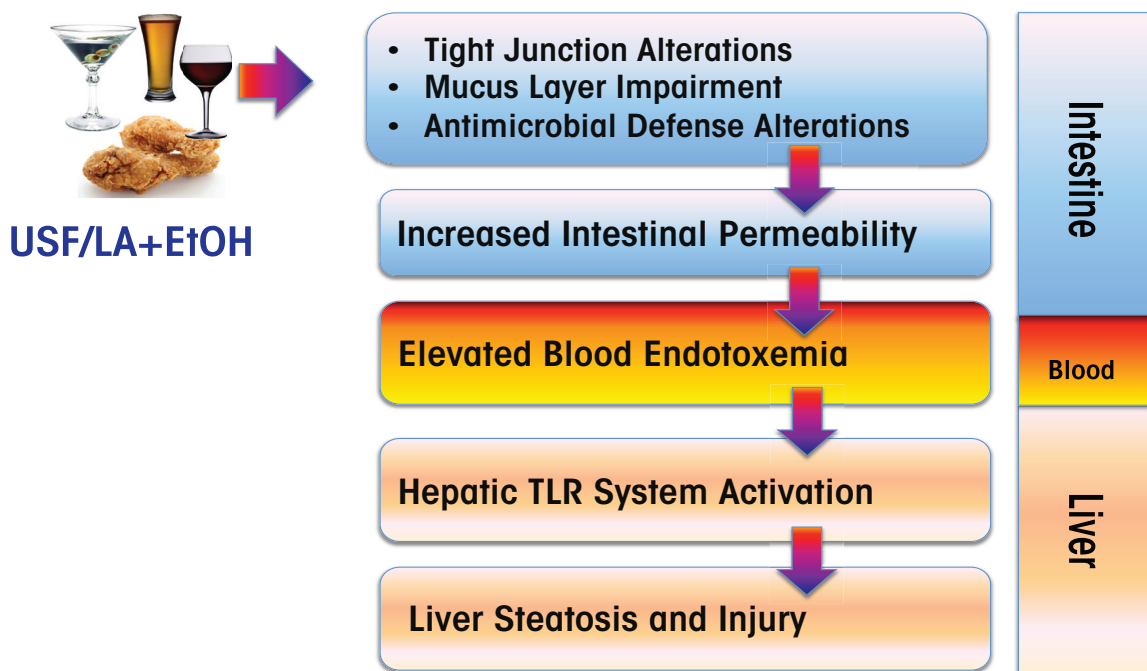


Figure 4 Alcohol (EtOH) consumption combined with dietary intake of unsaturated fatty acids (USFs) (e.g., linoleic acid [LA]) can have numerous deleterious effects on the intestine, blood, and liver. In the intestine, this combination changes the bacterial composition (microbiome) and interferes with various aspects of the body’s defense systems, thereby increasing intestinal permeability. This leads to endotoxemia and liver injury.

NOTE: TLR = toll-like receptor.

- The animals that received EtOH+USF showed increased gut permeability and elevated endotoxemia compared with those that received EtOH+SF (Kirpich et al. 2012) (figure 5A).
- Compared with EtOH+SF, a chronic EtOH+USF diet triggered an intestinal proinflammatory response characterized by increased levels of several cytokines, including tumor necrosis factor- α and monocyte chemoattractant protein-1. In addition, the intestinal mucus layer and antimicrobial defenses were altered (Kirpich et al. 2013).
- Intestinal inflammation was positively correlated with the EtOH+USF-triggered disruption of the intestinal tight junctions (figure 5B). Even in the absence of alcohol, a USF diet resulted in downregulation of intestinal expression of tight-junction protein mRNA compared with an SF diet. Alcohol further suppressed tight-junction proteins in animals receiving EtOH+USF, but did not affect intestinal tight junctions in the EtOH+SF group (Kirpich et al. 2013) (figure 5B).
- Unlike EtOH+SF, dietary EtOH+USF caused alterations in gut microbiota (Bull-Otterson et al. 2012; Kirpich et al. 2016) (figure 5C).² The observed microbiota and intestinal barrier changes were associated with significant liver steatosis, inflammation, and injury in EtOH+USF-fed mice (figure 5D). These adverse effects of ethanol on the liver were markedly attenuated by a SF diet containing medium-chain triglycerides.

² The EtOH+USF-induced changes in gut microbiota were characterized by the decrease of certain bacteria (i.e., the *Bacteroidetes* phylum) with a proportional increase in others (i.e., gram-negative *Proteobacteria* and gram-positive *Actinobacteria* phyla). The bacterial genera that showed the biggest expansion were the gram-negative, alkaline-tolerant *Alcaligenes* and gram-positive *Corynebacterium* (Bull-Otterson et al. 2013).

Thus, it is clear that the interactions of dietary fat and alcohol are important in mediating alcohol-induced intestinal and liver injury.

Similarly, in mice, zinc deficiency associated with chronic alcohol intake led to markedly decreased tight-junction proteins and increased endotoxemia. Zinc supplementation corrected these effects through multiple mechanisms, including zinc-finger function and epigenetic mechanisms (Zhong et al. 2015). In summary, an important component of alcohol-induced organ inflammation/injury arises in the gut and may be modified by nutrition.

Liver Injury

Patients with severe alcoholic hepatitis almost invariably demonstrate some form of malnutrition. Probably the most detailed information concerning malnutrition in ALD comes from two large studies from the Veterans Health Administration (VA) Cooperative Studies Program in patients with alcoholic hepatitis (Mendenhall et al. 1984, 1986, 1995*a, b*). In these studies, almost 50 percent of the patients' energy intake was derived from alcohol. Although they frequently showed no inadequate calorie intake, the patients often exhibited insufficient intake of protein and critical micronutrients. The severity of liver disease generally correlated with the severity of malnutrition. During treatment, the patients received a balanced 2,500-kcal hospital diet (monitored by a dietitian) that they were encouraged to consume. Investigators found that voluntary oral food intake correlated in a step-wise fashion with 6-month mortality data. Thus, patients who voluntarily consumed more than 3,000 kcal per day had virtually no mortality, whereas those who consumed less than 1,000 kcal per day had a 6-month mortality of more than 80 percent (Mendenhall et al. 1995*a*). Moreover, the degree of malnutrition correlated with the development of serious complications, such as encephalopathy,

ascites, and hepatorenal syndrome (Mendenhall et al. 1995*a*).

Initial interest in nutrition therapy for ALD was stimulated by Patek and colleagues (1948) who demonstrated that a "nutritious diet" improved the 5-year outcome of patients with alcoholic cirrhosis compared with historic control subjects. Subsequently, nutritional supplementation through a feeding tube was shown to significantly improve liver function in inpatients with ALD compared with inpatients who ate a hospital diet (Kearns et al. 1992). Probably the most important data supporting nutrition therapy came from a multicenter study by Cabré and colleagues (2000), who randomly assigned patients with severe alcoholic hepatitis to receive either the glucocorticoid prednisone (40 mg daily) or a liver-specific formula containing 2,000 calories per day through a feeding tube.³ The 1-month mortality was the same in both groups, but the 1-year mortality was significantly lower in the enteral-nutrition group than in the glucocorticoid group, mainly because they experienced fewer infectious complications. This study clearly documented the importance of enteral nutrition in severe alcoholic hepatitis. Oral/enteral nutrition is preferable over parenteral nutrition because of lower costs, risk of sepsis from the parenteral nutrition line, preservation of the integrity of the gut mucosa, and prevention of bacterial translocation and multiple-organ failure.

Enteral nutrition supplements also have been shown to improve nutritional status and immune function in outpatients with alcoholic cirrhosis as well as to reduce hospitalization. The concept of an outpatient late-evening snack (prior to bedtime) was established after studies demonstrated altered energy metabolism in people with liver cirrhosis. These patients exhibit depleted hepatic glycogen stores, which force the body to depend on fat and protein stores, leading to catabolism during an overnight fast.

³ This polymeric enteral solution was enriched in branched-chain amino acids, energy dense (1.3 kcal/ml), and low in fat and sodium.

A randomized controlled trial demonstrated that provision of a late-evening nutritional supplement (compared with daytime supplements) over a 12-month period could improve body protein stores in patients with cirrhosis. The nighttime snack resulted in body protein accrual equivalent to about 2 kg of lean tissue sustained over 12 months, whereas this benefit was not observed with daytime snacks. Thus,

late-evening snacks are valuable nutritional interventions in outpatients with alcoholic cirrhosis (Plank et al. 2008).

Many types of nutritional supplements have yielded positive effects in animal models of ALD, especially antioxidants. However, human studies using specific nutrients or combination therapy are limited and generally have shown equivocal or negative results. Larger, well-designed studies are required.

Lung Injury

Chronic alcohol abuse alters the phenotype of the lung and makes it more susceptible to subsequent challenges, such as bacterial infection and acute lung injury. One of the mechanisms that contribute to increased susceptibility to infection and injury is alcohol-induced oxidative stress. Oxidative

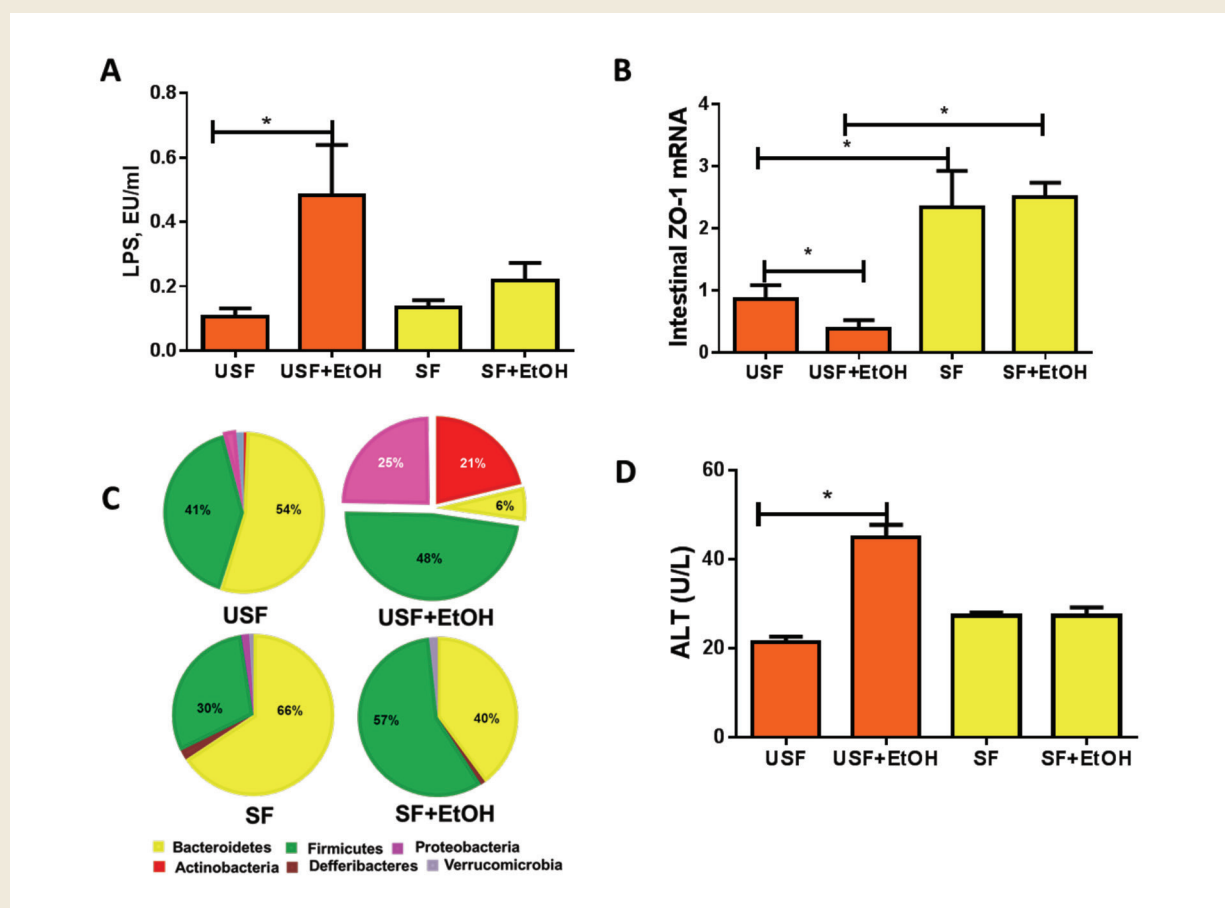


Figure 5 Effects of saturated fat (SF) and unsaturated fat (USF) diets on endotoxemia, intestinal tight junctions, gut microbiome, and liver injury in response to chronic alcohol (EtOH) feeding. **(A)** Plasma endotoxin levels assessed by plasma lipopolysaccharide (LPS) measurement. Alcohol feeding significantly increases LPS levels in the plasma when combined with a USF diet. **(B)** Levels of the mRNA for the tight-junction protein zonula occludens-1 (ZO-1) in the intestine. Animals receiving a USF diet showed greater disruption of tight junctions (i.e., lower ZO-1 levels) than animals receiving a SF diet; this effect was exacerbated with alcohol feeding. **(C)** Comparative analysis of the relative abundance of different phyla of gut bacteria in mice fed ethanol and different types of dietary lipids. The phyla abundance is indicated by the color bars. **(D)** Liver injury was evaluated by plasma alanine aminotransferase (ALT) activity. In animals receiving a USF diet, but not in those receiving a SF diet, alcohol feeding caused significant liver injury.

NOTE: Horizontal bars indicate statistically significant differences.

stress is defined as an imbalance between oxidants and antioxidants, and the way cells sense and respond to such an imbalance is a key determinant of disease initiation/progression or resolution. Oxidant-sensing and -signaling pathways rely primarily on proteins with reactive thiol-containing cysteine residues. The reactivity of a given protein thiol can be fine tuned by its local redox environment—that is, by the ratio of reduced versus oxidized molecules in the cell. This redox environment largely is controlled by two low-molecular-weight thiol-disulfide redox couples: one composed of the amino acid cysteine (Cys), which is the reduced partner of the pair, and its disulfide cystine (CySS), which serves as the oxidized partner. The other redox pair comprises glutathione (GSH) as the reduced partner and its disulfide GSSG as the oxidized partner. The two pairs are related but have different roles. Cys is one of the three component amino acids making up GSH, so it is not surprising that they share similar chemical properties. However, these redox control systems are compartmentalized; GSH/GSSG provides control mechanisms within cells and in the lung-lining fluid, whereas Cys/CySS predominates in the extracellular fluids of plasma and interstitium. The extracellular Cys/CySS redox state has been shown to have a direct effect on the production of two important proinflammatory cytokines, namely production of transforming growth factor β by lung fibroblasts (Ramirez et al. 2007) and interleukin-1 β by monocytes (Iyer et al. 2009).

Accumulating evidence suggests that the Cys/CySS and GSH/GSSG redox couples can be controlled by the diet. Dietary supplementation with the cysteine precursors N-acetylcysteine or procysteine has been used extensively to counteract the effects of oxidative stress. Although the effects of these cysteine precursors usually are attributed to enhanced GSH synthesis, they also are effective even when given in combination with a GSH-synthesis inhibitor

(e.g., buthionine sulfoximine) (Lailey et al. 1991). Recent studies showed that supplementing the diet with a combination of cysteine and methionine could prevent oxidation of the plasma Cys/CySS redox couple and decrease circulating levels of proinflammatory interleukin-1 β in endotoxin-challenged mice (Iyer et al. 2009). Similar diets also can alter the plasma Cys/CySS redox state in humans (Jones et al. 2011). It will be interesting to determine whether this type of dietary intervention can protect against lung injury in chronic alcoholics.

Zinc deficiency, particularly within immune cells in the lungs (i.e., alveolar macrophages), also contributes to increased susceptibility to bacterial infection in chronic alcoholics (Mehta et al. 2011). Studies in rats showed that chronic alcohol feeding decreased bacterial clearance from lung and oxidized Cys/CySS in the alveolar space. Dietary zinc supplementation blocked both of these effects (Mehta et al. 2011).

Brain Injury

Prenatal alcohol exposure can result in a range of detrimental effects, including damage to the developing brain, that are collectively known as FASD. Early autopsy studies, as well as more recent magnetic resonance imaging studies in both animal models and humans have revealed a variety of brain abnormalities, including reduced brain size (i.e., microcephaly) and anomalies of specific brain structures (e.g., the cerebrum, cerebellum, hippocampus, basal ganglia, and corpus callosum) after prenatal alcohol exposure (Lebel et al. 2011; Lipinski et al. 2012). These ethanol-induced brain insults contribute to the learning deficits, impairment in memory, difficulties with motor planning, and problems in regulating emotions and behavior observed in children with FASD.

Alcohol can damage the developing embryo through multiple mechanisms. Oxidative stress seems to play an important role in ethanol-induced

programmed cell death (i.e., apoptosis) and morphological abnormalities (Chen et al. 2013). In addition, accumulating evidence suggests that changes in epigenetic regulation are involved in the pathogenesis of FASD. For example, in animal studies, prenatal alcohol exposure increased the proportion of offspring with an unusual coat color by inducing hypermethylation of a specific gene, *Avylocus* (Kaminen-Ahola et al. 2010). Moreover, recent studies demonstrated that microRNA 125b can prevent ethanol-induced apoptosis of certain embryonal cells (i.e., neural crest cells) by targeting two specific genes called *Bak1* and *PUMA* (Chen et al. 2015).

It also is well known that nutritional deficiencies contribute to the pathogenesis of FASD and to ethanol-induced damage to the developing brain. Heavy maternal alcohol consumption results in deficiency in nutrients that are critical for fetal development and maternal health, including vitamins A and D, thiamin, folate, and zinc (Dreosti 1993). Moreover, as in adult brains, DHA deficiency occurred in the developing brain of animals prenatally exposed to ethanol. Finally, studies have shown that diets low in nutrients exacerbate alcohol-induced brain damage in the offspring (Nacach et al. 2009).

Maternal nutrient supplementation may decrease the risk of FASD and serve as a potential intervention for FASD. Some nutritional interventions target oxidative stress. For example, antioxidant supplements, such as vitamins C and E, can reduce oxidative stress, cell death, and behavioral impairments in animals prenatally exposed to ethanol. Studies in the adult brain have demonstrated that ethanol-induced neuro-inflammation and degeneration can be countered by dietary DHA. Similarly, an ω -3-enriched diet that contains 24.6 percent DHA has been shown to reduce ethanol-induced oxidative stress in the developing brain (Patten et al. 2011), consistent with the relationship between dietary fat and organ injury discussed earlier. Other nutritional

Glossary

Ascites: Accumulation of fluids in the abdominal cavity.

Cardiomyopathy: A condition of the heart muscle wherein it becomes enlarged, thick, or rigid. In rare cases, the muscle tissue in the heart is replaced with scar tissue.

Cell-Mediated Immunity: Part of the immune response that involves the activation of phagocytes, antigen-specific cytotoxic T-lymphocytes, and the release of various *cytokines* in response to a foreign molecule (i.e., antigen).

Cheilitis: Inflammation affecting the lips; this inflammation may include the skin around the mouth (i.e., perioral skin), the vermilion border, and/or the labial mucosa.

CpG Islands: Short DNA sequences that contain high levels of the normally rare cytosine–guanine sequence among the nucleotide sequence; they are targets of *DNA methylation* and are involved in the regulation of gene transcription.

Cytokines: A broad and loose category of small proteins (~5–20 kDa) that are important in cell signaling. Their release has an effect on the behavior of cells around them. They can be either proinflammatory or anti-inflammatory in their effects.

DNA Methylation: Epigenetic mechanism of regulation of gene expression, in which a strand of DNA is modified by addition of a methyl group (CH₃) to any cytosine located directly before a guanine.

Encephalopathy: A syndrome of overall brain dysfunction that can have many different organic and inorganic causes.

Enteral Nutrition: Delivery of nutrients in liquid form directly into the stomach or intestine.

Epigenetic: Heritable or nonheritable changes in phenotype or gene expression caused by mechanisms other than changes in the underlying DNA sequence; epigenetic changes can alter the appearance and structure of the DNA or the histone proteins around which the DNA is wound (e.g., *DNA methylation*, *histone acetylation*), thereby influencing gene expression.

Glossitis: Inflammation of the tongue.

Glycogen: Large, branched carbohydrate molecule consisting of glucose residues; constitutes the major carbohydrate reserve of animals and is stored primarily in liver and muscle.

Hepatorenal Syndrome: Functional kidney failure, but without pathological changes to the kidneys that is associated with cirrhosis and *ascites*.

Histones: Protein structures around which DNA strands are wrapped.

Histone Acetylation: *Epigenetic* modification of *histones* that involves the addition of an acetyl group.

Humoral Immunity: Immunity mediated by proteins called antibodies.

Interstitialium: The space between cells in a tissue or organ.

Metallothionein: Cysteine-rich proteins that can bind to heavy metals (e.g., zinc) through the *thiol* groups of their cysteine components. They participate in the uptake, transport, and regulation of zinc and can help control *oxidative stress*.

Methionine: An essential amino acid that can supply methyl groups for various metabolic reactions.

Micronutrient: Any essential dietary element required only in small quantities (e.g., trace minerals).

Myopathy: Muscular disease in which the muscle fibers do not function for any one of many reasons, resulting in muscular weakness.

Oxidative Stress: An imbalance between oxidants (e.g., free radicals) and antioxidants that can lead to excessive oxidation and cell damage.

Parenteral Nutrition: Intravenous administration of nutrients.

Pellagra: A clinical niacin deficiency syndrome characterized by dermatitis, inflammation of the mucous membranes, diarrhea, and psychic disturbances (e.g., depression, irritability, anxiety, disorientation, or hallucinations).

Glossary (*continued*)

Petechiae: Small, nonraised, perfectly round, purplish red spots caused by bleeding in the skin layer or beneath the mucous membranes.

Purpura: Any of a group of conditions characterized by small hemorrhages in the skin, mucous membranes, or serous membranes.

Redox Environment: The balance between oxidants and antioxidants in a cell or organ; often used to describe the balance of oxidized and reduced nicotinamide adenosine dinucleotide (NAD and NADH) in a biological system such as a cell or organ.

S-adenosylmethionine (SAM): Common co-substrate involved in methyl group transfers, transsulfuration, and aminopropylation. Although these anabolic reactions occur throughout the body, most SAM is produced and consumed in the liver.

Scurvy: Condition caused by vitamin C deficiency and characterized by weakness, anemia, spongy gums, and bleeding from the mucous membranes.

Steatosis: Abnormal accumulation of lipids in the functional cells of various tissues (e.g., in the liver).

Thiol: Any organic compound containing a thiol (-SH, or sulfhydryl) group; often have strong odors resembling garlic or rotten eggs.

Tight Junction: An intercellular junction between epithelial cells, at which the adjacent cell membranes are joined tightly together, forming a belt-like seal; these junctions limit the passage of small molecules and ions between cells.

Zinc-Finger Protein: A protein containing a small structural motif that is characterized by the coordination of one or more zinc ions in order to stabilize the fold.

interventions may work through epigenetic modulations. Supplementation with nutrients that act as methyl donors, including folic acid and choline, may modulate epigenetic profiles and alter the expression of genes important for neurodevelopment. Thus, prenatal folic acid supplementation attenuated ethanol-induced malformations, growth retardation, and neuronal loss (Wang et al. 2009), whereas prenatal and postnatal supplementation with choline reduced ethanol-induced malformations and behavioral impairment (Thomas et al. 2010). Furthermore, recent studies have shown that sulforaphane, a chemical that is abundant in broccoli sprouts and which can inhibit enzymes involved in epigenetic modifications (i.e., DNA methyltransferase and histone deacetylases), can diminish ethanol-induced apoptosis in neural crest cells through induction of nuclear factor (erythroid-derived 2)-like 2 (Nrf2) (Chen et al. 2013). These findings highlight the potential of nutrient supplementation in preventing or attenuating brain damage associated with FASD, improving cognitive

function in children with FASD, and attenuating brain damage in adults.

Immune Dysfunction

Excessive alcohol consumption has deleterious effects on the immune system. Several clinical and experimental studies have suggested that long-term alcohol use can lead to the dysregulation of both cell-mediated and humoral immunity (Barve et al. 2002). Epidemiologic studies have documented that alcohol-induced impairment of the immune system leads to increased susceptibility to opportunistic infections and development of certain tumors (Barve et al. 2002). Although many types of immune cells are affected by alcohol, including neutrophils, natural killer cells, and monocytes/macrophages, several observations suggest that the major effect of ethanol involves the impairment of thymus-derived lymphocytes (T lymphocytes or T cells). Because a subgroup of T-lymphocytes (i.e., CD4+ T cells) are the central regulators of the

immune system, including cell-mediated and humoral immunity, loss of their survival and function constitutes a critical part of alcohol-induced immune dysfunction.

A number of experimental animal models of ethanol abuse have established that chronic alcohol administration decreases the absolute numbers of CD4+ T cells in the thymus, spleen, lymph nodes, and periphery, as well as the immune function of these cells (Barve et al. 2002). Similarly, patients with AUD exhibit significantly reduced numbers of CD4+ T cells (Barve et al. 2002). Although other clinical complications in alcoholic patients can negatively influence the immune system, recovery of the CD4+ T-cell count was noted after alcohol withdrawal in several studies, suggesting that ethanol can directly affect CD4+ T-cell survival (Barve et al. 2002). Moreover, experimental and clinical studies have documented that alcohol intake can cause depletion of CD4+ T cells, and the mechanisms underlying this effect are only beginning to be understood. Research has indicated that ethanol

can potentially act as a cofactor and exacerbate clinical conditions that cause CD4+ T-cell depletion by enhancing activation-induced, fatty acid synthase-mediated apoptosis (Ghare et al. 2014). In addition to affecting CD4+ T-cell numbers, ethanol also has a major effect on T-cell function by decreasing the production of the cytokine, interleukin-2, which is critical for the clonal expansion of CD4+ T cells (Ghare et al. 2011).

In subjects with AUD, the combined effects of alcohol metabolism and compromised nutrition led to major nutrient disturbances, including deficiency of the critical nutrient metabolite, SAM. Studies found that levels of SAM as well as of methionine adenosyltransferase (MAT II), the enzyme that converts methionine to SAM, were markedly reduced in cultured CD4+ cells exposed to alcohol. This resulted in a significant upregulation of expression and activity of several enzymes involved in apoptosis, leading to increased apoptotic cell death (Hote et al. 2008). Moreover, restoration of intracellular SAM levels via SAM supplementation considerably attenuated this apoptotic death in T cells, implying a causal/protective role for SAM in T-cell survival (Hote et al. 2008).

Overall, these findings have begun to provide critical molecular insights into epigenetic mechanisms underlying the alcohol- and nutrient (SAM)-status-induced immunotoxicity in human CD4+ T cells. Because there currently is no Food and Drug Administration-approved therapy for the treatment of immune suppression associated with chronic alcohol abuse, these findings have the potential to facilitate the development of nutrient (SAM)-based therapy in alcoholic patients.

Conclusions

Alterations in nutrition and nutrient metabolism are common in chronic alcoholics and may contribute to alcohol-induced organ injury. Conversely,

nutritional supplementation may prevent the development or attenuate the progression of alcohol-induced organ injury. Nutritional supplements may alleviate a nutrient deficiency or act as pharmacologic agents. Such nutrients also may have epigenetic effects. Nutritional supplementation as a therapy is especially attractive because there are currently no Food and Drug Administration-approved therapies for most forms of alcohol-induced organ injury and nutrient supplements are readily available.

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The authors declare that they have no competing financial interests.

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Alcohol Misuse and Kidney Injury: Epidemiological Evidence and Potential Mechanisms

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Chronic alcohol consumption is a well-known risk factor for tissue injury. The link between alcohol use disorder (AUD) and kidney injury is intriguing but controversial, and the molecular mechanisms by which alcohol may damage the kidneys are poorly understood. Epidemiological studies attempting to link AUD and kidney disease are, to date, inconclusive, and there is little experimental evidence directly linking alcohol consumption to kidney injury. However, studies conducted primarily in other organs and tissues suggest several possible mechanisms by which alcohol may promote kidney dysfunction. One possible mechanism is oxidative stress resulting from increased production of reactive oxygen species, which leads to an excessive amount of free radicals, which in turn trigger tissue injury and increase inflammation. In addition, AUD's effect on other major organs (liver, heart, intestines, and skeletal muscle) appears to promote unfavorable pathological processes that are harmful to the kidneys. Notably, these mechanisms have not yet been validated experimentally in the kidney. Additional research is needed to clarify if alcohol does indeed promote kidney injury and the mechanisms by which alcohol-induced kidney injury may occur.

Key words: Alcoholic nephropathy; nephrotoxicity; acetaldehyde; proteinuria; glomerular filtration rate (GFR); glomerulonephritis; alcohol use disorder (AUD); kidney injury

Alcohol use disorder (AUD) is a substantial public health problem, affecting 15.7 million people age 12 and older in the United States (Center for Behavioral Health Statistics and Quality 2016). In 2012, 5.9 percent of all global deaths were attributable to alcohol—7.6 percent for men and 4.0 percent for women. Moreover, alcohol-attributable deaths have increased worldwide, making alcohol the fifth leading risk factor for premature death and disability in 2010 and the first among people ages 15 to 49 (World Health Organization 2014).

Among the major consequences of chronic AUD that contribute to alcohol-related morbidity and mortality are liver cirrhosis, liver cancer, pancreatitis, and cardiovascular complications. To date, the epidemiological evidence connecting AUD and an increased

incidence of chronic kidney disease is controversial. However, several preclinical studies suggest that alcohol consumption has a profound effect on the kidney and imply that there may be an independent pathologic entity, which we refer to here as “alcoholic kidney injury.”

Studies conducted primarily in other organs and tissues suggest several possible mechanisms by which alcohol may promote kidney dysfunction. In particular, alcoholic kidney injury may be associated with a complex interaction of ethanol-induced oxidative stress and pro-inflammatory alterations. This may be complicated by the interplay between the kidneys and other organs, including the liver, intestines, skeletal muscle, and cardiovascular system. This

brief synopsis reviews the evidence in support of these hypotheses.

Kidney Diseases and AUD: Lessons From Epidemiology

It is well established that cardiovascular diseases (including hypertension and ischemic heart disease) and diabetic microvascular complications are major risk factors for the development of chronic kidney diseases (Briasoulis et al. 2012; Carlsson et al. 2005; Reynolds et al. 2003; Ronksley et al. 2011). In turn, heavy alcohol consumption is implicated in the development of these cardiac diseases, with chronic, heavy drinkers at higher risk than those who consume small to moderate amounts of alcohol.

That said, epidemiological data have yet to confirm a relationship between alcohol consumption and chronic kidney disease. A recent meta-analysis (Cheungpasitporn et al. 2015) found little support for such a relationship. The researchers performed an extensive literature search using online databases (MEDLINE, EMBASE and Cochrane Databases) to identify studies investigating the association between high alcohol consumption and chronic kidney disease, end-stage renal disease, or proteinuria (i.e., excess protein in the urine that indicates kidney damage). Their analysis included 20 studies representing a total of 292,431 patients. The researchers reported that the pooled risk ratios of chronic kidney disease, proteinuria, and end-stage renal disease in patients with high alcohol consumption were 0.83, 0.85, and 1.00, respectively, indicating decreased risk or no risk of kidney disease in heavy alcohol consumers (Cheungpasitporn et al. 2015).

Other studies report similar findings, showing that the incidence of kidney disease is comparable or even lower in heavier drinkers (more than 210 g/week alcohol consumption) than in those who drink moderately (70–210 g/week alcohol consumption) (Buja et al. 2011; Knight et al. 2003; Koning et al. 2015; Reynolds et al. 2008; Sato et al. 2014; Yamagata et al. 2007). In contrast, some studies find that heavy alcohol consumption may predict poorer outcome in patients with chronic kidney diseases (Kronborg et al. 2008; Shankar et al. 2006; White et al. 2009). For example, White and colleagues (2009) reported that heavier drinkers (those consuming more than 30 g of alcohol/week) were at higher risk of incident albuminuria, which is typically a symptom of kidney disease. Japanese (Yamagata et al. 2007) and Italian (Buja et al. 2011) cohort studies revealed a U-shaped association between alcohol consumption and incidence of proteinuria. It is possible that the contradictory findings are the result of varying effects of different types of alcoholic beverages on the kidney,

or the result of different alcohol consumption patterns in different countries. In addition, the self-reporting nature of drinking behaviors and the amount of alcohol consumed may bias some of the conclusions as shown, for example, by Parekh and Klag (2001), who found that people who drink heavily underreport their alcohol consumption.

Potential Mechanisms of Alcoholic Kidney Injury: Lessons From Experimental Studies

If alcohol consumption does in fact influence kidney disease, the question remains: How? There is direct and indirect evidence for several possible mechanisms. These changes are caused either by alcohol itself or by excessive amounts of the products formed when cells break down (or metabolize) alcohol, including acetaldehyde, NADH, and free radicals. These alcohol-related pathophysiologic changes in cells have been linked to damage in many organs and may play a role in kidney damage. In addition, complex interactions between organs may further complicate and accentuate the development of kidney pathology in people with AUD (see figure).

Oxidative Stress

Free radicals (also called reactive oxygen species [ROS]) are one of the by-products of alcohol metabolism and are known to cause cellular damage, unless the body can use antioxidants to clean them up. Oxidative stress occurs when the body cannot detoxify free radicals as fast as they are being produced, and it is pivotal in triggering alcohol-related tissue injury. Studies suggest that several mechanisms produce ROS in alcohol-damaged organs, including the liver (Cederbaum et al. 2009), heart (Tan et al. 2012; Varga et al. 2015), and kidney (Latchoumycandane et al. 2015). The mechanisms producing ROS in organs include nonenzymatic mechanisms

such as mitochondrial electron transport chain malfunction (Gyamfi et al. 2012; Mantena et al. 2008) and enzymatic mechanisms that involve enzymes such as NADPH oxidases (Kono et al. 2000) and the enzyme CYP2E1 (Lu and Cederbaum 2008). CYP2E1 is of particular interest when thinking about potential mechanisms for alcohol-related kidney damage. The body mainly metabolizes alcohol using the enzyme alcohol dehydrogenase, which is expressed primarily in the liver. However, during chronic ethanol consumption, the body also uses CYP2E1 in the liver as well as the kidneys. Interestingly, studies find that CYP2E1 induction is much more robust in the kidneys compared with the liver (Roberts et al. 1994; Zerilli et al. 1995). This massive induction of CYP2E1 in the kidneys results in oxidative stress that modifies phospholipids in cell membranes. Such modified phospholipids may in turn activate immune cells called neutrophil granulocytes, which further aggravates oxidative stress, promoting a vicious cycle (Latchoumycandane et al. 2015).

Studies suggest that ethanol consumption may increase renal expression of other potential sources of free radicals involving a family of enzymes called nitric oxide synthases (Tirapelli et al. 2012). Nitric oxide synthase stimulates the production of nitric oxide, which, if produced excessively, can react with other molecules and create free radicals that trigger tissue damage in the kidneys (Pacher et al. 2007; Szalay et al. 2015). Tirapelli and colleagues (2012) showed that ethanol consumption increased the expression of two nitric oxide synthases. However, it is still unclear exactly how ethanol upregulates nitric oxide synthases, or whether it does so directly or indirectly. It may be that toxins released from the intestines into blood circulation because of ethanol's effects on the digestive system activate the expression of nitric oxide synthase. Another theory suggests that both enzymes may undergo the process of uncoupling due to oxidation or lack of critical coenzymes (e.g.,

tetrahydrobiopterin). Uncoupling eventually leads to generation of damaging ROS like superoxide anion, instead of the vasorelaxant nitric oxide that maintains normal blood flow in the kidney.

Alcohol-Metabolism Derived Intermediaries

Along with oxidative stress, increasing evidence suggests that some nonoxidative mechanisms also factor into alcohol-related organ damage. Specifically,

ethanol metabolism produces fatty acid ethyl esters in various organs (Laposata and Lange 1986), which can cause ethanol-induced organ damage. Calabrese and Rizza (1999) found that ethanol induced a significant increase in the levels of fatty acid ethyl esters. They measured the highest levels in the heart, followed by kidney, brain, and liver.

Due to the metabolism of ethanol, significant amounts of acetate are produced and subsequently incorporated into acetyl-coenzyme-A, a molecule

that participates in metabolism of proteins, lipids, and carbohydrates. This leads to the reprogramming of systemic metabolism. Protein acetylation—adding an acetyl group to a protein—is integral to regulating processes controlled by mitochondria, including fatty acid metabolism and antioxidant defense (Choudhary et al. 2014). Our current understanding is that the balance of lysine acetylation and deacetylation (the removal of an acetyl group) of key proteins (e.g., of the master regulator of mitochondrial biogenesis, PGC-1 alpha) serves, at least in part, to trigger a switch in metabolic status in conditions of over-nutrition or undernutrition (Bai et al. 2015; Ghanta et al. 2013; Jeninga et al. 2010). A recent study demonstrated that ethanol induces mitochondrial protein hyperacetylation (excessive modification by acetylation of the lysine residues of a protein) in the kidney, which might interfere with the function of some mitochondrial proteins involved in alcohol metabolism or defense against oxidative stress (e.g., superoxide dismutase 2, aldehyde dehydrogenase 2, glutathione peroxidase). This could also be a significant factor contributing to ethanol-induced mitochondrial dysfunction in the kidneys (Harris et al. 2015).

Alcohol-Induced Intestinal Damage

Alcohol-induced intestinal damage and increased mucosal translocation of bacterial endotoxin are crucial in the initiation and progression of alcoholic liver injury and in the pathogenesis of other alcohol-related diseases (Bala et al. 2014; Purohit et al. 2008). (For an in-depth discussion of alcohol and the digestive tract, see the article by Keshavarzian in this issue.) The direct role of alcohol-related endotoxin release in alcoholic kidney injury has not yet been studied. However, it is possible that activation of the innate immune system due to endotoxins released by a leaky gut plays a central role in the development of renal

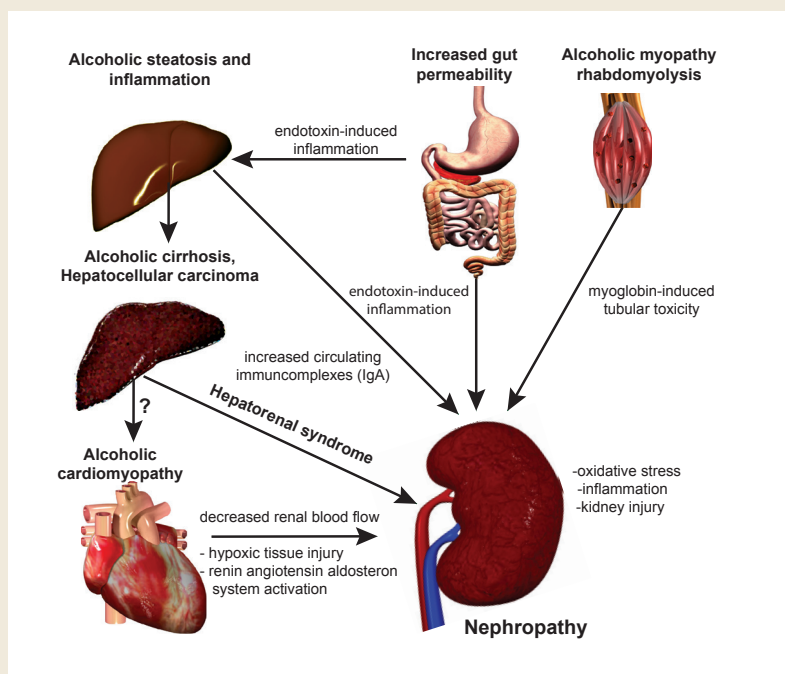


Figure Possible mechanism for alcohol-induced kidney injury. Chronic alcohol consumption induces profound injury in several organs that may affect and aggravate the deleterious effect of ethanol on the kidney. Ethanol itself markedly induces the expression of the microsomal ethanol oxidation system (CYP2E1), producing reactive oxygen species as a byproduct. Increased gastrointestinal permeability and endotoxin load may lead to alcoholic steatohepatitis resulting in excessive immunoglobulin A (IgA) load (due to increased intestinal production and decreased hepatic IgA clearance). IgA deposits may accumulate in the kidney, leading to glomerulopathy. Renal microcirculatory alterations in advanced liver cirrhosis leads to hepatorenal syndrome. Alcohol-induced skeletal muscle damage leads to excessive amounts of circulating myoglobin, causing renal tubular injury as a result of increased oxidative stress. Due to the development of alcoholic cardiomyopathy, chronic renal hypoxia develops, activating the renin-angiotensin-aldosterone system (RAAS), which in turn leads to further free radical production and to the propagation of fibrotic pathways.

damage, as it does for liver damage (Zhang et al. 2008).

Substantial experimental and clinical evidence suggests that increased intestinal permeability and endotoxin release caused by excessive alcohol consumption leads to higher levels of circulating immunoglobulin A (IgA), an antibody critical to the immune response of mucous membranes. The kidney is particularly sensitive to an increased IgA load. In fact, IgA glomerulonephritis—acute inflammation of the kidney caused by an IgA immune response—is one of the most common types of primary glomerulonephritis worldwide (D’Amico 1987). This IgA-related kidney disease leads to clinical symptoms of renal injury and eventually progresses into renal failure (Amore et al. 1994; Bene et al. 1988; Pouria and Feehally 1999). Experimental studies suggest that heavy alcohol consumption induces IgA kidney disease (Smith et al. 1990). In addition, rats given intragastric infusions of a commercial whiskey (1.5 ml/100 gm body weight) 3 times a week along with a nutrient-deficient diet develop a more severe form of IgA nephropathy (Amore et al. 1994).

Evidence also exists that alcohol-related damage to the liver, in particular advanced liver cirrhosis, leads to hepatorenal syndrome (HRS)—a deterioration in renal function related to impaired circulation. The underlying mechanisms involved in the development and progression of HRS are incompletely understood, although it is plausible that the altered balance between vasoconstrictor and vasodilator factors plays a significant role (Lenz 2005).

Alcoholic Skeletal Myopathy: A Potential Indirect Mechanism

Severe AUD is frequently associated with various acute or chronic muscle symptoms, including difficulties with gait, muscle cramps, pain, and overall reduced muscle mass. In fact, biochemical lesions in the muscles and the resulting myopathy develop

independently of any peripheral neuropathy, macro- and micronutrient malnutrition, and overt liver disease in people with AUD. In chronic alcoholic myopathy, a person’s entire muscle mass may be reduced by up to one-third. It is the most common skeletal muscle disorder in the industrialized world, present at varying severity in approximately half of alcohol misusers (Preedy et al. 2001). To date, studies have not examined whether there is a direct link between acute alcoholic myopathy and kidney injury. However, several lines of research suggest there might be a connection.

Although the mechanism of alcoholic myopathy is not fully understood, it is likely that disruption of mitochondria-related energy homeostasis is important in promoting muscle cell (myocyte) injury (Eisner et al. 2014). In rare cases in malnourished chronic alcoholics, acute alcoholic myopathy, also termed acute alcoholic necrotizing myopathy or alcoholic rhabdomyolysis, also may occur, which may lead to reversible or irreversible acute kidney injury (Haller and Knochel 1984; Hewitt and Winter 1995; Muthukumar et al. 1999; Sofat et al. 1999).

A few studies have linked rhabdomyolysis and myoglobin toxicity with acute kidney injury, supporting a possible association among alcohol use, alcohol-related acute myopathy, and kidney damage. For example, Belliere and colleagues (2015) showed a link between rhabdomyolysis and excessive macrophage infiltration in the kidney, which in turn led to pro-inflammatory marker expression and consequent tissue injury (Belliere et al. 2015). Another study by Plotnikov and colleagues (2009) showed that mitochondria isolated from rat kidneys were damaged by oxidative stress when incubated with myoglobin. This finding suggests that rhabdomyolysis and myoglobin toxicity may trigger oxidative stress in the kidney via mitochondrial injury.

Alcoholic Cardiomyopathy: Another Potential Confounder

Several epidemiological studies have shown that mild alcohol consumption benefits cardiovascular health (Coate 1993; Kannel and Ellison 1996) by reducing the risk of coronary heart disease (Mukamal et al. 2006). In contrast, heavy drinking leads to the development of nonischemic dilated cardiomyopathy (Klatsky 2007) and significantly increases the risk of sudden cardiac death (Hookana et al. 2011).

Chronic or acute heart failure can lead to chronic or acute dysfunction in the kidneys, known as cardiorenal syndrome (Cleland et al. 2012). The complex renal pathophysiological response leads to fluid buildup in tissues, ischemic injury, peripheral vasoconstriction, and activation of the hormone system that helps regulate blood flow (called the renin-angiotensin-aldosterone system, or RAAS) (Palazzuoli and Ronco 2011). The overactivation of RAAS further aggravates oxidative stress in chronic alcoholism (Ungvari et al. 2004). As a consequence, oxidative stress not only propagates kidney failure, but it also contributes to the progression of chronic heart failure (Pacher et al. 2005) and leads to a vicious cycle in alcohol-induced cardiovascular complications.

Conclusions

As noted above, there is much to learn about alcoholic kidney disease and the complex interplay among multiple organs affected by alcohol consumption. Although research suggests several potential mechanisms by which alcohol may directly or indirectly affect the kidneys, they have not yet been validated experimentally. Future research will hopefully explore these hypotheses to provide a better understanding of alcoholic kidney injury. This article highlights the effects of other organs on kidney and renal function; however, it should be noted that alcoholic kidney injury itself may have negative metabolic consequences. One such

complication is impaired vitamin D metabolism (Shankar et al. 2008), which may influence the function of several other organs, creating a vicious cycle.

The treatment of alcoholic kidney injury is still largely symptomatic, despite accumulating knowledge about underlying mechanisms. Both preclinical and human studies highlight the central role of oxidative stress and inflammation in triggering and driving the pathological processes associated with alcoholic kidney injury. Early diagnosis of this condition and rigorous abstinence from alcohol are very important for slowing down the progression of the disease and allowing the kidneys to regenerate.

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Alcohol and Puberty

Mechanisms of Delayed Development

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Adolescence represents a vulnerable period for developing youth. Alcohol use and misuse are especially problematic behaviors during this time. Adolescents are more sensitive to alcohol and less tolerant of its detrimental effects than are adults. Research in humans and animals has revealed that early alcohol consumption can result in delayed pubertal development. Animal studies have shown that alcohol detrimentally affects neuroendocrine systems within the hypothalamic region of the brain that are associated with the normal, timely onset of the pubertal process. To effectively restore development and shorten recovery time associated with the adverse effects of alcohol on puberty, researchers must first understand the molecular and physiological mechanisms by which alcohol interferes with critical hypothalamic functions.

Key words: Alcohol consumption; alcohol use and misuse; adverse effects; adolescence; puberty; development; brain; hypothalamus; hypothalamic function; neuroendocrine system

Despite efforts to prevent underage alcohol use, drinking does occur as early as the 6th grade. According to a recent national survey, 9.7 percent of 8th graders and 21.5 percent of 10th graders reported using alcohol at least once in the previous 30 days (Johnston et al. 2016). This is important because people who begin drinking between ages 11 and 14 are at increased risk for developing alcohol use disorder (DeWit et al. 2000), compared with those who begin drinking at later ages. These high-risk age groups also are exactly within the pubertal time frame. Some of the younger adolescents may not have begun the pubertal process. Others, however, are subject to the process being slowed or halted by alcohol, thus impeding further development. Following a brief summary of alcohol's effects on puberty in humans, this review describes the neuroendocrine processes that control puberty and research using animal models to assess the effects of prepubertal alcohol exposure.

Early research demonstrated that alcohol use by adolescent boys causes suppressed serum levels of growth hormone (GH), luteinizing hormone (LH), and testosterone (Diamond et al. 1986; Frias et al. 2000*a,b*), as well as lower bone den-

sity (Fehily et al. 1992; Neville et al. 2002). In adolescent girls, alcohol use caused suppressed serum GH and estradiol (E_2) levels (Block et al. 1993; Frias et al. 2000*b*). Other studies found evidence for disruptions in stature, weight distribution, and a risk for nutritional deficiencies (Block et al. 1991; Yamamoto et al. 1991). More recently, studies in girls have shown that prepubertal alcohol use was associated with delayed breast development (Peck et al. 2011) and onset of menarche (Richards et al. 2011). This research suggested that prepubertal girls who use alcohol have four times the chance of delayed onset of puberty than those who do not (Peck et al. 2011). This finding is confirmed in animal models, which show that alcohol acts within the hypothalamic region of the brain to suppress key puberty-related genes and hormones responsible for the normal timing of development.

Basic Neuroendocrine Control of Puberty

The onset of puberty results from a complex series of interactions between nerve cells (i.e., neurons) and glial cells (i.e., nonneuronal brain cells) within the hypothalamus that are governed by metabolic signals, as well as genetic and environmental influences. Although age at puberty varies widely between and among mammalian species, the main event that signals puberty onset is basically similar, in that it relies on the increased pulsatile secretory activity of a hypothalamic neuropeptide, luteinizing hormone–releasing hormone (LHRH). This event occurs through the enhanced developmental responsiveness of the LHRH-producing neurons and their nerve terminals to excitatory inputs, such as insulin-like growth factor-1 (IGF-1) (Hiney et al. 1996; Wilson 1998) and the kisspeptins (Kp), a family of neuropeptide products of the *KiSS-1* gene (Navarro et al. 2004; Shahab et al. 2005), as well as leptin (Dearth et al. 2000; Lebrethon et al. 2000), transforming growth factor α (Ojeda et al. 1990), and excitatory amino acids (Claypool et al. 2000; Gay and Plant 1987; Urbanski and Ojeda 1990).

In addition to the development of excitatory inputs, the timing of puberty is influenced by a concomitant and gradual removal of prepubertal inhibitory inputs, such as γ aminobutyric acid (GABA) and the opioid peptides β endorphin and dynorphin (Lehman et al. 2010; Navarro et al. 2009; Srivastava et al. 2015; Terasawa and Fernandez 2001).

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This alteration, often referred to as a “brake” on the pubertal process, is responsible for keeping prepubertal LHRH secretion low. As LHRH secretion increases, it drives the timing of puberty in both sexes by stimulating pituitary gonadotropin secretions, which in turn stimulate gonadal steroid synthesis and secretions for further maturation of the hypothalamus and reproductive organs. Although all of the excitatory and inhibitory influences noted above have been shown to be involved in the pubertal process, the mechanism-of-action portion of this review will concentrate on the most current findings about some of these modulators in relation to their upstream and downstream influences on the pubertal process.

Overall Effects of Alcohol on Puberty-Related Hormones and Indices of Pubertal Development

Initial studies using both female and male rodents revealed that chronic alcohol administration caused delayed puberty (Anderson et al. 1987; Bo et al. 1982; Ramaley 1982). Over the years, researchers have attempted to correlate the timing of puberty with specific puberty-related hormones following chronic prepubertal alcohol exposure. In female rats, alcohol caused delayed vaginal opening and the age at first estrus (Dees and Skelley 1990; Emanuele et al. 2002), as well as suppressed serum levels of GH and LH but not follicle-stimulating hormone (FSH) (Dees and Skelley 1990). In this regard, the differential effects of alcohol on LH and FSH were not surprising, because this previously had been shown in adult rats (Dees and Kozlowski 1984). Significantly, several studies have shown that prepubertal alcohol exposure in females caused suppressed circulating levels of E_2 (Bo et al. 1982; Dees and Skelley 1990; Emanuele et al. 2002), a clear indication of impaired ovarian development and activity. Although less is known about the prepubertal effects of alcohol in males, it has been shown to cause an early suppression in serum LH (Cicero et al. 1990) and to reduce the serum levels of GH and testosterone. Prepubertal alcohol use also can lead to lower testicular weight and smaller secondary sex organs (Anderson et al. 1987; Cicero et al. 1990; Emanuele et al. 1999; Tentler et al. 1997).

Additional research conducted in an animal model that more closely resembled humans, female rhesus monkeys, found that chronically administered alcohol resulted in suppressed GH, LH, and E_2 (Dees et al. 2000), exactly as described above in immature female rats. Furthermore, these actions were associated with the altered development of a regular monthly pattern of menstruation (Dees et al. 2000).

In addition to the effects of alcohol on GH and LH, research has shown that prepubertal alcohol administration caused suppressed serum IGF-1 in immature female rats (Emanuele et al. 2002; Srivastava et al. 1995) and rhesus monkeys (Dees et al. 2000), thereby reducing the amount of peptide available to the prepubertal hypothalamus. This is relevant because IGF-1 normally can act centrally to influence both the hypothalamic–pituitary–gonadal axis

and the hypothalamic–pituitary GH axis at puberty. Specifically, IGF-1 has been shown to act at the hypothalamic level to stimulate LHRH/LH secretion (Hiney et al. 1991, 1996) and advance the time of puberty in female rodents (Danilovich et al. 1999; Hiney et al. 1996). The ability of IGF-1 to regulate GH through its actions on hypothalamic growth hormone–releasing hormone and somatostatin (i.e., somatotropin release–inhibiting factor), the latter being a GH-release inhibitor, have been well documented (for review, see Bercu 1996).

It is important to note that the central control of these two hypothalamic systems is complex and interrelated, especially regarding the important integrative and bidirectional influences of IGF-1 on their respective neuro-secretions. Although a detailed discussion of these basic interrelationships is beyond the scope of this review, it also is worth noting that alcohol can affect both of these systems at multiple levels. For example, in addition to the aforementioned alcohol-related suppression of LHRH/LH resulting in suppressed serum E_2 , alcohol also causes altered hypothalamic growth hormone–releasing hormone synthesis and secretion (Dees et al. 1990). This then results in decreased pulsatile GH release (Dees et al. 1988), which in turn downregulates IGF-1 synthesis by liver hepatocytes (Srivastava et al. 2002). The resulting alcohol-induced suppression in circulating IGF-1 (Srivastava et al. 1995) causes suppressed body growth and interferes with the maturation and function of several organ systems. Furthermore, the accompanying reduction in circulating IGF-1 to feedback on the hypothalamus further reduces the secretion of LH and GH (for review, see Dees et al. 2009).

All of the above hormones are critical for puberty. However, alcohol’s suppression of the pituitary secretion of LH has become a primary focus of research on pubertal onset, because this gonadotropin is regulated by LHRH, the hypothalamic peptide responsible for beginning the pubertal process. Researchers now are examining whether the alcohol-induced effect to suppress LH is a result of a hypothalamic or pituitary site of action.

The Hypothalamic Site of Alcohol’s Actions

Studies in female rats, which showed increased hypothalamic LHRH content after chronic prepubertal alcohol administration (Dees et al. 1990), offered the first indirect evidence that alcohol affects this part of the brain. Subsequently, alcohol was shown to block the stimulatory effects of norepinephrine (Hiney and Dees 1991), IGF-1 (Hiney et al. 1998), leptin (Hiney et al. 1999), and *N*-methyl-DL-aspartic acid (NMA) (Nyberg et al. 1993) on the in vitro release of prepubertal LHRH. Although important, these collective observations did not rule out the possibility that alcohol also may act at the level of the pituitary.

To definitively assess the site of alcohol action, prepubertal rhesus monkeys that had been chronically exposed to alcohol were subjected to hypothalamic and

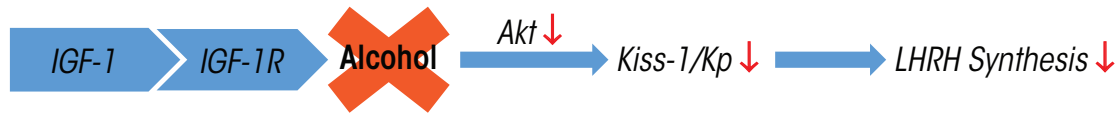


Figure 1 Alcohol blocks the ability of insulin-like growth factor-1 (IGF-1) to induce the *KiSS-1* gene and therefore suppresses production of kisspeptins (Kp), a family of neuropeptide products of *KiSS-1*, by inhibiting IGF-1 receptor (IGF-1R)-induced phosphorylation of Akt, a transduction signal that mediates the actions of IGF-1. Suppressed Kp production subsequently results in reduced synthesis of luteinizing hormone–releasing hormone (LHRH).

SOURCE: Hiney et al. 2010.

pituitary response tests (Disson et al. 2004). The hypothalamic stimulation test showed that the NMA-induced LH secretion observed in the non-alcohol-treated monkeys was blocked in the alcohol-treated monkeys. This is significant, because NMA causes LH release by first stimulating hypothalamic LHRH secretion and does not act at the pituitary level. Three weeks later, these same animals were given LHRH to test pituitary responsiveness. Results indicated that the LH response to the peptide was the same in both non-alcohol-treated and alcohol-treated monkeys, conclusively demonstrating the hypothalamic site of action.

Mechanisms of Action

Upstream Effects of Alcohol on LHRH Synthesis

The majority of LHRH-synthesizing neurons are localized within the brain preoptic area and the region just posterior to it referred to as the anterior hypothalamic area. This latter area also contains the anteroventral periventricular (AVPV) nucleus. Neurons in the AVPV nucleus produce kisspeptins, which regulate prepubertal LHRH synthesis and are critical for the onset of puberty (de Roux et al. 2003; Keen et al. 2008; Navarro et al. 2004; Shahab et al. 2005). Thus, research focused on discerning which factors affect prepubertal *KiSS-1* expression. Chronic prepubertal alcohol exposure was shown to cause suppressed *KiSS-1* gene expression in the AVPV nucleus of female rats, an action associated with a decrease in the usual level of phosphorylated Akt (Srivastava et al. 2009). Akt is a transduction signal that mediates the actions of IGF-1 (Cardona-Gomez et al. 2002), a peptide known to activate puberty in rats and rhesus monkeys (Hiney et al. 1996; Wilson 1998). Understanding IGF-1's ability to regulate *KiSS-1* was essential to further research. In studies with rats, an injection of IGF-1 directly into the brain's third ventricle caused the upregulation of prepubertal *KiSS-1* gene expression in the AVPV nucleus 6 hours later (Hiney et al. 2009). Subsequently, alcohol was shown to block the IGF-1 induction of *KiSS-1* in the

AVPV nucleus by inhibiting IGF-1 receptor (IGF-1R)-induced phosphorylation of Akt (Hiney et al. 2010). Figure 1 depicts this alcohol action, which leads to suppressed Kp and, subsequently, suppression of LHRH synthesis.

Further investigation will determine whether the suppressed Akt activity occurred directly at the level of Kp-containing neurons or through an interneuron or glial cell that also expresses the IGF-1R. However, the fact that alcohol can interfere with this pathway to LHRH synthesis is important, because once the onset of puberty begins, the synthesis of this peptide must keep pace with its release to drive the pubertal process.

Downstream Effects of Alcohol on LHRH Release

Alcohol is known to alter several downstream signals in the hypothalamus that collectively reduce LHRH release at puberty. Although the numerous excitatory substances mentioned above influence LHRH at puberty, the role of *KiSS-1* and Kp also are noteworthy. *KiSS-1* expression increases in the hypothalamus as puberty approaches (Navarro et al. 2004), and Kp is a potent stimulator of prepubertal LHRH secretion (Keen et al. 2008; Navarro et al. 2004). By suppressing prepubertal *KiSS-1*/Kp (Srivastava et al. 2009), alcohol contributes to decreased LHRH secretion at a time when increases are needed as puberty approaches. In addition, alcohol has been shown to stimulate the release of GABA and the opioid peptides (Lomniczi et al. 2000), which, as stated above, are known inhibitors of LHRH release. Alcohol also can activate the hypothalamic–pituitary–adrenal axis (Rivier 1996), and the hormones involved in the stimulation of this stress axis can suppress LH secretion (Kinsey-Jones et al. 2009; Li et al. 2015). Furthermore, the newly described gene *Lin28b* also is associated with the brake on puberty, and its expression has been shown to gradually decrease as puberty approaches (Sangiao-Alvarellos et al. 2013).

Recent research assessed whether alcohol would alter the normal pubertal rise in Kp and decrease in Lin28b protein. Chronic alcohol exposure reversed these actions within the brain region known as the medial basal hypothalamus

(MBH) in prepubertal female rats by suppressing Akt, *Kiss-1*, and Kp (Srivastava et al. 2009, 2015), while stimulating the synthesis of Lin28b (Srivastava et al. 2015). In addition, research showed that Lin28b induced dynorphin (DYN) synthesis and that alcohol stimulated DYN release (Srivastava et al. 2015). DYN inhibits Kp and LHRH secretion (Lehman et al. 2010; Navarro et al. 2009). Because the MBH contains neurons that coexpress Kp and DYN, these observations are relevant to the control of prepubertal LHRH secretion. Figure 2 illustrates the simultaneous and differential effects of alcohol on the excitatory Kp and inhibitory Lin28b pathways. Although LHRH neurons are not localized within the MBH of the rat, they are in primates, including humans. Therefore, both

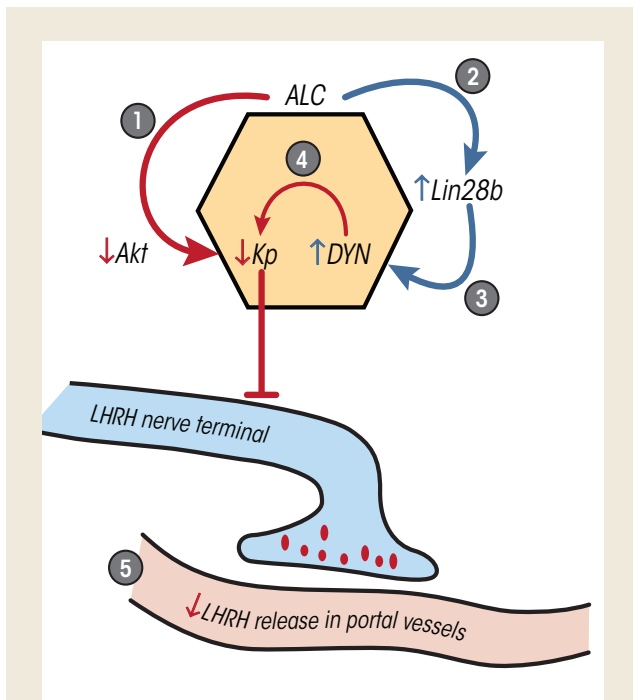


Figure 2 Schematic showing the effects of alcohol (ALC) on critical pathways within the hypothalamus that contribute to the control of luteinizing hormone-releasing hormone (LHRH) secretion. (1) Alcohol inhibits Akt, a transduction signal that mediates the actions of insulin-like growth factor-1 (IGF-1). This results in suppressed synthesis of kisspeptins (Kp), peptides that stimulate LHRH secretion. (2) Alcohol prevents the normal pubertal decline in the expression of Lin28b, a gene associated with the brake on puberty, by stimulating its synthesis. (3) Lin28b then stimulates synthesis of dynorphin (DYN), a peptide that inhibits Kp. (4) Alcohol stimulates the release of inhibitory DYN to suppress Kp. (5) The suppressed Kp ultimately results in decreased LHRH release. Red indicates suppression/inhibition; Blue indicates stimulation. For clarity, other factors contributing to LHRH release are not shown.

the release and synthesis of LHRH in the MBH of primates may be affected by alcohol.

In addition to alcohol's actions on neuronal inputs controlling prepubertal LHRH secretion discussed above, alcohol may affect neuronal-to-gial and glial-to-gial inputs facilitating LHRH release within the MBH. LHRH secretory activity can be modulated by a specific neuronal-gial gene family that synthesizes signaling proteins involved in bidirectional communications at puberty (Ojeda et al. 2010). Chronic prepubertal alcohol exposure decreases the synthesis of glial protein tyrosine phosphatase- β , which is required for binding to the neuronal components contactin and contactin-associated protein-1. This finding demonstrates that alcohol can alter these interactions and interfere with glial-neuronal communications (Srivastava et al. 2011a).

Glial-to-gial interactions also are affected by alcohol. Once released, glial-derived epidermal growth factor and transforming growth factor α (TGF α) both bind to the erbB1 receptor on adjacent glial cells and stimulate the release of prostaglandin E_2 (PGE $_2$) (Ma et al. 1997), a well-known stimulator of LHRH secretion. Alcohol exposure initially was shown to inhibit PGE $_2$ release induced by epidermal growth factor/TGF α (Hiney et al. 2003). In addition, glial-derived IGF-1 binds to IGF-1R on adjacent glial cells, which produce TGF α , and alcohol exposure altered the synthesis and release of TGF α (Srivastava et al. 2011b) and PGE $_2$ (Hiney et al. 1998; Srivastava et al. 2011b), thereby resulting in decreased prepubertal LHRH secretion. Furthermore, specialized glial cells within the MBH known as tanycytes release glial-derived TGF β 1, causing retraction of their processes and allowing for better entry of LHRH into the system of blood vessels that connect the hypothalamus with the pituitary (i.e., hypophyseal portal system) (Prevot et al. 2003). Alcohol blocks IGF-1 from stimulating the synthesis and release of TGF β 1 by altering the IGF-1R synthesis and Akt phosphorylation, therefore further contributing to diminished LHRH secretion (Hiney et al. 2014).

Conclusion

Alcohol use and misuse by adolescents increases the risk for altered neuro-endocrine function, potentially modifying the timing of pubertal development. This review highlights results of research with animal models showing the site and mechanisms by which alcohol causes puberty-related problems. These studies demonstrate that alcohol acts within the hypothalamus to alter the expression and function of excitatory and inhibitory puberty-related genes and neuro-hormones, which are critical for the timely increase in LHRH secretion and the onset of puberty. More research in this field is needed and would no doubt promote a better understanding of normal mechanisms controlling events leading to increased LHRH release at puberty, as well as the cause-and-effect relationships by which alcohol can differentially affect them.

Advancing knowledge in this area will allow researchers to begin to identify potential treatment substances that may lessen the impact and shorten the recovery time of adolescents who show signs of delayed development associated with alcohol use and misuse. It also is significant that delayed puberty is known to be associated with altered gonadal steroid production, which is needed for the development and function of several body systems. Furthermore, delayed pubertal development correlates with other health concerns such as altered bone density or height and weight issues, as well as psychological problems. Thus, the neuroendocrine consequences of alcohol use can result in far-reaching adolescent health concerns.

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Pathophysiology of the Effects of Alcohol Abuse on the Endocrine System

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Alcohol can permeate virtually every organ and tissue in the body, resulting in tissue injury and organ dysfunction. Considerable evidence indicates that alcohol abuse results in clinical abnormalities of one of the body's most important systems, the endocrine system. This system ensures proper communication between various organs, also interfacing with the immune and nervous systems, and is essential for maintaining a constant internal environment. The endocrine system includes the hypothalamic–pituitary–adrenal axis, the hypothalamic–pituitary–gonadal axis, the hypothalamic–pituitary–thyroid axis, the hypothalamic–pituitary–growth hormone/insulin-like growth factor-1 axis, and the hypothalamic–posterior pituitary axis, as well as other sources of hormones, such as the endocrine pancreas and endocrine adipose tissue. Alcohol abuse disrupts all of these systems and causes hormonal disturbances that may result in various disorders, such as stress intolerance, reproductive dysfunction, thyroid problems, immune abnormalities, and psychological and behavioral disorders. Studies in both humans and animal models have helped shed light on alcohol's effects on various components of the endocrine system and their consequences.

Key words: Alcohol consumption; alcohol use, abuse, and dependence; harmful effects of alcohol; pathophysiology; endocrine system; hypothalamus; pituitary gland; hormones; hormonal disturbances; endocrine pancreas; endocrine adipose tissue; immune system; humans; animal models

Alcohol abuse can result in clinical abnormalities of one of the body's most important systems, the endocrine system. Together with the nervous system, the endocrine system is essential for controlling the flow of information between the different organs and cells of the body. The nervous system is responsible for rapid transmission of information between different body regions, whereas the endocrine system, which is composed of a complex system of glands that produce and secrete hormones directly into the blood circulation, has longer-lasting actions. Together, the nervous system and the endocrine system ensure proper communication between various organs of the body to maintain a constant internal environment, also called homeostasis. Almost every organ and cell in

the body is affected by the endocrine system. Its hormones control metabolism and energy levels, electrolyte balance, growth and development, and reproduction. The endocrine system also is essential in enabling the body to respond to, and appropriately cope with, changes in the internal or external environments (e.g., changes in the body's temperature or in the electrolyte composition of the body's fluids) as well as to respond to stress and injury.

Both acute and chronic exposure to alcohol may have differential direct and indirect effects on endocrine functions. Alcohol intoxication induces hormonal disturbances that can disrupt the body's ability to maintain homeostasis and eventually can result in various disorders, such as cardiovascular diseases, reproductive deficits, immune

dysfunction, certain cancers, bone disease, and psychological and behavioral disorders. Alcohol use has been shown to affect many hormone systems, including the hypothalamic–pituitary–adrenal (HPA) axis, the hypothalamic–pituitary–gonadal (HPG) axis, the hypothalamic–pituitary–thyroid (HPT) axis, the hypothalamic–pituitary–growth hormone/insulin-like growth factor-1 (GH/IGF-1) axis, and the hypothalamic–posterior pituitary (HPP) axis. After a brief overview of the hormones of the hypothalamus and pituitary gland, this article discusses the adverse effects of both acute and chronic alcohol exposure on the different components of these hormone systems based on recent findings from human and animal studies. In addition, alcohol influences the release

and actions of the pituitary hormone prolactin (outlined in the sidebar “Alcohol and Prolactin”) as well as of hormones produced and released in other tissues, such as the endocrine pancreas and the adipose tissue (reviewed in the sidebar “Alcohol and Other Endocrine Tissues”).

Hormones of the Hypothalamus and Pituitary Gland

The hypothalamic–pituitary axis can be considered the coordinating center of the endocrine system. The hypothalamus is the main neural control center, also known as the “master switchboard,” which coordinates nervous and endocrine system functions. The hypothalamus consolidates inputs derived from higher brain centers, various environmental cues, and endocrine feedback. Neurons within the hypothalamus produce and secrete releasing hormones, such as corticotropin-releasing factor (CRF), luteinizing hormone–releasing hormone (LHRH), thyrotropin-releasing hormone (TRH), and growth hormone–releasing hormone (GRH), as well as inhibiting hormones, such as somatostatin and dopamine, directly into the blood vessel connecting the hypothalamus with the pituitary gland (i.e., the hypothalamic–hypophyseal portal vein). These hormones then control the synthesis and release of hormones in the pituitary gland. The pituitary gland comprises two sections—the adenohypophysis, or anterior lobe, and the neurohypophysis, or posterior lobe. In response to signals from the hypothalamus, the anterior pituitary produces and secretes trophic hormones, which are hormones that have a growth effect on the organs or tissues they are targeting. They include, among others, adrenocorticotropic hormone (ACTH), thyroid-stimulating hormone (TSH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin, and growth hormone (GH) and modulate the functions of several peripheral endocrine glands (i.e., adrenal glands, thyroid, and gonads)

and tissues (e.g., breast, muscle, liver, bone, and skin) (see the table).

The posterior or neurohypophyseal lobe of the pituitary contains the terminals of certain neurons (i.e., magnocellular vasopressin- and oxytocin-producing neurons) originating in two specific sections (i.e., the paraventricular nuclei [PVN] and supraoptic nuclei) of the hypothalamus. These neurons secrete primarily two hormones from the posterior pituitary into the systemic blood: arginine vasopressin (AVP), which controls the renal water handling and cardiovascular functions, and oxytocin, which regulates milk ejection during lactation and uterine contractions during birth. Evidence also indicates that both AVP and oxytocin act not only as hormones but also as neuromodulators and neurotransmitters within the central nervous system (de Wied et al. 1993; Stoop 2014). However, AVP and oxytocin also can be produced in another group of neurons in the PVN and supraoptic nuclei (i.e., in the parvocellular neurons) and released into the hypothalamic–hypophyseal portal vessels to reach the anterior pituitary. There, AVP acts synergistically with CRF to promote secretion of ACTH (Plotsky 1991). In contrast, oxytocin acts on specialized cells in the anterior pituitary to promote prolactin secretion (Sarkar and Gibbs 1984).

Alcohol and the HPA Axis

Normal Functioning of the HPA Axis

The HPA axis (figure 1) is one of the endocrine pathways most sensitive to the effects of alcohol abuse. This hormone system controls the stress-response pathways and regulates many of the body’s physiological processes, such as metabolic, cardiovascular, and immune functions. It integrates physical and psychosocial stimuli to allow the body to maintain homeostasis. In response to stress (i.e., psychological, physical, or infectious stressors) or

other homeostatic challenges, neurons in the PVN of the hypothalamus synthesize and secrete CRF and AVP. At the anterior pituitary, CRF binds to CRF1 receptors and stimulates specific cells (i.e., corticotropic cells) to synthesize and secrete a peptide called proopiomelanocortin (POMC). POMC can be cleaved into several smaller peptides, including ACTH; β -endorphin (BEP); and three similar peptides called α -, β -, and γ -melanocyte stimulating hormones. The POMC in the anterior pituitary primarily is processed into ACTH, whereas BEP mainly is derived from POMC produced in the hypothalamus (i.e., the ventromedial arcuate nucleus). At the same time, the AVP binds to V1b receptors, potentiating the effects of CRF on ACTH production in the anterior pituitary.

ACTH then is released into the systemic circulation, where it binds to specific receptors (i.e., melanocortin type 2 receptors) on cells in an area called the zona fasciculata in the outer layer (i.e., cortex) of the adrenal glands that are located on top of the kidneys. There, ACTH stimulates the production of glucocorticoid hormones—mainly cortisol in humans and corticosterone in rodents. These hormones then initiate a cascade of biological responses that help counteract the altered homeostatic state. Glucocorticoids achieve their effects by binding to widely distributed high-affinity mineralocorticoid receptors and low-affinity glucocorticoid receptors on their target cells. These receptors then translocate to the cell nucleus, where they bind to specific DNA sequences called glucocorticoid response elements of genes that are responsive to glucocorticoids, thereby positively or negatively regulating the expression of those genes.

The activity of the HPA axis is regulated through several feedback mechanisms. The principal protection against overactivation of the HPA axis involves the glucocorticoids (e.g., cortisol) through a negative feedback loop. Thus, glucocorticoids bind to mineralocorticoid (type 1) receptors and glucocorticoid (type 2) receptors in

Alcohol and Prolactin

Prolactin, also known as luteotropin, is a polypeptide hormone produced and secreted by specialized cells in the anterior pituitary called lactotropes. As the name indicates, prolactin is involved in the maintenance of lactation by the mammary glands. However, prolactin also has been implicated in a plethora of other biological functions or responses, such as mammary-gland development; reproduction; immune functions; and behavioral functions, including learning, memory, and adaptation. Prolactin is regulated by numerous mechanisms, including both inhibitory and stimulatory signals from the hypothalamus. The main hypothalamic factor responsible for inhibition of prolactin release is dopamine. Thus, prolactin secretion is controlled by a short-loop inhibitory feedback effect, whereby elevated prolactin levels in the circulation stimulate the hypothalamus to release dopamine, which then acts on the pituitary to stop further prolactin release. Dopamine also can block prolactin release directly at the level of lactotropes. In addition to dopamine, γ -aminobutyric acid released by hypothalamic neurons inhibits prolactin release. Conversely, several hypothalamic factors stimulate prolactin release from the anterior pituitary, including thyrotropin-releasing hormone, vasoactive intestinal peptide, oxytocin, β -endorphin, neurotensin, substance P, serotonin, and prostaglandins.

Several reports have indicated that chronic alcohol use can cause excessive levels of prolactin in the blood (i.e., hyperprolactinemia) in both men and women. For example, persistent hyperprolactinemia was observed in women with alcohol use disorder (AUD) and no clinical evidence of alcoholic liver cirrhosis who reported

an average daily alcohol intake of 170 g (i.e., approximately 12 standard drinks) for 2 to 16 years (Valimaki et al. 1984). Elevated prolactin levels also were reported in women with AUD and admitted for alcoholism treatment who reported drinking an average of 84 g of alcohol (i.e., approximately 7 standard drinks) per day for at least 7 years (Seki et al. 1991). Alcohol-induced hyperprolactinemia also was evident in postmenopausal women (Gavaler 1994) and in men with AUD (Soyka et al. 1991).

Studies in nonhuman primates and laboratory animals have confirmed an alcohol-induced hyperprolactinemia. For example, acute ethanol administration increased serum prolactin levels in male (Seilicovich et al. 1985) and female (Dees and Kozlowski 1984) rats. Similarly, chronic self-administration of alcohol (3.4 g/kg/day) in female monkeys was associated with an increase in plasma prolactin levels (Mello et al. 1988) as well as apparent enlargement (i.e., hyperplasia) of the pituitary as demonstrated by immunocytochemical examination (Mello et al. 1983). Ethanol also increased plasma prolactin levels and pituitary weight both in female rats with normal menstrual cycles and in rats whose ovaries had been removed (i.e., ovariectomized rats) and promoted estradiol-induced development of prolactin-producing benign tumors (i.e., prolactinomas) in the pituitary (De et al. 1995). Finally, ethanol increased basal and estradiol-mediated proliferation of lactotropic cells in primary cultures of mixed anterior pituitary cells, but failed to do so in cultures of only lactotropic cells, indicating that ethanol's effects on proliferation require cell-to-cell

communication between lactotropic and other pituitary cells (De et al. 2002).

The inhibitory action of hypothalamic dopamine on pituitary prolactin secretion is mediated by the dopamine G-protein-coupled D2 receptors (D2R), which interact with regulatory molecules called G-proteins and specifically a subtype called adenylyl-cyclase-inhibitory Gi/Go (Ben-Jonathan et al. 2001; Sarkar 2010). There are two isoforms of the D2R, a long (D2L) and a short (D2S) isoform.¹ Chronic exposure to ethanol increases the expression of prolactin mRNA and of D2L mRNA but decreases expression of D2S both in the pituitary of Fischer-344 rats and in primary cultures of anterior pituitary cells (Oomizu et al. 2003). In addition, exposure of ovariectomized rats to ethanol for 2 to 4 weeks reduced the expression of two other G-proteins, Gi2 and Gi3 (Chaturvedi and Sarkar 2008). Similar results were found in experiments using various cell culture models (Sengupta and Sarkar 2012).

Finally, ethanol treatment had differential effects on various G-proteins in cells expressing only D2S or D2L, eliciting a marked increase in Gs expression and a decrease in Gi3 expression in D2S cells but a moderate increase in Gs and marked increase in Gi3 expression in D2L (Sengupta and Sarkar 2012). Taken together these studies indicate that ethanol diminishes dopamine's ability to inhibit prolactin secretion by altering the processing (i.e., splicing) of D2R mRNA, promoting the increase of the D2L isoform, as well as by differentially altering the expression of various Gi and Gs proteins in lactotropic cells.

¹ The D2S isoform results from an exclusion of the sixth exon of the D2R gene in the mature transcript.

Alcohol and Prolactin (continued)

Ethanol exposure affects prolactin production not only in adults but also in the developing fetus. Fetal alcohol exposure from day 7 to day 21 of gestation increased pituitary weight, pituitary prolactin mRNA and protein content, and prolactin plasma levels in female rats compared with control animals (Gangisetty et al. 2015). These changes are associated with decreased D2R mRNA and protein. This decrease seems to be related to reduced activity of the gene resulting from epigenetic modifications of the D2R gene. Thus, fetal ethanol exposure increased methylation of a regulatory element (i.e., the promoter) of the D2R gene, thereby reducing transcription. In addition, ethanol exposure increased the mRNA levels for several methylating enzymes and enzymes called histone deacetylases that modify the proteins (i.e., histones) around which the DNA is wound, which also interfere with transcription (Gangisetty et al. 2015). The role of these processes in ethanol-induced modifications of prolactin levels was confirmed by the finding that treatment with agents that prevent DNA methylation and/or histone deacetylase activity normalized D2R mRNA expression, pituitary weight, and plasma prolactin levels in fetal alcohol-exposed rats (Gangisetty et al. 2015).

Ethanol affects prolactin levels not only through its impact on D2R but also through changes in the production and secretion of growth factors in the pituitary that help control lactotropic cell proliferation. Specifically, ethanol exposure of ovariectomized rats for 2 to 4 weeks decreased the levels of growth-inhibitory molecules (e.g., transforming growth factor beta-1 [TGF β -1]) and increased the levels of growth-stimulatory factors,

such as TGF β -3 and basic fibroblast growth factor, in the pituitary gland; similar results were found in isolated cell cultures enriched for lactotropes and exposed to ethanol for 24 hours (Sarkar and Boyadjieva 2007).

These and other studies (Gavaler 1994; Mello et al. 1989; Seki et al. 1991; Valimaki et al. 1984) clearly have demonstrated that chronic alcohol consumption is a positive risk factor for the development of prolactinomas and hyperprolactinemia. Common manifestations of hyperprolactinemia in women include lack of menstrual cycles (i.e., amenorrhea) and excessive or spontaneous secretion of milk (i.e., galactorrhea). Men with hyperprolactinemia typically show hypogonadism, with decreased sex drive, reduced sperm production, and impotence, and may also exhibit breast enlargement (i.e., gynecomastia), although they very rarely produce milk.

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the hypothalamus, hippocampus, and pituitary. This binding decreases CRF, AVP, and ACTH production (figure 1). An additional negative feedback mechanism involves the BEP produced from POMC, which is synthesized in the ventromedial arcuate nucleus of the hypothalamus after stress activation. CRF release by cells from the PVN of the hypothalamus activates this BEP synthesis and release, which then inhibits further CRF release, creating a negative feedback cycle (Plotsky et al. 1991). These feedback processes help to maintain the cortisol concentration within a narrow physiological window and

switch off the stress response (Myers et al. 2012; Wynne and Sarkar 2013).

A second component of the stress response is the fight-or-flight response of the sympathetic nervous system, which acts as the first line of defense against stressors. In a stressful situation, a brain region called the amygdala sends out a stress signal to the hypothalamus, which induces the activation of the sympathetic nervous system and the release of the neurotransmitter acetylcholine from preganglionic sympathetic nerves. Acetylcholine, in turn, stimulates the release of the catecholamine hormones epinephrine and norepinephrine from the inner layer

(i.e., medulla) of the adrenal gland.¹ These hormones facilitate an immediate reaction by triggering physiological changes, such as increased heart rate and respiration, and provide the body with a burst of energy through the release of sugar (i.e., glucose) and fat into the bloodstream as energy sources that help the body to respond to the stressors and fight off the threat. This part of the stress response also is regulated by BEP produced from POMC in the hypothalamus, which not only

¹ Norepinephrine also is released from postganglionic neurons of the sympathetic nervous system.

Table Summary of Important Hormones, Their Sites of Production, the Hormone System They Belong to, and Their Main Functions or Target Organs

Site of Production	Hormone	Hormone System	Main Function or Target Organ
Hypothalamus	Corticotropin-releasing factor	Hypothalamic–pituitary–adrenal axis	Anterior pituitary gland
	Luteinizing hormone–releasing hormone	Hypothalamic–pituitary–gonadal axis	Anterior pituitary gland
	Thyrotropin-releasing hormone	Hypothalamic–pituitary–thyroid axis	Anterior pituitary gland
	Growth hormone–releasing hormone	Growth hormone/insulin-like growth factor-1	Anterior pituitary gland
	Somatostatin	Growth hormone/insulin-like growth factor-1, Hypothalamic–pituitary–thyroid axis	Anterior pituitary gland
	Dopamine	Prolactin	Anterior pituitary gland
Anterior Pituitary Gland	Adrenocorticotropic hormone	Hypothalamic–pituitary–adrenal axis	Adrenal cortex
	Thyroid-stimulating hormone	Hypothalamic–pituitary–thyroid axis	Thyroid
	Follicle-stimulating hormone	Hypothalamic–pituitary–gonadal axis	Gonads (ovaries, testes)
	Luteinizing hormone	Hypothalamic–pituitary–gonadal axis	Gonads (ovaries, testes)
	Growth hormone	Growth hormone/insulin-like growth factor-1	Growth and repair of all cells
	Prolactin	Prolactin	Breast
Hypothalamus/ Posterior Pituitary Gland	Arginine vasopressin	Hypothalamic–pituitary–adrenal axis	Blood vessels and kidney
	Oxytocin	Oxytocin	Uterus, mammary glands, male reproductive organs
Adrenal Glands	Glucocorticoids (cortisol, corticosterone)	Hypothalamic–pituitary–adrenal axis	Body stress, metabolism, glucose maintenance
Ovary (Follicle)	Estrogen (estrone, estradiol, estriol)	Hypothalamic–pituitary–gonadal axis	Female reproductive glands and tissues, bones, heart
Ovary (Corpus Luteum)	Progesterone	Hypothalamic–pituitary–gonadal axis	Maintenance of pregnancy and preparation of breast tissue
Testes	Testosterone	Hypothalamic–pituitary–gonadal axis	Masculinity, sperm production, bone
Thyroid	Thyroxine (T4) Triiodothyronine (T3)	Hypothalamic–pituitary–thyroid axis	Heart rate, temperature, metabolism
Pancreas	Insulin	Pancreas	Lower blood sugar
	Glucagon	Pancreas	Increase blood sugar

modulates CRH release but also can help decrease the stress response and return the body to a state of homeostasis.² BEP binds with high specificity to different receptors (i.e., μ - and δ -opioid receptors), thereby inhibiting the sympathetic nervous system response to stress. BEP produced from pituitary POMC in response to hypothalamic CRF and AVP, in contrast, circulates in the periphery and has less impact on sympathetic nervous system function (Wynne and Sarkar 2013).

Alcohol's Effects on the HPA Axis

Considerable lines of evidence indicate that alcohol consumption affects the stress-response pathways and the HPA axis. Acute exposure to alcohol activates the HPA axis, leading to a dose-related increase in circulating ACTH and glucocorticoids and inducing anxiolytic-like responses (Richardson et al. 2008; Varlinskaya and Spear 2006). Jenkins and Connolly (1968) showed that plasma cortisol levels significantly increased in healthy subjects at alcohol doses exceeding 100 mg/dL. Similarly, healthy men who were in the top percentile of self-reported alcohol consumption had higher levels of excreted cortisol in urine (Thayer et al. 2006). In addition, these researchers reported that the inhibitory control of the HPA axis was impaired in heavy drinkers. Finally, people with a family history of alcohol use disorder (AUD) exhibited hyperresponsiveness of the stress response mediated by the HPA axis (Uhart et al. 2006; Zimmermann et al. 2004).

Similar findings were obtained in animal studies, where acute ethanol administration to rats increased plasma ACTH and corticosterone levels by enhancing CRF release from the hypothalamus (Rasmussen et al. 2000; Rivier and Lee 1996). Neutralization of circulating CRF using specific antibodies inhibited ethanol's stimulatory actions on ACTH and corticosterone secretion (Rivier and Lee 1996).

Additional studies of chronic alcohol administration found an association between HPA axis response and level of alcohol consumption (Richardson et al. 2008). In these analyses, the HPA response after several weeks of daily 30-minute self-administration of alcohol was highest in the animals with the lowest level of consumption (<0.2 mg/kg/session) and most blunted in animals with the highest level of consumption (~1.0 mg/kg/session). Furthermore, chronic alcohol exposure was associated with anxiety-producing-like (i.e., anxiogenic-like) behaviors (King et al. 2006). These studies clearly indicate that chronic exposure to alcohol attenuates basal ACTH and corticosterone levels and increases anxiogenic-like behaviors.

Various mechanisms have been proposed for the blunted HPA axis responsiveness to chronic alcohol consumption. Several of these focus on the relationship between alcohol and CRF expression:

- Alcohol dependence has been shown to be associated with a decrease in CRF mRNA expression (Richardson et al. 2008) as well as reduced responsiveness of the pituitary to CRF (Sarnyai et al. 2001).
- Animal studies using mice that produced no CRF (i.e., CRF knockout mice) found that when the animals were exposed to ethanol (in a continuous- or a limited-access paradigm), they consumed twice as much ethanol as their counterparts with a functional CRF gene. In addition, the knockout mice exhibited a reduced sensitivity to the locomotor-stimulant and rewarding effects of ethanol (Olive et al. 2003).
- Mice lacking a functional CRF1 receptor progressively increased their ethanol intake when subjected to repeated stress; this effect seemed to persist throughout their life (Sillaber et al. 2002).

Numerous studies have suggested that genetically determined differences in

the HPA axis stress response, glucocorticoid signaling, and the BEP and opioid system also may be involved in the predisposition for, as well as development and progression of, AUD. However, a discussion of this evidence and the proposed mechanisms is beyond the scope of this article.

The HPA Axis, Alcohol, and the Immune System

AUDs often are associated with chronic systemic inflammation and high levels of circulating proinflammatory cytokines. Alcohol may induce inflammation through both direct and indirect mechanisms. For example, alcohol metabolism results in the production of reactive oxygen species (ROS) and cell damage that can trigger the production of proinflammatory cytokines (Haorah et al. 2008). Alcohol also may damage the bacterial flora in the gut as well as the intestinal walls, leading to the release and transfer into the blood of bacterial lipopolysaccharides, which play a key role in alcohol-mediated inflammation (Purohit et al. 2008; Wang et al. 2010). A bidirectional interaction between the HPA axis and the immune system also may contribute to alcohol-induced inflammatory reactions. Thus, by binding to their receptors, glucocorticoids can interfere with certain signaling pathways that repress transcription of many inflammatory proteins (Barnes 2006).

In addition, CRF and ACTH have immuno-potentiating and proinflammatory properties (figure 1) (Besedovsky and del Rey 1996). Conversely, interleukins (ILs) and cytokines produced by activated immune cells (i.e., macrophages) can act on the HPA axis and induce CRF and ACTH secretion in an adaptive feedback mechanism (Bateman et al. 1989; Blalock and Costa 1989). This bidirectional interaction between the HPA axis and immune function is essential for survival and for maintaining the body's homeostasis. However, excessive alcohol exposure compromises HPA axis and immune functions by altering cytokine

² Note that BEP also acts as an endogenous opioid peptide with pain-relieving (i.e., antinociceptive) effects.

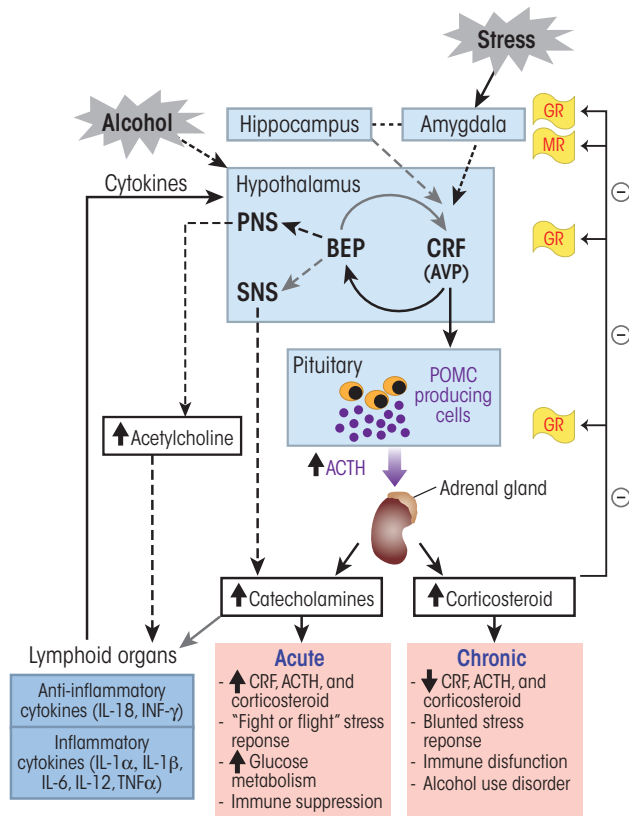


Figure 1 Alcohol's effects on the hypothalamic–pituitary–adrenal (HPA) axis and the stress response. Alcohol can stimulate neurons in the paraventricular nucleus (PVN) of the hypothalamus to release corticotropin-releasing factor (CRF) and arginine vasopressin (AVP). Stress sensed in the amygdala also elicits a similar activation of this stress response pathway. In the anterior pituitary, CRF stimulates the production of proopiomelanocortin (POMC), which serves as the prohormone for adrenocorticotropic hormone (ACTH). AVP potentiates the effects of CRF on ACTH release from the anterior pituitary. ACTH stimulates cells of the cortical portion of adrenal glands to produce and release glucocorticoid hormones (i.e., cortisol). High levels of glucocorticoids inhibit CRF and ACTH release through a negative feedback by binding to glucocorticoid receptors (GRs) and mineralocorticoid receptors (MRs) in various brain regions. Neurons in the arcuate nucleus of the hypothalamus release β-endorphin (BEP), which also regulates CRF release. BEP also acts on the autonomous nervous system and inhibits the sympathetic nervous system (SNS) stress response. CRF, ACTH, and glucocorticoids also act on different organs of the immune system and stimulate cytokine production and release into the general circulation. These cytokines then reach the brain where they trigger a neuroimmune response that sensitizes the stress-response pathway. Acute exposure to alcohol stimulates the HPA-axis stress response and induces suppression of cytokine production. In contrast, chronic exposure to alcohol induces a blunted HPA-axis stress response characterized by an absence of negative feedback control of this pathway and an increase in proinflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF α), leading to stress intolerance, immune dysfunction and alcohol use disorder.

levels in a variety of tissues, including the brain, with the specific effect on cytokine production depending on the length of exposure. For example, acute exposure to ethanol is associated with suppressed production of certain cytokines (e.g., tumor necrosis factor alpha [TNF α] and IL-1 β) (Pruett et al. 2004), whereas chronic exposure induces an increase in the production of proinflammatory cytokines, such as TNF α (Mandrekar et al. 2009; Nagy 2004). The increase in innate immune signaling molecules in the brain associated with chronic alcohol consumption can affect cognitive function and promote alcohol use behaviors.

It has been speculated that dysregulations of HPA axis function caused by chronic alcohol exposure mediates these effects on the immune system (figure 1). Several studies clearly have demonstrated that ethanol exposure during the developmental period induced neurotoxicity and permanent impairments in the HPA axis that were associated with immune dysfunction (Hellemans et al. 2010; Kuhn and Sarkar 2008; Sarkar et al. 2007). Macrophages residing in the brain (i.e., microglia) play an important role in these neurotoxic effects of alcohol (Boydjjeva and Sarkar 2010; Fernandez-Lizarbe et al. 2009).

Alcohol and the HPG Axis

Normal Functioning of the HPG Axis

Reproductive function is regulated by a cascade of events that are under the control of the HPG axis. The hypothalamus produces and secretes LHRH, also called gonadotropin-releasing hormone, into the hypothalamic–pituitary portal network. At the anterior pituitary, LHRH stimulates the production and secretion of FSH and LH from gonadotropic cells into the general circulation. These gonadotropins regulate the development of follicles (i.e., folliculogenesis) in females and of sperm (i.e., spermatogenesis) in males. Moreover, each month during the

follicular phase of the menstrual cycle, FSH stimulates the development of a dominant follicle in the ovary, which then produces and secretes the hormone estradiol. The rise in estradiol through a feedback mechanism is responsible for the surge in LH and FSH levels that occurs in the middle of the menstrual cycle. LH then induces ovulation and the development of the corpus luteum, which in turn produces and secretes progesterone, an important hormone that helps maintain pregnancy. In the testes, in contrast, LH stimulates testosterone production and release, whereas FSH controls spermatogenesis. HPG axis function is controlled through feedback mechanisms, where testosterone, estrogen, and progesterone control their own production by acting on the hypothalamus and anterior pituitary to inhibit or stimulate the release of LHRH, LH, and FSH (Sarkar 1983).

Alcohol's Effects on the HPG Axis

Numerous studies have documented alcohol's diverse deleterious effects on the HPG axis and its hormones (figure 2). The resulting HPG dysfunction observed in people with AUD can be associated with diverse outcomes, including a decreased libido, infertility, and gonadal atrophy. It also is important to note that these deleterious effects are not limited to adult drinkers but may also affect adolescents in puberty who begin to consume alcohol. For more information, see the sidebar "Alcohol's Effects on the Hypothalamic–Pituitary–Gonadal Axis During Puberty."

In women, alcohol use can cause a multitude of reproductive disorders, such as irregular menstrual cycles, absence of ovulation (i.e., anovulation), increased risk of spontaneous abortions, and early menopause. Alcohol intake, even as little as five drinks per week, was associated with decreased fecundability in healthy women ages 20–35 (Jensen et al. 1998). Other studies (Mendelson et al. 1988) found that 50 percent of social (i.e., about

3.84 drinks per day) and 60 percent of heavy (i.e., about 7.81 drinks per day) healthy, nondependent drinkers exhibited significant disturbances of their reproductive hormones and menstrual cycle compared with occasional drinkers (i.e., about 1.22 drinks per day). In addition, social drinkers had anovulatory cycles, and 3 of 5 heavy

drinkers exhibited excessive levels of prolactin in the blood (i.e., hyperprolactinemia) (Mendelson et al. 1988). Studies have shown that alcohol intake consistently induces an increase in estradiol levels in humans (Mendelson and Mello 1988; Muti et al. 1998) and rodents (Emanuele et al. 2001a), possibly as a result of decreased steroid

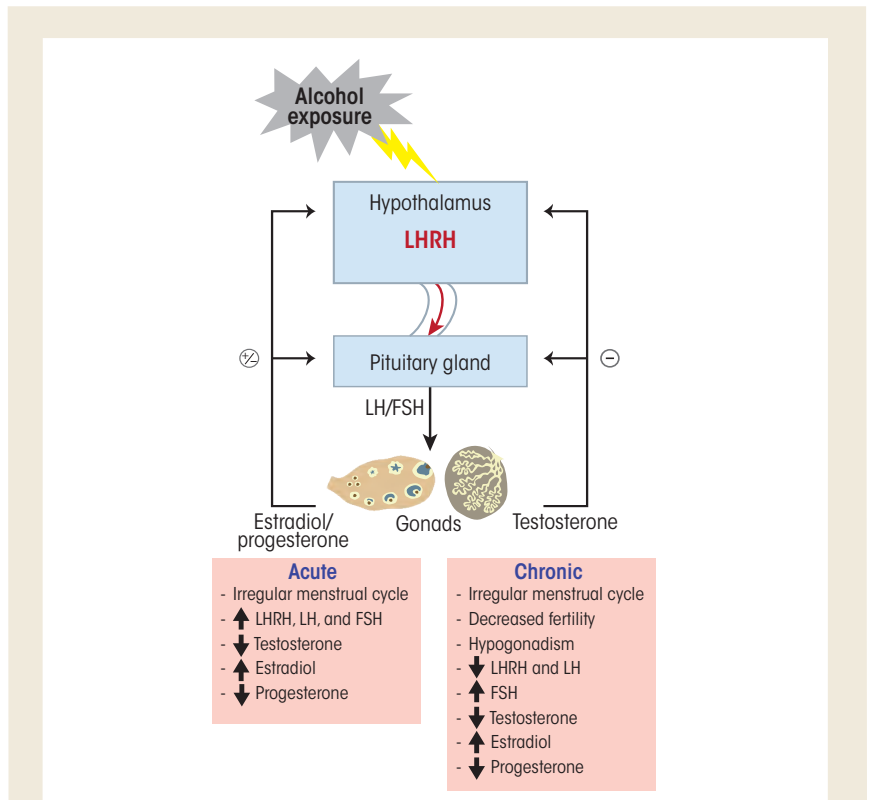


Figure 2 Alcohol's effects on the hypothalamic–pituitary–gonadal (HPG) axis. Neurons in the hypothalamus release luteinizing hormone–releasing hormone (LHRH) to the hypophyseal-portal blood system. LHRH then stimulates the secretion of gonadotropins (i.e., LH and FSH). During the ovary's follicular phase, FSH stimulates the development of a dominant follicle, which produces and secretes estradiol. Estradiol then stimulates an LH and FSH surge during midcycle of the menstrual cycle. LH stimulates ovulation and the development of the corpus luteum, which then produces and secretes progesterone. In the testis, LH stimulates testosterone production and release, while FSH controls spermatogenesis. HPG axis function is controlled through feedback loop mechanisms. Testosterone inhibits LHRH, LH, and FSH secretion through negative feedback, whereas estradiol and progesterone both can have negative- and positive-feedback actions, depending on the stage of the ovarian cycle, and can inhibit or stimulate the release of LHRH, LH, and FSH. Acute alcohol exposure results in increased LHRH, LH, FSH, and decreased testosterone and progesterone. Chronic alcohol exposure, in contrast, induces a decrease in LHRH, LH, testosterone, and progesterone and an increase in estradiol and FSH. These alcohol-induced hormonal dysregulations cause a multitude of reproductive disorders, such as menstrual cycle irregularity, decreased fertility, and hypogonadism.

catabolism (Sarkola et al. 1999). These increased estradiol levels could in part explain alcohol's negative effects on menstrual cycle regularity. Moreover, chronic alcohol has inhibitory actions on LHRH-producing neurons. Thus, exposure to 100 mM ethanol directly inhibited LHRH release from incubated medial basal hypothalamic sections, and this effect was reversed by naltrexone (Lomniczi et al. 2000). These results suggest that alcohol's effect on LHRH release involves the stimulation of BEP-releasing neurons, which prevent LHRH release by inhibiting nitric oxide synthase. Other studies have shown that long-term moderate alcohol consumption can decrease the number and quality of a woman's oocytes (i.e., ovarian reserve), which was associated with increased FSH levels (Li et al. 2013).

Extensive research in animals and humans also has documented the deleterious effects of alcohol on male reproductive function, including reduced testosterone levels (figure 2). Acute alcohol intake decreased the circulating levels of LH and testosterone as a result of diminished release of hypothalamic LHRH (Cicero et al. 1982; Dees et al. 1983; Rowe et al. 1974). In contrast, chronic alcohol consumption significantly increased FSH, LH, and estrogen levels but decreased testosterone and progesterone levels in men with AUD compared with men without AUD (Muthusami and Chinnaswamy 2005). The AUD group also had significantly lower semen volume, sperm count, motility, and number of morphologically normal sperm (Muthusami and Chinnaswamy 2005). Several mechanisms may contribute to alcohol's effects on the various hormones involved in the male HPG axis:

- The activity of the enzyme aromatase, which converts androgens to estrogens, especially in the liver, is increased by ethanol (Purohit 2000). This mechanism may explain why alcohol abuse results in hypo-

gonadism even in the absence of liver disease.

- In men with AUD and cirrhosis, a decrease in IGF-1 bioavailability as a result of liver disease contributes at least in part to the elevated circulating levels of estradiol and estrone (Martinez-Riera et al. 1995) and the development of hypogonadism (Castilla-Cortazar et al. 2000) since IGF-1 can stimulate testosterone synthesis and spermatogenesis (Roser 2008).
- ROS produced during alcohol metabolism may cause cell damage in the testes (Emanuele et al. 2001*b*). The testicular alcohol-inducible cytochrome P450 2E1, which is involved in the generation of ROS as well as hydroxyl ethyl free radicals, was shown to be elevated in testes of rats chronically exposed to ethanol (Shayakhmetova et al. 2013).
- The alcohol metabolite acetaldehyde can disrupt testosterone production by inhibiting protein kinase C, a key enzyme in testosterone synthesis (Chiao and Van Thiel 1983).
- Nitric oxide, which is synthesized in the testes by nitric oxide synthase, is another proposed player in the alcohol-induced reduction of testosterone production. Inhibition of nitric oxide synthase prevents the alcohol-induced decrease in testosterone (Adams et al. 1992).

Alcohol and the HPT Axis

Normal Functioning of the HPT Axis

The HPT axis is responsible for maintaining normal circulating levels of the thyroid hormones thyroxin (T4) and its active form, triiodothyronine (T3). These two hormones affect every cell and organ in the body, primarily regulating different metabolic processes

that influence how cells use different energetic compounds (i.e., proteins, fats, and carbohydrates). When circulating levels of thyroid hormones are low, the hypothalamus responds by releasing TRH, which then stimulates thyrotropic cells in the anterior pituitary to produce and secrete TSH. This hormone, in turn, promotes the synthesis and secretion of T4 and T3 from the follicular cells of the thyroid gland. Iodine is essential to T4 and T3 production, with T4 containing four, and T3 containing three, iodine atoms. Although both T4 and T3 are secreted by the thyroid following TSH stimulation, 80 percent of circulating T3 is derived from the conversion of T4 by enzymes called deiodinases in the liver. Like the HPA and HPG axes, the HPT axis is regulated by negative-feedback loops where T4 and T3 act back on the hypothalamus and the pituitary to control their own release by inhibiting TRH and TSH secretion.

Alcohol's Effects on the HPT Axis

Numerous studies have described HPT axis dysfunction in people with AUD (see figure 3). For example, these individuals consistently exhibit a reduced or absent response of TSH to TRH (Sellman and Joyce 1992). A blunted TSH response also was observed during early withdrawal and was positively correlated with severity of withdrawal symptoms; in fact, it may be an important predictor of relapse (Pienaar et al. 1995). However, conflicting changes in peripheral thyroid hormones in response to alcohol exposure and withdrawal have been reported. T4 and T3 circulate in two forms, a protein-bound inactive form and a free, readily available active form. Some studies found normal concentrations of total plasma T4 (tT4) during early withdrawal (Majumdar et al. 1981), whereas others found significantly reduced tT4 levels (Valimaki et al. 1984). The levels of free T4 and T3, however, were lower in people with AUD during withdrawal and early

Alcohol's Effects on the Hypothalamic–Pituitary–Gonadal Axis During Puberty

Little research has assessed the effects of alcohol use on the hypothalamic–pituitary–gonadal (HPG) axis during puberty in humans. Initiation and progression of puberty are controlled by signals from the central nervous system that stimulate the pulsatile diurnal secretion of luteinizing hormone-releasing hormone (LHRH) from the hypothalamic–pituitary portal system (Sarkar and Fink 1979; Sarkar et al. 1976). LHRH then triggers the pituitary to secrete luteinizing hormone (LH) and follicle-stimulating hormone (FSH), resulting in subsequent ovarian maturation (Plant 2015). During childhood, the LHRH surge is repressed through inhibitory signals in the hypothalamus mediated by γ -aminobutyric acid and opioid peptides (Terasawa and Fernandez 2001). During puberty, however, LHRH release is triggered by a variety of stimulatory agents, such as insulin-like growth factor-1 (IGF-1) (Hiney and Dees 1991), norepinephrine (Sarkar et al. 1981), leptin (Dearth et al. 2000), transforming growth factor alpha (Ojeda et al. 1990), and kisspeptins (Navarro et al. 2005).

Human studies have documented that moderate alcohol consumption induces disruptions in normal hormone levels during puberty, including a decrease in estrogen levels in adolescent girls that was sustained for long periods of time (Block et al. 1993). Similar, alcohol abuse induced a significant reduction in testosterone, LH, and FSH levels in adolescent boys (Diamond et al. 1986). Animal studies on rodents and monkeys have helped to understand and identify the mechanisms involved in these alcohol-mediated

disruptions of puberty-related processes. Bo and colleagues (1982) reported that alcohol administration to prepubertal female rats induced a marked delay in vaginal opening. This delay could be prevented by naltrexone, an antagonist of the opioid receptors (Emanuele et al. 2002), suggesting that alcohol's effects during puberty partly may result from an increased opioid restraint on the normal progression of pubertal processes. Another proposed mechanism for the alcohol-induced decrease in LH secretion during puberty is that even though the hypothalamus produced more LHRH, the release of the hormone to the pituitary gland was diminished (Dees and Skelley 1990). This effect may result, at least in part, from altered release of prostaglandin E2 (Hiney and Dees 1991), which normally mediates stimulation of LHRH release by norepinephrine. In addition, alcohol exposure induces an increase in hypothalamic growth hormone (GH)-releasing hormone content that also is associated with diminished release of the hormone and, therefore, reduced ability to stimulate GH secretion from the anterior pituitary (Dees and Skelley 1990). These effects of alcohol exposure on GH were associated with a decrease in circulating IGF-1, which could explain the growth impairments observed in animals exposed to alcohol (Srivastava et al. 1995). In studies in rhesus macaques, administration of alcohol (2 g/kg) for 12 months to immature females resulted in suppression of the nightly increase in circulating GH that occurs during late juvenile development (Dees et al. 2000). This effect was associated with a

significant decline in circulating IGF-1, LH, and estrogen and was most pronounced at 32 months of age. The reduced hormone levels affected the monthly pattern of menstruation in the rhesus macaques and induced a lengthening of the intervals between menses in the alcohol-exposed monkeys (Dees et al. 2000).

Taken together, these findings clearly show that the activities of the HPG and GH/IGF-1 axes during puberty are closely interconnected. This is further demonstrated by observations that estrogen can stimulate GH secretion (Mauras et al. 1996) and that IGF-1 can stimulate LHRH secretion (Hiney and Dees 1991), suggesting that activation of the HPG axis leads to both sexual maturation and a growth spurt mediated through estrogen-induced stimulation of the GH/IGF-1 axis. Therefore, alcohol-induced disturbances in the activity of the HPG axis during this critical stage of human development could have far-reaching consequences on reproductive function as well as growth that might persist through adult life.

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abstinence compared with nonalcoholic healthy control subjects (Hegedus et al. 1988). Additional analyses identified a significant positive correlation between free T₃ and alcohol-seeking behaviors in alcohol-dependent individuals (Aoun et al. 2015), supporting the hypothesis of a relationship between alcohol dependence and thyroid dysfunction. This thyroid dysfunction can recover after longer periods of abstinence, with thyroid hormones and the TSH response to TRH returning to normal levels (Pienaar et al. 1995). Moreover, people who relapsed and returned to their alcohol-drinking behavior again exhibited lower T₄ and T₃ levels and a blunted TSH response to TRH (Heinz et al. 1996). Animal studies have yielded similar results. Chronic exposure of adult male rats to ethanol (10 percent weight/volume) for 40 days induced a significant decrease in total T₄ and T₃, free T₄ and T₃, as well as basal TSH levels (Mason et al. 1988).

Several mechanisms have been proposed to explain the blunted TSH response to TRH in people with AUD. For example, several studies suggest

that the number of TRH receptors in the pituitary is reduced as a result of increased TRH secretion (Aoun et al. 2015; Herman 2002). A role for increased TRH section in blunting the TSH response also is supported by observations that abstinent patients with AUD who had a severely blunted TSH response to TRH showed increased levels of TRH in the cerebrospinal fluid (Adinoff et al. 1991). In rats, chronic alcohol exposure induced an increase in TRH mRNA in neurons of the PVN, but the animals no longer responded to peripheral stimulation of thyroid hormone secretion by exposure to cold (Zoeller et al. 1996). This suggests that chronic exposure to ethanol induces dysfunction of the thyroid gland, which then is no longer able to properly respond to TRH stimulation.

Direct actions of ethanol on thyroid hormone metabolism, specifically on the activity of enzymes that catalyze the conversion of T₄ to T₃ (i.e., 5′II deiodinase) or inactivate T₃ to 3,3′-T₂ (i.e., 5-II deiodinase), also have been proposed. In a study comparing “behaviorally dependent” and ethanol-exposed but “nondependent” rats,

Baumgartner and colleagues (1997) found that the activity of 5′II deiodinase was elevated in the frontal cortex in both groups of rats. The activity of 5-II deiodinase, however, was only inhibited in the amygdala of the rats that were behaviorally dependent on ethanol but was normal in the non-dependent rats. As a result, intracellular T₃ levels were increased, and this increase of intracellular T₃ in the amygdala might be involved in the development of dependence behaviors to alcohol (Baumgartner et al. 1997). The role of changes in thyroid hormone levels in the development of AUD also is supported by findings that a functionally significant genetic variant (i.e., single nucleotide polymorphism) in the deiodinase type II (D2) gene was associated with drinking behavior in alcohol-dependent individuals (Lee et al. 2015).

Chronic alcohol use also had a direct toxic effect on the thyroid gland, inducing a dose-dependent significant reduction in thyroid volume and increase in thyroid fibrosis in alcohol-dependent individuals (Hegedus et al. 1988). These effects were associated with

reductions in total and free T3 levels, although the concentrations of total and free T4 as well as of TSH remained unchanged (Hegedus et al. 1988). In contrast to these effects of chronic alcohol use on thyroid hormones, moderate alcohol consumption was shown to reduce the risk of developing thyroid cancer. Several studies, including the large NIH–AARP Diet and Health Study that followed 490,000 participants (males and females) over 7.5 years, have shown a significant reduction in the risk of developing all types of thyroid cancers in people who consumed two or more alcoholic drinks per day, especially in men. However, the effects differed between different subtypes of thyroid cancer, with a stronger inverse association for papillary thyroid cancer (relative risk = 0.58) compared with follicular thyroid cancer (relative risk = 0.86) (Meinhold et al. 2009). Furthermore, in a study of 4,649 healthy individuals who were exposed to increasing levels of alcohol, Knudsen and colleagues (2001) found an association between a reduced thyroid gland volume and a lower risk of developing goiter or solitary nodules.

Alcohol and the GH/IGF-1 Axis

Normal Functioning of the GH/IGF-1 Axis

Like the other hormone systems discussed so far, the GH/IGF-1 axis is under the control of the hypothalamus. Growth hormone–releasing hormone (GHRH) secreted from cells in the arcuate and ventromedial nuclei of the hypothalamus into the hypophyseal portal system acts on somatotrophic cells in the anterior pituitary, stimulating them to synthesize and release GH into the general circulation. GH is essential to the growth of all tissues in the body. It stimulates protein synthesis and increases fat metabolism to provide the necessary energy for growth. GH binds to specific receptors on target tissues and directly affects cell

function or it stimulates IGF-1 production and secretion, especially from the liver, the principal production site for this factor. IGF-1 then is either released into the general circulation, where it is bound to large circulatory binding proteins that regulate its delivery to target tissues, or it mediates the anabolic effects of GH through paracrine and autocrine mechanisms. At birth, plasma IGF-1 levels are at 50 percent of the adult levels and gradually increase throughout childhood

with a spike during puberty, when IGF-1 plays a critical role in reproductive-organ maturation and long-bone growth. After puberty, the levels again decrease slowly to reach the adult level. IGF-1 can control its own secretion through negative feedback at the level of the hypothalamus and pituitary by reducing GH synthesis and release.

Another hormone called somatostatin, which is secreted from the PVN of the hypothalamus, also acts on the pituitary and inhibits GH secretion.

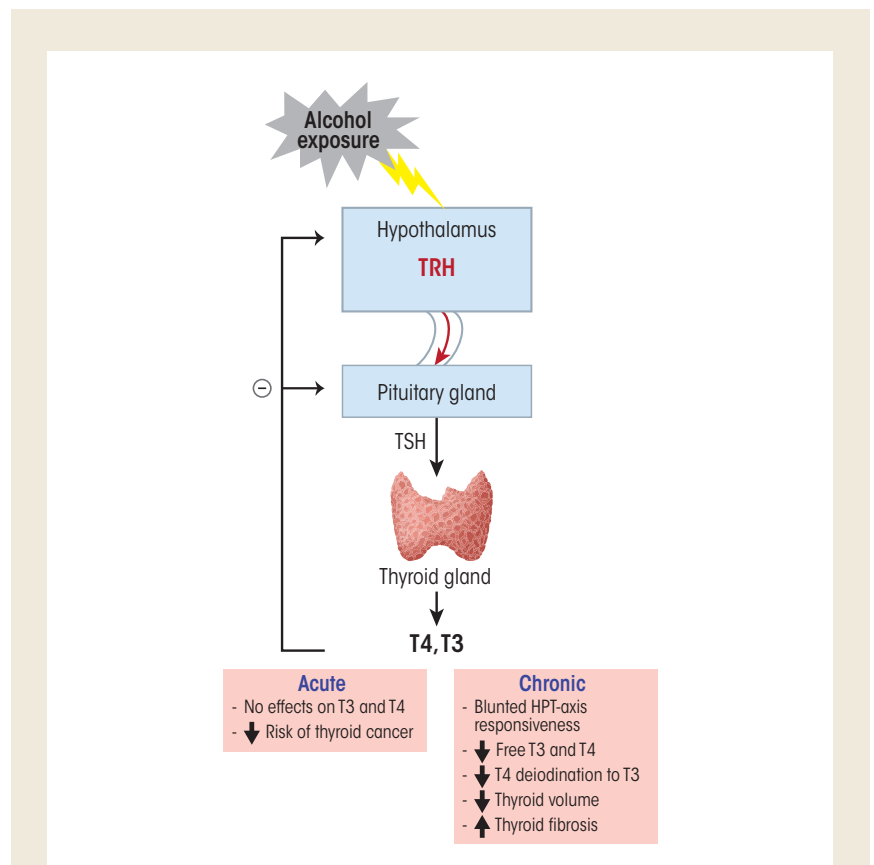


Figure 3 Alcohol’s effects on the hypothalamic–pituitary–thyroid (HPT) axis. Thyrotropin-releasing hormone (TRH) released from neurons in the hypothalamus stimulates thyrotropic cells in the anterior pituitary to produce and secrete thyroid-stimulating hormone (TSH). TSH then stimulates the synthesis and secretion of thyroxine (T4) and its active form, triiodothyronine (T3), from the follicular cells of the thyroid gland. Circulating T3 comes from conversion of T4 by enzymes called deiodinases in the liver. T3 and T4 can control their own release by negative feedback at the hypothalamus and the pituitary and inhibit TRH and TSH release. Acute alcohol exposure has no effect on HPT-axis function. However, chronic alcohol exposure leads to a blunted TSH response to TRH, as well as to decreased free T3 and T4, decreased deiodination of T4 to T3, decreased thyroid volume, and increased thyroid fibrosis.

Thus, the amount of GH secreted by the anterior pituitary is tightly regulated by GHRH, IGF-1, and somatostatin. Together, GH and IGF-1 regulate important physiological processes in the body, such as pre- and postnatal growth and development (Giustina et al. 2008) and carbohydrate and lipid metabolism (Moller and Jorgensen 2009).

Alcohol's Effects on the GH/IGF-1 Axis

Numerous studies in both humans and experimental animals have shown that acute and chronic alcohol exposure has a variety of effects on the GH/IGF-1 axis (figure 4). For example, alcohol exposure reduces circulating GH and IGF-1 levels. Acute exposure

of healthy men to ethanol (1.5 g/kg) reduced the nightly peak of GH secretion (Valimaki et al. 1987). This effect did not seem to be mediated through a direct action of ethanol on the pituitary that would have rendered it less sensitive to GHRH, because intravenous injection of exogenous GHRH induced an increase in GH secretion in both ethanol-exposed (1 g/kg) and control men (Valimaki et al. 1987). Similarly, De Marinis and colleagues (1993), using an agent that can stimulate GHRH secretion (i.e., clonidine), demonstrated that the pituitary response to GHRH was intact in abstinent alcoholics. Other studies evaluated alcohol's effects on numerous other factors that regulate GH secretion either through direct actions on the anterior pituitary or by modulating GHRH and somatostatin release from the hypothalamus. The analyses demonstrated that during early abstinence, the GH response to these different secretagogues, which include such neurotransmitters as dopamine, nor-epinephrine, acetylcholine, γ -aminobutyric acid (GABA), and serotonin, also is altered. For example, men with AUD exhibited impairments both in the serotonin-mediated stimulation of GH secretion (Coiro and Vescovi 1995) and in melatonin's effect on basal and hypoglycemia-induced GH secretion (Coiro and Vescovi 1998) during early abstinence. Moreover, intravenous injection of 10 mg diazepam, an allosteric modulator of GABA receptor function, had no effect on GH secretion in men with AUD who had maintained a 5-week abstinence, whereas control subjects without AUD showed a striking increase of GH secretion in response to diazepam (Vescovi and Coiro 1999). Finally, alcohol interferes with the normal release pattern of GH. The hormone normally is secreted in a pulsatile manner, with the major secretory episode of GH occurring shortly after sleep onset, during the first period of slow-wave sleep. Studies have identified a consistent and robust relationship between slow-wave sleep and increased GH

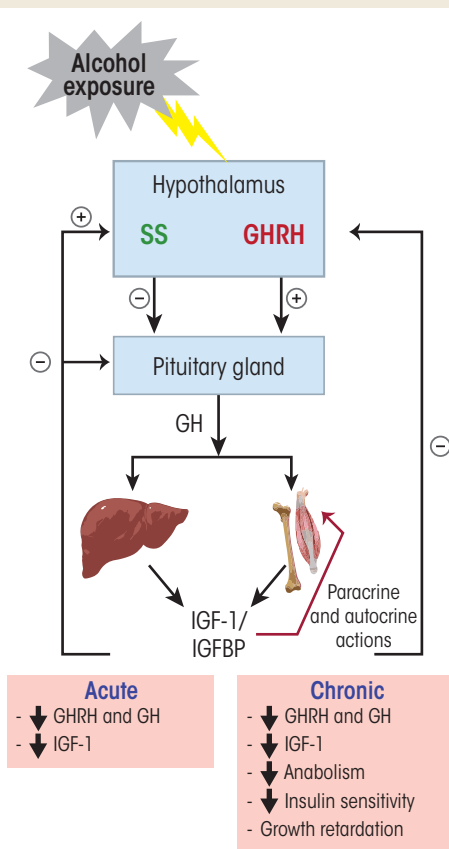


Figure 4 Alcohol's effects on the growth hormone–insulin-like growth factor-1 (GH/IGF-1) axis. Growth hormone (GH)-releasing hormone (GHRH) secreted from neurons in the hypothalamus acts on somatotrophic cells in the anterior pituitary and stimulates the production and release of GH into the circulation. GH can act on target tissues and directly affect their function or it can stimulate IGF-1 production and secretion from these target tissues, especially from the liver. IGF-1 then is either released into the general circulation, where it circulates bound to IGF binding proteins (IGFBP), or it can mediate GH anabolic effects on target tissues through paracrine and autocrine actions. Through negative feedback at the hypothalamus and pituitary, IGF-1 can reduce GHRH and GH secretion. Somatostatin (SS), secreted in the paraventricular nucleus of the hypothalamus, also acts on the pituitary and inhibits GH secretion. IGF-1 stimulates SS secretion. Acute and chronic alcohol exposure leads to decreased GHRH, GH, and IGF-1 secretion.

Alcohol and Other Endocrine Tissues

In addition to the brain areas and organs involved in the main hormone axes in the body that are discussed in this article, several other tissues also produce and secrete hormones that regulate crucial body functions, including the pancreas and fat (i.e., adipose) tissue. Alcohol exposure also can interfere with these hormonal systems.

The Endocrine Pancreas

The pancreas, which lies behind the stomach, serves two major functions. First, acinar cells secrete digestive enzymes into the small intestine, thereby supporting digestion. Second, islet cells dispersed throughout the whole pancreas have an endocrine activity by producing hormones (i.e., insulin and glucagon) that regulate blood glucose levels. These islet cells can be further subdivided into α - and β -cells. The α -cells produce glucagon, which raises blood glucose levels by stimulating the liver to metabolize glycogen into glucose molecules and to release the glucose into the blood. In addition, glucagon stimulates the adipose tissue to metabolize triglycerides into glucose, which then is released into the blood. Conversely, the β -cells of the pancreas produce insulin, which lowers blood glucose levels after a meal by stimulating the absorption of glucose by liver, muscle, and adipose tissues and promoting the storage of glucose in the form of glycogen in these tissues. The endocrine function of the pancreas primarily is controlled by both the sympathetic and the parasympathetic divisions of the autonomic nervous system.

Alcohol's Effects on the Endocrine Pancreas

Heavy alcohol drinking can induce the development of inflammation of the pancreas (i.e., pancreatitis), most commonly in acinar cells. However, the inflammatory aspect of this disease also can damage islet cells and, therefore, the endocrine pancreas (Apte et al. 1997). Chronic alcohol consumption also is a risk factor for the development of pancreatic cancer, with moderate to heavy consumption increasing the risk both alone and in combination with other risk factors, such as tobacco and obesity (de Menezes et al. 2013; Haas et al. 2012). One type of pancreatic cancer called ductal adenocarcinoma has a very aggressive behavior with a 5-year survival rate of less than 4 percent (Welsch et al. 2006).

Chronic alcohol consumption also is a known independent risk factor for the development of type 2 diabetes (Hodge et al. 1993; Holbrook et al. 1990; Wei et al. 2000). This syndrome is characterized by impaired glucose metabolism with high blood glucose levels (i.e., hyperglycemia) and peripheral insulin resistance. The relationship between alcohol consumption and the risk of type 2 diabetes is "U" shaped—that is, risk is lower with moderate alcohol consumption than with either abstinence or high alcohol consumption. Thus, the risk was reduced by 30 percent in moderate drinkers compared with abstainers, whereas no risk reduction was observed in heavy drinkers consuming 48 grams of ethanol (i.e., 3 to 4 drinks) per day or more (Koppes et al. 2005). Moderate alcohol use may have protective effects by enhancing peripheral insulin sensitivity (Conigrave et al. 2001; Tomie Furuya et al. 2005).

Some studies have shown that moderate alcohol consumption improves peripheral insulin sensitivity without affecting insulin secretion from pancreatic β -cells (Avogaro et al. 2004), whereas others determined a reduced basal insulin secretion rate associated with a lower fasting plasma glucagon concentration (Bonnet et al. 2012). The beneficial metabolic effects of moderate alcohol use on insulin sensitivity and glucose homeostasis therefore might explain the significant reduction in the risk of development of type 2 diabetes and of cardiovascular disorders (Avogaro et al. 2004; Bantle et al. 2008).

Heavy alcohol consumption, in contrast, has several detrimental effects resulting in impaired control of blood glucose levels. In addition to its effects on peripheral tissues, such as adipose tissue and the liver, where it induces insulin resistance, heavy drinking also negatively affects pancreatic β -cell function. In a study by Patto and colleagues (1993), chronic drinkers exhibited a decreased insulin-secretion response to glucose compared with the control group. When the investigators measured the total integrated response values for secreted insulin and for C-peptide¹ following oral or intravenous glucose administration in these two groups, both values were significantly lower in the chronic drinkers compared with the control group. Moreover, in both groups the total integrated response value for insulin was significantly higher after oral glucose administration than after

¹ C-peptide is a chain of 31 amino acids that during insulin synthesis connects the two parts, or chains, of the insulin molecule in a precursor molecule. During final processing of the insulin molecule, the C-peptide is removed to yield the functional insulin molecule with its two chains.

Alcohol and Other Endocrine Tissues (*continued*)

intravenous administration, suggesting a potentiating incretin² effect on insulin secretion. These findings clearly indicate that chronic alcohol exposure induces a β -cell dysfunction and not an enteroinsular incretin dysfunction, because the decrease in insulin response compared with the control group also was observed when glucose was administered intravenously.

Animal studies demonstrated that mice exposed to chronic alcohol for 8 to 10 weeks developed impairments in fasting glucose levels and exhibited an increase in β -cell apoptosis, which were associated with diminished insulin secretion (Kim et al. 2010). The investigators suggested that alcohol exposure led to a down-regulation and inactivation of the enzyme glucokinase, which acts as a β -cell sensor for blood glucose levels. Glucokinase is involved in glucose metabolism that leads to increased production of adenosine-triphosphate, a necessary step in insulin secretion by β -cells. The researchers also detected a decrease in the glucose transporter Glut2 in β -cells as well as a decrease in insulin synthesis, further exacerbating the effects of chronic alcohol exposure.

More recently, Wang and colleagues (2014) reported that intraperitoneal administration of ethanol (3g/kg body weight) to mice resulted in an impaired glucose metabolism, which was associated with decreased expression of two subunits (i.e., α 1 and δ -subunits) of the type A gamma-aminobutyric acid (GABA)

receptors on pancreatic β -cells. This could account at least for part of the alcohol-induced impairment in β -cell function, because activation of GABA receptors in pancreatic β -cells increases insulin secretion (Bansal et al. 2011), has a protective and regenerative effect on β -cells, and decreases cell apoptosis in cultured islet cells (Dong et al. 2006). The investigators further showed that acute treatment of cultured rat β -cells (i.e., the INS-1 cell line) with 60 mM ethanol interfered with GABA-mediated cell activation as well as insulin secretion and that these effects could be prevented by pretreating the cultured cells with GABA (100 mM), further supporting the theory that alcohol's effects on β -cells and insulin production are mediated at least in part by GABA signaling (Wang et al. 2014). In addition, experiments in another cultured β -cell line indicated that heavy alcohol consumption may induce β -cell dysfunction in type 2 diabetes by increasing the production of reactive oxygen species and inducing apoptosis in the cells (Dembele et al. 2009).

All of these studies clearly show that heavy alcohol consumption has deleterious effects on pancreatic β -cell function and glucose homeostasis. However, more studies are needed to specify the mechanisms by which chronic alcohol affects β -cell function.

Endocrine Adipose Tissue

There are two types of adipose tissue—white adipose tissue (WAT) and brown adipose tissue (BAT)—that differ in their morphology and function. For a long time, WAT had been considered a passive reservoir for energy storage. Over the last decade, however, numerous studies have

demonstrated that WAT is a dynamically active endocrine organ that can produce and secrete biologically active peptides and proteins called adipokines, which have autocrine, paracrine, and endocrine actions. In fact, WAT may be the largest endocrine organ in mammals and can be found in individual pads in different locations throughout the body, both near other organs (i.e., viscerally) and under the skin (i.e., subcutaneously). Depending on its location, WAT synthesizes and secretes different sets of adipokines (Coelho et al. 2013). Since the discovery of leptin (Zhang et al. 1994), multiple adipokines released by WAT have been identified, including hormones, growth factors, and cytokines (Coelho et al. 2013).

WAT also expresses several receptors that allow it to respond to signals from other hormone systems and from the central nervous system. Through these different communication pathways, WAT can influence the function of many tissues, such as hypothalamus, pancreas, skeletal muscle, and immune system. In addition, WAT can coordinate numerous important biological processes through its various adipokines, such as food intake and body weight (leptin), glucose homeostasis (adiponectin and resistin), lipid metabolism, pro- and anti-inflammatory functions (tumor necrosis factor alpha [TNF α] and interleukin-6 [IL-6]), as well as reproductive functions (Campfield et al. 1996; Coelho et al. 2013).

BAT, on the other hand, is present at birth but is almost absent in adult mammals. Brown adipocytes are smaller than white adipocytes, have numerous mitochondria, and specialize in heat production through oxidation of fatty acids (i.e., thermogenesis). However, recent

² Incretin is a hormone secreted by the wall of the intestine that acts on the pancreas to regulate insulin production after glucose administration. This so-called enteroinsular signaling pathway can therefore only occur after oral glucose administration, which results in increased glucose levels in the intestine, but not after intravenous administration, which bypasses the intestine.

Alcohol and Other Endocrine Tissues (*continued*)

direct and indirect evidence also suggests a potential endocrine role for BAT (Villarroya et al. 2013). Thus, BAT was shown to release factors such as IGF-1, fibroblast growth factor-2, IL-1 α , IL-6, bone morphogenetic protein-8b, and lipocalin prostaglandin D synthase that primarily have autocrine or paracrine actions (Villarroya et al. 2013). The only known endocrine factor released by BAT is the active thyroid hormone T3. Upon thermogenic activation, the type II thyroxine 5'-deiodinase enzyme, which is expressed specifically in BAT, converts T4 into T3 (de Jesus et al. 2001).

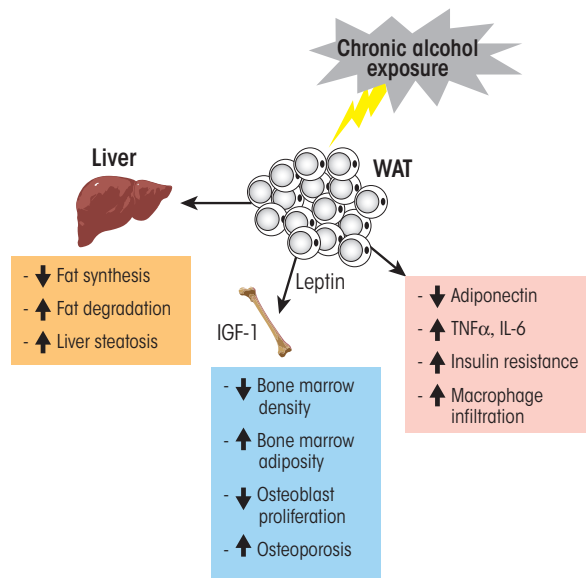
Alcohol's Effects on Endocrine Adipose Tissue

Although the results have not been consistent, numerous studies have shown that alcohol consumption can change adipokine levels. For example, studies found that leptin levels were increased (Nicolas et al. 2001; Obradovic and Meadows 2002), decreased (Calissendorff et al. 2004), or remained unchanged (Beulens et al. 2008; Strbak et al. 1998) by alcohol exposure. Another adipokine is adiponectin, which is produced and secreted exclusively by WAT and has antidiabetogenic and anti-inflammatory effects. Its production and actions are regulated by TNF α , with the two compounds suppressing each other's production and antagonizing each other's actions in target tissues (Maeda et al. 2002). Moderate alcohol consumption can increase adiponectin plasma levels, which is associated with a significant increase in insulin sensitivity (Sierksma et al. 2004; Thamer et al. 2004); the extent of this effect, however, depends on the

frequency of alcohol administration. In a study comparing the effects of exposure of high-fat-fed rats to 5 g/kg body weight ethanol per day delivered either by twice-daily administration via a gastric tube or through free-access drinking, Feng and colleagues (2012) demonstrated greater improvement of insulin sensitivity with twice-daily ethanol administration. Accordingly, adiponectin plasma levels were significantly increased in the twice-daily administration group compared with the free-access group. The researchers suggested that ethanol concentrations in the blood might be an important fac-

tor influencing adiponectin secretion and, consequently, insulin sensitivity.

One proposed mechanism for the adiponectin-mediated improvement in insulin sensitivity is that the increase in adiponectin causes a decrease in plasma levels of TNF α (Ouchi et al. 2000; Yokota et al. 2000). Conversely, decreasing adiponectin levels would be expected to result in increasing TNF α levels. High circulating TNF α levels, in turn, have been implicated in the development of peripheral insulin resistance (Hotamisligil et al. 1995). Chronic alcohol consumption can significantly decrease adiponectin



Alcohol and the endocrine white adipose tissue (WAT). WAT is a dynamically active endocrine organ that produces and secretes adipokines, including hormones, growth factors, and cytokines. These factors, through autocrine, paracrine, and endocrine actions, can influence the function of many tissues and coordinate numerous important biological processes such as food intake, glucose homeostasis, lipid metabolism, and pro- and anti-inflammatory functions. Acute and moderate alcohol exposure induces an increase in circulating adiponectin levels, which is associated with decreased insulin resistance. Chronic alcohol exposure induces a decrease in adiponectin, an increase in macrophage infiltration and proinflammatory cytokine secretion (e.g., tumor necrosis factor alpha (TNF α) and interleukin-6 [IL-6]) and insulin resistance. Chronic alcohol exposure also increases the risk of fatty liver (i.e., steatosis).

Alcohol and Other Endocrine Tissues (*continued*)

levels (Xu et al. 2003).³ Thus, male rats that had received ethanol for 4 weeks exhibited significantly decreased mRNA levels of adiponectin and retinol binding protein 4 but increased mRNA levels of monocyte chemoattractant protein 1, TNF α , and IL-6 in epididymal adipose tissue. These changes were associated with increased macrophage infiltration into adipose tissue and the development of insulin resistance (see figure) (Kang et al. 2007).

In addition, studies have suggested that reduced adiponectin expression could play an important role in the development of alcohol-induced liver damage (Xu et al. 2003). Alcoholic fatty liver (i.e., steatosis) is one of the most prevalent forms of chronic liver diseases caused by alcohol abuse; it is characterized by the excessive accumulation of fat in the liver and can progress to more severe forms of liver injury, such as steatohepatitis, fibrosis, and cirrhosis. Adiponectin's protective effects on the liver are believed to be mediated through its actions on hepatic signaling molecules involved in enhanced fat oxidation and reduced lipid synthesis (Rogers et al. 2008; Xu et al. 2003). A recent study assessed the serum concentrations of total adiponectin, leptin, and resistin in male and female patients with chronic alcohol abuse and different degrees of liver dysfunction (Kasztelan-Szczerbinska et al. 2013). The analyses found elevated total levels of adiponectin and resistin in patients with alcoholic liver disease (ALD) compared with control subjects. Also, women with ALD had

lower leptin levels than did control subjects, whereas there were no significant differences in leptin concentrations in males with and without ALD. Gender-related differences in serum leptin concentrations may influence the clinical course of ALD, which differs in males and females. It is possible that metabolic alterations caused by ethanol in the course of ALD, by differentially modulating leptin secretion, may be responsible for different clinical presentations of the disease in females and males (Kasztelan-Szczerbinska et al. 2013). However, more studies are needed to help with our understanding of the adipose tissue pathology associated with alcohol abuse.

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³ The increased TNF α levels associated with decreased adiponectin also may play a role in the development of liver disease. TNF α production was increased in adipose tissue at early stages of alcoholic fatty liver, resulting in increases in both circulating and local TNF α levels (Lin et al. 1998).

Alcohol and Other Endocrine Tissues (continued)

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secretion as well as between sleep disturbances and decreased GH secretion (Van Cauter et al. 2004). Alcohol-dependent individuals have been shown to have lower levels of slow-wave sleep power that was associated with lower levels of GH release compared with normal control subjects (Lands 1999).

Similar findings have been obtained in animal studies. In a rat model of binge ethanol exposure, intraperitoneal injection of one dose of ethanol resulted in a significant decline of GH serum levels at 0.5, 1.5, and 3 hours compared with saline-injected control rats (Emanuele et al. 1992). In a model

of chronic alcohol exposure, rats receiving 5 percent ethanol in a liquid diet for 4.5 months showed a significant decrease in circulating IGF-1 levels (Sonntag and Boyd 1988). Similarly, chronic 6-day administration of 5 percent ethanol to awake rats resulted in a 75 to 90 percent decrease in spontaneous GH secretion (Soszynski and Frohman 1992). In addition, ethanol treatment was associated with significant declines in IGF-I serum levels and GHRH mRNA levels, whereas somatostatin or GH mRNA levels did not change (Soszynski and Frohman 1992). These results suggest that chronic ethanol

affects GH secretion primarily at the hypothalamic level where it induces impairments in GHRH gene expression. However, the responsiveness of the anterior pituitary to a GHRH challenge was the same in both saline- and ethanol-injected animals (Dees et al. 1988).

As mentioned earlier, the GH/IGF-1 pathway regulates carbohydrate and lipid metabolism. Recent studies have suggested that alcohol-induced changes in the circulating levels of IGF-1 and GH might contribute to the alcohol-mediated development of glucose intolerance and type 2 diabetes.

Glossary

Anabolic: Pertaining to the metabolic processes by which organisms convert substances into other components the body needs.

Apoptosis: Specific pattern of reactions resulting in the death of single cells; also referred to as programmed cell death.

Autocrine: A mode of hormone action in which a hormone binds to receptors on, and affects the functions of, the cell type that produced it.

Autonomic Nervous System: Part of the nervous system that connects the central nervous system to the organs and controls involuntary bodily functions, such as respiration and digestion.

C-peptide: Part of the precursor molecule of insulin that gets excised during the final processing of the insulin molecule; has no physiologic activity.

Epididymal: Pertaining to the epididymis—the elongated, cordlike structure along the rear of the testis that provides for storage, transit, and maturation of sperm.

Epigenetic: Altering the activity of genes without changing their DNA sequences (e.g., through chemical modification of the DNA or the histone proteins around which the DNA is coiled).

Fecundability: The probability that a woman becomes pregnant in a certain period of time.

Glycogen: A large, highly branched molecule consisting of chains of glucose molecules; constitutes the major carbohydrate reserve of animals and is stored primarily in liver and muscle.

Insulin Resistance: Impairment of the normal physiological response to insulin that may be the result of a variety of abnormalities; occurs in diabetes mellitus.

Paracrine: A mode of hormone action in which a hormone binds to receptors on, and affects the functions of, nearby cells of a different type from the cell type that produced it.

Parasympathetic Nervous System: Part of the *autonomic nervous system* that operates to help the body conserve energy and resources in a relaxed state.

Promoter: Segment of DNA usually in front of a gene that acts as a controlling element in the expression of that gene.

Reactive Oxygen Species: Biologically active, partially reduced derivatives of molecular oxygen that are produced by normal metabolic processes and which can damage the cells or their components.

Sympathetic Nervous System: Part of the *autonomic nervous system* that stimulates organs and blood vessels to help the body react to stressful situations.

Total Integrated Response: A measure of the area under the curve of the insulin or glucose response to an oral glucose challenge used to determine *insulin resistance*.

In a rat model of type 2 diabetes (i.e., the type-2 diabetic Otsuka Long-Evans Tokushima Fatty rat model), alcohol administration significantly decreased IGF-1 serum levels and increased GH serum levels compared with nondiabetic control rats (Kim et al. 2013). These effects on IGF-1 and GH might contribute to the alcohol-mediated exacerbation of type 2 diabetes in the rats.

Alcohol and the HPP Axis

The HPP axis includes two neuropeptides—AVP and oxytocin—both of which are produced by cells whose cell bodies are located in the hypothalamus but that extend to the posterior pituitary,

where they release their hormones. AVP can be produced by two types of cells (i.e., magnocellular and parvocellular cells). Magnocellular neurosecretory cells produce the AVP that is found in peripheral blood. This AVP is secreted in response to osmotic stimuli and is involved in regulating the concentration of dissolved molecules (i.e., osmolality) in the body fluids by retaining water in the body and constricting blood vessels (Iovino et al. 2012; Verbalis 1993). In contrast, AVP produced by the parvocellular system is secreted following psychological stress and is involved in potentiating the action of CRF on ACTH release (Romero and Sapolsky 1996). Some AVP also may be released directly into

the brain, and accumulating evidence suggests it plays an important role in social behavior, sexual motivation and pair bonding, and maternal responses to stress (Insel 2010). In the context of chronic alcohol use, AVP is involved in the disturbed water balance observed in actively drinking people with AUD and during acute withdrawal (Döring et al. 2003; Ehrenreich et al. 1997). AVP also may affect cognitive function, because treatment of alcoholic patients with memory deficits by using AVP analogs resulted in improved cognitive performance (Laczi 1987). Finally, studies in rodents have suggested that AVP may play a role in the development and maintenance of alcohol tolerance (Hoffman 1994).

Like AVP, oxytocin is produced by both magnocellular and parvocellular neurons of the hypothalamus. It functions both as a peripheral hormone and as a signaling molecule in the central nervous system (Buijs 1983). In its role as a peripheral hormone, oxytocin is released into the circulation from the posterior pituitary, enhancing uterine contractions during labor and, together with prolactin, enhancing milk release during lactation (Leng et al. 2015). Maternal alcohol use before or during lactation can interfere with the proper function of both prolactin and oxytocin (Heil and Subramanian 1998). In the central nervous system, oxytocin is released by a variety of neurons. Some of these are neurons whose cell bodies are in the hypothalamus and that extend to limbic and forebrain areas, where they release oxytocin from their terminals. Other oxytocin-releasing neurons are located outside the hypothalamus, in the amygdala and bed nucleus of the stria terminalis (Ross and Young 2009). Oxytocin may be a major contributor to alcohol tolerance and dependence (Hoffman and Tabakoff 1981; McGregor et al. 2012). Moreover, recent studies have demonstrated that peripheral administration of oxytocin can reduce ethanol consumption in rats (MacFadyen et al. 2016) and that intranasal oxytocin administration blocks alcohol withdrawal in humans (Pedersen et al. 2013).

Conclusion

Alcohol's deleterious effects on the endocrine system have far-reaching consequences that can result in serious physiological and behavioral disorders. Alcohol abuse not only causes hormonal disturbances, but because these disturbances permeate every organ and tissue in the body, can result in various debilitating disorders, such as stress intolerance, disturbed water balance and body osmolality, reproductive dysfunction, thyroid problems, immune abnormalities, diabetes, cardiovascular disease, cancer, and psychological and

behavioral disorders. The different components of the endocrine system, particularly the HPA axis, HPG axis, HPT axis, GH/IGF-1 axis, and HPP systems, normally communicate with each other as well as with the nervous and immune systems in response to external environmental cues and help maintain homeostasis and health. These coordinated bidirectional interactions rely on the production and release of chemical messengers, such as neurotransmitters, hormones, and cytokines, that mediate the communications between the different systems. Alcohol abuse disrupts the release of these chemical signals and negatively affects the communication pathways. A better understanding of the mechanisms involved in alcohol's effects on the bidirectional interactions between the HPA, HPG, HPT, and GH/IGF-1 axes; the HPP system; and the immune system will help pave the way for the development of effective therapeutic tools for AUD.

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Alcohol and the Lung

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Among the many organ systems affected by harmful alcohol use, the lungs are particularly susceptible to infections and injury. The mechanisms responsible for rendering people with alcohol use disorder (AUD) vulnerable to lung damage include alterations in host defenses of the upper and lower airways, disruption of alveolar epithelial barrier integrity, and alveolar macrophage immune dysfunction. Collectively, these derangements encompass what has been termed the “alcoholic lung” phenotype. Alcohol-related reductions in antioxidant levels also may contribute to lung disease in people with underlying AUD. In addition, researchers have identified several regulatory molecules that may play crucial roles in the alcohol-induced disease processes. Although there currently are no approved therapies to combat the detrimental effects of chronic alcohol consumption on the respiratory system, these molecules may be potential therapeutic targets to guide future investigation.

Key words: Alcohol consumption; alcohol use disorder; alcoholic lung; lung; lung disease; lung injury; respiratory system; pulmonary system; alveolar macrophage; antioxidant

Few social practices have had a longer or more complicated history in human civilization than the consumption of alcohol. As documented in academic writings, but even more commonly in art and music, humans have consumed alcohol for thousands of years, and drinking is either a celebrated facet of social activities or a proscribed practice, depending on the local moral or religious views. Although alcohol intoxication has been described in various written recordings since antiquity, it is only relatively recently that its true effects on lung health have been recognized. In the latter years of the 18th century, the first Surgeon General of the United States of America, Benjamin Rush (for whom the medical school in Chicago is named), noted that excessive alcohol consumption was associated with pneumonia (see Happel and Nelson 2005; Mehta and

Guidot 2012). More than a century later, William Osler wrote that alcohol abuse was the most important risk factor for pneumonia (see Happel and Nelson 2005; Mehta and Guidot 2012). As modern medicine evolved throughout the 20th century, it became abundantly clear that alcohol use disorder (AUD) rendered people more susceptible to a wide variety of lung infections, including bacterial pneumonias and tuberculosis, and increased morbidity and mortality. In a now-classic modern citation, Perlino and Rimland (1985) coined the term “alcoholic leukopenic pneumococcal sepsis syndrome” when they published a case series of patients with underlying AUD who suffered from pneumococcal pneumonia and sepsis associated with leukopenia that was associated with a mortality of more than 80 percent. Excessive alcohol consumption seems to increase

susceptibility to pneumonia through multiple mechanisms. The major factors include an increased risk of aspiration, abnormalities in the way particles are eliminated from the conducting airways through the mucus (i.e., in mucociliary clearance), and impaired activity of one branch of the immune system (i.e., innate immunity) within the lower airways (for reviews, see Joshi and Guidot 2007; Mehta and Guidot 2012).

Even more recently, researchers have identified an association between underlying AUD and acute respiratory distress syndrome (ARDS). ARDS is a severe form of acute lung injury that occurs as a complication of diverse insults, including sepsis, massive aspiration, and trauma; it has a mortality rate of 30 percent to 50 percent, even with state-of-the-art modern medical care in an intensive care unit (Villar et

al. 2011; Wang et al. 2014; Ware 2006; Ware and Matthay 2000). In 1996, a seminal study demonstrated for the first time that AUD independently conferred an approximately twofold increase in risk of developing ARDS (Moss et al. 1996). A subsequent prospective study focusing only on patients with severe sepsis revealed that the relative risk of developing ARDS was closer to fourfold higher in those with an underlying AUD;¹ this effect was independent of factors such as age, smoking, severity of illness, and nutritional status (Moss et al. 2003). Other investigators have confirmed these associations (Iribarren et al. 2000; Licker et al. 2003; Spies et al. 1996; von Heymann et al. 2002). Taken together, all of these findings indicate that drinking patterns that define AUD are associated with a significantly increased risk of serious lung infections and acute lung injury and thereby contribute to the deaths of tens of thousands of Americans every year, and many more worldwide.

This review first will discuss key aspects of the epidemiology and pathophysiology of AUD and lung health, before focusing more in-depth on lung infections and acute lung injury, which comprise the majority of alcohol-related lung diseases. The article also will briefly review some of the experimental therapies that hold promise for decreasing the enormous morbidity and mortality caused by the “alcoholic lung” in our society.

Alcohol and the Airways

The potential influence of alcohol consumption on airway health and disease has been documented for a long time. Chronic alcohol ingestion constantly subjects the drinker’s airways to high concentrations of alcohol vapor, as best evidenced by the use of alcohol breath tests (i.e., Breathalyzer). The volatile nature of alcohol is exploited in this common field sobriety test,

which is reliably used as a surrogate to quantify blood alcohol concentrations. Interestingly, the alcohol vapor found in the airways is not caused by inhalation but is a result of the ready diffusion of alcohol from the airway blood supply across the airway epithelium and into the airways themselves (George et al. 1996). This process explains why alcohol vapor in the breath may be used to determine blood alcohol concentration. The alcohol then is deposited and metabolized in the airways. This process leads to the formation of reactive aldehydes (e.g., acetaldehyde), which in turn can interact and form harmful adducts with proteins and DNA (Sapkota and Wyatt 2015). The formation of these adducts may disrupt normal cellular functions, induce inflammation, and impair healing. Taken together, these findings demonstrate that the airways—including the oral cavity and extending all the way to the alveolar space—are subjected to high concentrations of alcohol and its deleterious metabolites during intoxication.

Within the upper airways, chronic alcohol consumption leads to several alterations. First, chronic heavy drinking often is associated with poor tooth development and arrangement (i.e., dentition) as well as poor oral hygiene, and although these usually are attributed to poor nutritional and lifestyle choices, clinical studies have established that they also result, to some extent, from the direct effects of alcohol exposure on the upper airway. Specifically, alcohol decreases saliva production in the salivary glands located in front of the ears (i.e., the parotid glands) (Dutta et al. 1989), thereby eliminating an important mucosal defense within the oral cavity. As a result, heavy drinkers are susceptible to dental caries and gingivitis (Friedlander et al. 2003), conditions that may be exacerbated by concurrent tobacco use, which is common in people with AUD. This alters the microenvironment of the mouth, making it more susceptible to colonization with certain bacteria, including gram-negative bacilli (Fuxench-Lopez and Ramirez-Ronda 1978). Moreover, acute alcohol

intoxication and the resulting decrease in the level of consciousness promotes aspiration of oral secretions into the lower airways because of diminished gag and upper-airway reflexes that would normally protect against this phenomenon. These modifications in the upper airways seem to contribute to the increased risk of lung infections, including those caused by more virulent gram-negative organisms, in chronic heavy drinkers.

This risk further is exacerbated by the negative effects of chronic alcohol ingestion on the lower airways. In particular, animal models have established that chronic excessive alcohol ingestion causes dysfunction of the mucociliary apparatus, an important host defense mechanism responsible for clearing harmful pathogens and mucus from the lower airways (Happel and Nelson 2005). An early experimental study in sheep investigating the effects of alcohol on ciliary beat frequency (CBF) demonstrated a dose-dependent effect, such that low alcohol concentrations actually stimulated CBF, whereas high concentrations impaired it (Maurer and Liebman 1988). Later mechanistic studies found that whereas short-term alcohol exposure causes a transient increase in CBF, chronic exposure desensitizes the cilia so that they cannot respond to stimulation (Wyatt et al. 2004). Alcohol-induced failure of the mucociliary system could interfere with the clearance of pathogens from the airways and thereby may contribute to the increased risk of pulmonary infections in people with chronic heavy alcohol use (Sisson 2007).

Although alcohol’s influences on upper and lower airway host defenses collectively are harmful, its role in causing specific diseases, such as asthma, within the conducting airways is less clear (Ayres 1987), despite some interesting historical references. For example, some documentation in Egyptian papyri dating back to about 2000 B.C.E. suggests the use of alcohol in the treatment of asthma (Leake 1952), although one cannot be certain of the accuracy of the asthma diagnosis in

¹The diagnosis of an AUD was based on the Short Michigan Alcohol Survey Test (Selzer et al. 1975).

these ancient writings. More than 1,000 years later, Hippocrates—who is regarded as the father of Western medicine—noted that wine has a variety of medicinal uses and is specifically beneficial for reducing sputum production (Lucia 1963); again, however, it is not clear if he was referring to asthma as we currently define the syndrome. Much more recently, Salter (1863) described the successful use of alcohol to treat three patients with intractable asthma who had failed all other treatments. In contrast, more modern epidemiological data suggest that chronic heavy drinking is associated with increased odds of all-cause mortality and hospitalization among patients with asthma, although a direct link between asthma control and alcohol use was not investigated (Sumino et al. 2014).

To supplement the various anecdotal reports of using alcohol in the treatment of airway diseases, early mechanistic investigations demonstrated that alcohol itself seems to have bronchodilating properties in asthmatics. However, the effects differed depending on the alcohol concentration used as well as on the route of administration (i.e., intravenous versus oral) (Ayres and Clark 1983*b*; Ayres et al. 1982; Brown 1947; Herxheimer and Stresemann 1963). Moreover, these observations directly conflict with findings that many asthmatics actually report exacerbations of their disease after alcohol ingestion (Ayres and Clark 1983*a*; Breslin et al. 1973; Vally et al. 2000). In an attempt to explain some of these discrepancies, Breslin and colleagues (1973) compared the effects of exposure to different types of alcohol in a clinical study. These analyses found that whereas pure alcohol did not appear to induce bronchial reactivity, some alcoholic beverages worsened asthma symptoms. These findings were the first to suggest that the nonalcohol components and additives of alcoholic beverages may be responsible for inducing asthma, rather than alcohol itself. Similar findings were seen in later studies that examined the effects of red wine in asthma (Dahl

et al. 1986; Vally et al. 2000). However, researchers have not yet been able to determine conclusively if alcohol ingestion has any clinically significant effects on asthma. For example, Bouchard and colleagues (2012) showed that alcohol exposure triggered asthma-like pulmonary inflammation in an allergen-sensitized mouse model. The alcohol-exposed mice exhibited increased numbers of certain inflammatory cells (i.e., eosinophils) in fluid obtained from the lungs (i.e., bronchoalveolar lavage fluid), increased production of the main component of mucus (i.e., mucin), and constriction of the small airways (i.e., decreased bronchiole patency). These effects were not seen in mice that were exposed to alcohol but were not allergen sensitized, suggesting that alcohol can be an important trigger for airway reactivity in the context of an underlying allergic component. In contrast, Oldenburg and colleagues (2012) demonstrated that alcohol actually reduced airway hyperresponsiveness and airway inflammation in a mouse model of allergic asthma.

One potential explanation for the disparate findings in the literature regarding alcohol's role in airway disease is that some forms (i.e., phenotypes) of asthma may be more sensitive to the effects of alcohol than others. One subtype of asthma called aspirin-sensitive asthma or aspirin-exacerbated respiratory disease represents less than 10 percent of all asthma cases (Stevenson and Szczeklik 2006) but accounts for a disproportionately high number of severe asthma cases and can be extremely difficult to diagnose and treat (Berges-Gimeno et al. 2002). Interestingly, alcohol-induced respiratory symptoms are more common in patients with aspirin-exacerbated respiratory disease than in aspirin-tolerant asthmatics (Cardet et al. 2014). These findings suggest that the potential irritant versus bronchodilator effects of alcohol may vary by disease subtype; however, further investigation is necessary to validate these observations.

Alcohol and Acute Lung Injury

ARDS is a severe form of lung injury characterized by fluid accumulation in the lung that is not related to heart problems (i.e., noncardiogenic pulmonary edema) as well as by flooding of the alveolar airspaces with protein-like (i.e., proteinaceous) fluid (Ware 2006; Ware and Matthay 2000). ARDS develops in response to inflammatory stresses, including sepsis, trauma, gastric aspiration, pneumonia, and massive blood transfusions (Ware and Matthay 2000). Originally described by Ashbaugh and colleagues (1967), ARDS is characterized by alveolar epithelial and endothelial barrier disruption, dysfunction of the lipoprotein complex (i.e., surfactant) coating the lung surfaces, and intense inflammation. Together, these alterations profoundly disrupt gas exchange and cause severe respiratory failure. Although much has been learned about the underlying pathophysiology of this syndrome over the past four decades, treatment of ARDS remains essentially supportive, and despite aggressive treatment in intensive care units and mechanical ventilation, the mortality rate for ARDS remains unacceptably high at 30 percent to 50 percent (Arcasoy et al. 2005; Villar et al. 2011; Wang et al. 2014; Ware and Matthay 2000).

The association between alcohol abuse and acute lung injury only has been identified within the past 20 years, when Moss and colleagues (1996) analyzed a patient database at the University of Colorado and reported that patients who were admitted to the hospital with a critical illness and who had underlying alcohol abuse were at about twofold-increased risk for developing ARDS. This original study was limited by the uncertain accuracy regarding the diagnosis of an underlying AUD in the database. However, a subsequent prospective study of 220 patients with septic shock, in whom a more precise diagnosis of an AUD was established using the Short Michigan Alcohol Screening Test, determined that the incidence of ARDS in patients

with AUD was 70 percent (46 of 66), compared with 31 percent (47 of 154) in patients without AUD ($P < 0.001$) (Moss et al. 2003). After controlling for potentially confounding variables, the relative risk of ARDS in alcoholic versus nonalcoholic patients was 3.7:1. Overall, 49 percent (46 of 93) of those patients that developed ARDS had an underlying AUD, consistent with the findings of the earlier study (Moss et al. 1996), in which 51 percent of the patients who developed ARDS were classified as alcoholics. If these findings are extrapolated to the population at large, then alcohol abuse contributes to the development of acute lung injury in tens of thousands of patients in the United States each year.

Alcohol-Related Mechanisms of Lung Injury

Disruption of the Epithelial Barrier

The recognition that excessive chronic alcohol ingestion has such a dramatic and independent effect on the risk of acute lung injury prompted a search for the underlying mechanisms. Because one of the cardinal features of ARDS is disruption of the alveolar epithelial barrier that regulates the fluid content of the airspace, this was a logical target for investigation. Maintaining the fluid balance of the alveolar space is critical for normal gas exchange. Acute lung injury involves the rapid development of noncardiogenic pulmonary edema, and patients with impaired alveolar epithelial fluid clearance are three times more likely to die from ARDS than patients with a maximal ability to clear lung fluid (Ware and Matthay 2001). Although the fluid balance in the lungs is regulated by the concerted actions of both epithelial and endothelial barriers (Mehta et al. 2004), it is the alveolar epithelium which primarily prevents protein and fluid flow into airspaces (Mutlu and Sznajder 2005). A pathological hall-

mark of ARDS is heterogeneous damage of the alveolar epithelium, with complete loss of the epithelial surface in some areas, whereas other alveoli remain relatively intact. Therefore, at a cellular level the extent of the alveolar epithelial damage may not be as widespread or as uniform as chest X-rays may suggest, and preservation and repair of the alveolar epithelium are key to survival.

In experimental animal models, chronic alcohol ingestion for as little as 6 weeks renders the lung susceptible to acute edematous injury (Holguin et al. 1998; Velasquez et al. 2002). In these same models, chronic alcohol ingestion produces a lasting defect in the ability of the alveolar epithelium to form and/or maintain a tight physical barrier; specifically, primary alveolar epithelial cells isolated from alcohol-fed animals form relatively leakier monolayers in culture, even if there is no alcohol in the culture medium (Guidot et al. 2000). In addition, the permeability of the alveolar epithelium to large proteins *in vivo* is increased approximately fivefold in the alcohol-fed rats (Guidot et al. 2000). The mechanisms by which alcohol impairs the alveolar epithelial barrier are still being investigated. Animal models suggest that chronic alcohol ingestion interferes with the expression and formation of tight junction complexes within the alveolar epithelium (see figure 1) (Fernandez et al. 2007). Tight junctions are closely associated areas of two cells where the membranes of the cells join together; they are critically necessary to form an impermeable barrier that can limit the passage of even very small molecules across cell layers (Koval 2013; Mitic et al. 2000; Schneeberger and Lynch 1992). Only a few studies of alcohol's effects on the alveolar epithelium have been conducted in humans. The findings indicate that people with AUD have impaired alveolar-capillary permeability at baseline and develop more pulmonary edema in the setting of ARDS compared with people without AUD (Berkowitz et al. 2009; Burnham et al. 2009).

The experimental evidence that alcohol can cause a profound defect in the physical barrier of the alveolar epithelium led to the question of why alcohol abuse alone, in the absence of an acute stress such as sepsis, does not cause pulmonary edema. Additional studies revealed that alcohol causes a concurrent, and perhaps compensatory, increase in salt and water transport across the epithelium. This transport is mediated by specific epithelial sodium channels located in the apical membrane and by protein pumps (i.e., Na/K-ATPase complexes) in the basolateral membrane of the epithelial cells. The expression and function of both the Na/K-ATPase complexes and epithelial sodium channels are increased in the alveolar epithelium of alcohol-fed animals (Guidot et al. 2000; Otis et al. 2008). Thus, as long as there are no additional stresses, the alcoholic lung seems to be able to limit edema formation by upregulating salt and water transport across the epithelium, thereby compensating for the marked increase in the leakage of fluid between cells (i.e., paracellular leakage) into the airways. In the presence of an acute inflammatory stress, such as sepsis or aspiration, however, the paracellular leak increases dramatically, and the alveoli flood with proteinaceous edema fluid that overwhelms the already upregulated transepithelial pumping mechanisms. This scenario is supported by findings in laboratory animals that even at baseline, the lungs of alcohol-exposed animals are unable to clear a salt and water challenge as efficiently, despite the compensatory increase in epithelial sodium channel and Na/K-ATPase function, reflecting the severe permeability defect in the paracellular barrier mechanisms (Guidot et al. 2000).

Reduced Antioxidant Levels

Another fundamental mechanism that appears to drive many of the pathophysiological manifestations of the alcoholic lung phenotype is a severe depletion of glutathione stores within

the alveolar space. Glutathione is the primary thiol antioxidant found in the alveoli; it serves an essential function in reactions catalyzed by the enzyme glutathione peroxidase, which clears harmful hydrogen peroxide and lipid hydroperoxides that readily form in the oxidizing environment of the lung. In both experimental animal models and humans, chronic alcohol ingestion causes a profound decrease of up to 80 percent to 90 percent in alveolar glutathione levels (Holguin et al. 1998; Moss et al. 2000). This glutathione depletion cannot be explained by dietary deficiency or smoking because it also occurs in experimental animals with an otherwise sufficient diet (Holguin et al. 1998); moreover, otherwise healthy smokers actually have increased glutathione levels within their alveolar space (Moss et al. 2000). Further analyses in experimental models found that alcohol-induced glutathione

depletion seems to mediate the defects in alveolar epithelial barrier function. For example, when glutathione precursors such as procysteine or S-adenosylmethionine (SAME) were added to the diet of alcohol-fed animals, the animals' alveolar epithelial barrier function was restored, and they no longer exhibited increased susceptibility to acute edematous injury compared with control-fed animals (Guidot et al. 2000; Holguin et al. 1998; Velasquez et al. 2002).

Another key function of the alveolar epithelium, namely the synthesis and secretion of surfactant—which is required to maintain alveolar integrity and gas exchange—also is impaired by chronic alcohol ingestion (Holguin et al. 1998). This impairment also is mediated by glutathione deficiency in the cells, and particularly in the mitochondria, and is reversible with dietary procysteine supplementation (Guidot

and Brown 2000). Although these animal models provide convincing evidence implicating glutathione depletion as a mediator of alveolar epithelial barrier dysfunction, additional studies in humans are necessary to confirm these findings.

The depletion of glutathione within the alveolar space of people with AUD explains many of the alcohol-related defects in the function of the alveolar epithelium as well as in the function of immune cells called macrophages (which will be discussed in the next section). But how does alcohol lower glutathione so profoundly? Glutathione levels are affected by oxidative stress and inflammation; however, lungs of alcohol-exposed animals show no gross evidence of inflammation or injury at baseline, and otherwise healthy alcoholics likewise have no indication of lung inflammation or oxidative stress. Without evidence of an oxidant assault

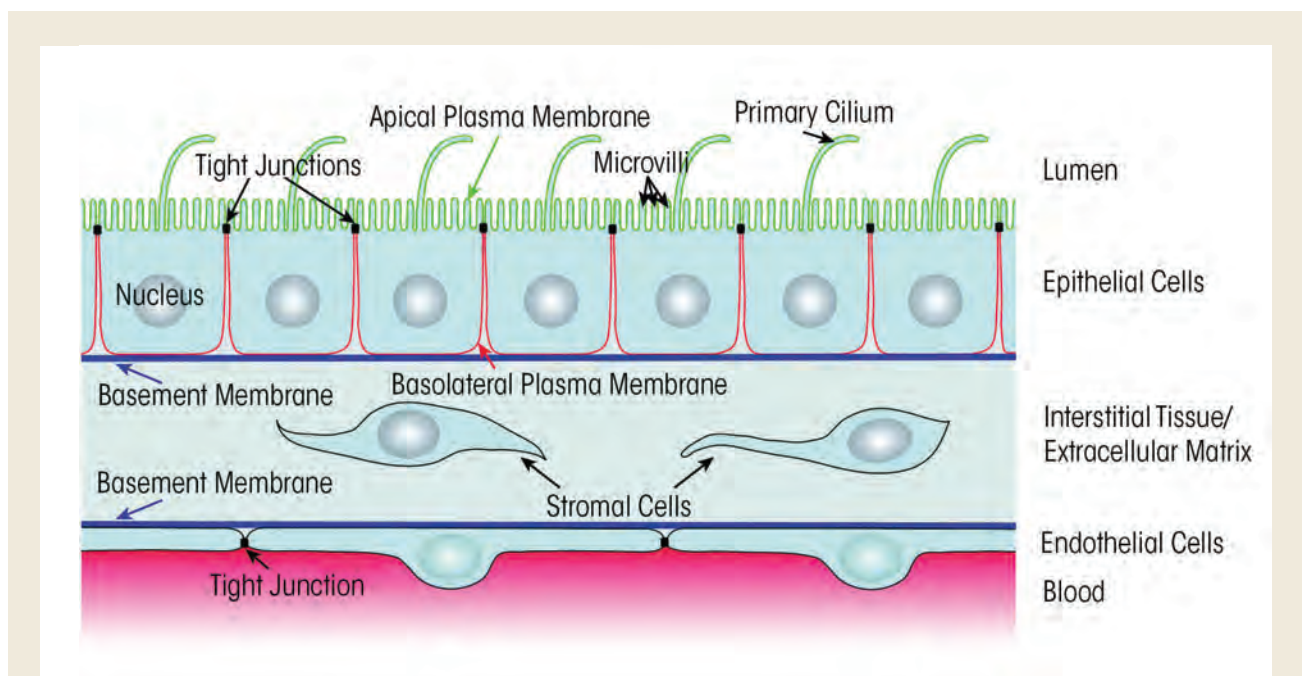


Figure 1 A representation of the alveolar space. In patients with alcohol use disorder (AUD), alterations occur in the tight junctions between alveolar epithelial cells so that protein-rich fluid from the blood can more easily traverse the interstitial tissue and enter the lumen of the alveoli that is normally dry. These and other changes in alveolar epithelial cells predispose people with AUD to developing acute respiratory distress syndrome (ARDS) that is characterized by pulmonary edema.

on the otherwise healthy alcoholic lung, the question remains why there is such overwhelming glutathione depletion. An intriguing answer comes from recent studies showing that, at least in experimental models, chronic alcohol ingestion inhibits the expression and function of a protein called Nrf2. This protein is a master transcription factor that binds to the antioxidant response element (ARE) in the regulatory (i.e., promoter) region of hundreds of antioxidant and immune-response genes (Jensen et al. 2013).

The alcohol-induced inhibition of Nrf2–ARE signaling is mediated at least in part by zinc. Specifically, Nrf2 function depends on adequate zinc levels, and alcohol interferes with the transporter molecules that mediate zinc absorption from the diet as well as its transport into the alveolar space (Joshi et al. 2009). Consistent with this proposed mechanism, dietary supplementation with zinc restores Nrf2 binding to the AREs, preserves the glutathione pool within the alveolar space, and enhances alveolar epithelial barrier function in alcohol-fed rats (Joshi et al. 2009; Mehta et al. 2011; for more information on the role of zinc deficiency and zinc supplementation see the article by Barve and colleagues). These observations suggest that chronic alcohol ingestion lowers antioxidant defenses by interfering with Nrf2-dependent production of antioxidants, including glutathione, and that this may be one mechanism by which alcohol increases the lung's susceptibility to oxidant stress and, consequently, such conditions as sepsis, pneumonia, or infections resulting from aspiration.

Other Mechanisms

The pathophysiological mechanisms discussed thus far undoubtedly are just components of a highly complex network of alcohol-induced cellular perturbations. Experimental animal models have elucidated other key aspects of the alcoholic lung, including the role of two signaling molecules,

transforming growth factor beta 1 (TGFβ1) and granulocyte/macrophage colony-stimulating factor (GM-CSF), both of which help control the growth and function of immune cells, such as macrophages. In healthy people there is relatively little TGFβ1 in the adult lung; instead, alveolar epithelial integrity and the function of alveolar macrophages are under the influence of GM-CSF. Chronic alcohol ingestion increases the expression of TGFβ1 in the lung (Bechara et al. 2004), and its activation during an acute stress situation, such as alcohol-induced transfer of toxic bacterial products from the intestine into the blood (i.e., endotoxemia), seems to further disrupt the already dysfunctional epithelial barrier function (Bechara et al. 2004). Moreover, chronic alcohol ingestion dampens the expression of GM-CSF receptors in alveolar epithelial cells and macrophages (Joshi et al. 2006). This relative imbalance in TGFβ1 and GM-CSF signaling in the alcoholic lung has important implications in the human lung epithelium, and critically ill patients with relatively higher ratios of TGFβ1 to GM-CSF in their alveolar space seem to have a higher mortality (Overgaard et al. 2015). The role of these two signaling molecules is supported by the observation that treatment with recombinant GM-CSF can rapidly restore alveolar epithelial function in alcohol-fed rats, both in vivo and in vitro (Pelaez et al. 2004).

Alcohol's effects on TGFβ1 also interface with its effects on antioxidant levels. Thus, in animal models the alcohol-induced increase in TGFβ1 decreased Nrf2 expression and function and interfered with the resolution of acute lung injury induced by bleomycin; this effect of alcohol could be mitigated by dietary supplementation with SAME (Sueblinvong et al. 2014; for more information on the role of SAME supplementation, see the article by Barve and colleagues). Interestingly, Nrf2 also regulates the expression of PU.1, a master transcription factor that mediates GM-CSF–dependent signaling (Staitieh et al. 2015). Accord-

ingly, alcohol-induced reduction of Nrf2 also inhibits binding of PU.1 to its nuclear targets, which can be improved by zinc treatment (Mehta et al. 2011). Thus, alcohol impairs epithelial barrier function in the lung through a complex set of mechanisms with several cycles and feedback mechanisms (see figure 2); however, future studies will almost certainly elucidate further details.

Alcohol, Alveolar Macrophages, and Pneumonia

Lung infections are major causes of morbidity and mortality worldwide. Data from the Centers for Disease Control and Prevention consistently show that pneumonia is one of the top 10 causes of death in the United States and remains the leading cause of death from an infection (Murphy et al. 2013). Alcoholism has been linked to pulmonary infections for over 200 years (Mehta and Guidot 2012). Additionally, recent studies have demonstrated that people who abuse alcohol are not only more likely to develop pneumonia, but also are susceptible to more severe forms of the disease, are more likely to experience complications, and require greater use of resources. A prospective study by Adamuz and colleagues (2011) examined features associated with increased use of health care services and risk for readmission in patients discharged with pneumonia. Interestingly, the only independent risk factor associated with increased health care utilization after discharge was alcohol abuse. Similarly, Chalmers and colleagues (2009) demonstrated in a multivariate regression model that alcohol abuse was among independent risk factors that were significantly associated with the development of certain complications (i.e., complicated parapneumonic effusion or empyema) in patients with community-acquired pneumonia.

Another experimental study using a pulmonary infection model of respiratory syncytial virus in mice found that

chronic alcohol ingestion caused not only more severe infections, but also influenced the levels of various signaling molecules (i.e., cytokines), inducing a more robust proinflammatory cytokine profile (Jerrells et al. 2007). In this particular study, pulmonary inflammation in alcohol-exposed mice persisted for more than 7 days after infection, compared with 3 to 5 days in the control animals. Moreover, some alcohol-exposed mice showed severe inflammation with hemorrhage and edema. These results corroborate findings that infection in the setting of alcohol exposure increases the risk of complications such as ARDS. Similarly, other studies showed that people with AUD not only are more prone to develop community-acquired pneumonia, but are likely to suffer from infections that portend a worse prognosis and are more likely to be caused by virulent microorganisms that are more challenging to treat (Chen et al. 2001; Fernandez-Sola et al. 1995).

The mechanisms by which chronic and excessive alcohol consumption increases susceptibility to pneumonia are multifactorial. In addition to the already-discussed alterations in bacterial colonization and impaired host defenses in the upper and lower airways, increasing evidence suggests that chronic alcohol ingestion negatively impacts the immune functions of alveolar macrophages in a manner that is similar to its effects on epithelial barrier function. The alveolar macrophage is the primary immune cell in the alveolar space and is responsible for maintaining homeostasis of the lower airways through phagocytosis of pathogens and removal of debris. Animal studies have shown that chronic alcohol exposure causes significant alveolar macrophage dysfunction, leaving these normally active immune cells poorly equipped to phagocytose or kill invading organisms (Brown et al. 2009; Joshi et al. 2009). Alveolar macrophages in alcohol-exposed animals also exhibit decreased production

of important chemokines and mediators, which impairs their ability to recruit other cell types, namely neutrophils, during times of stress and infection (Happel et al. 2004). Although the majority of data focuses on the effects of chronic alcohol ingestion, experimental evidence further suggests that even acute exposure has similar detrimental effects on alveolar macrophage immune function, although these defects readily resolve (Libon et al. 1993). Taken together, these alcohol-mediated defects in alveolar macrophage function contribute to increased vulnerability to pulmonary infections.

Mechanisms of Alcohol's Effects on Alveolar Macrophages

The precise mechanisms by which alcohol impairs alveolar macrophage immune function have yet to be elucidated; however, several observations indicate that the macrophages are subjected to an altered environment

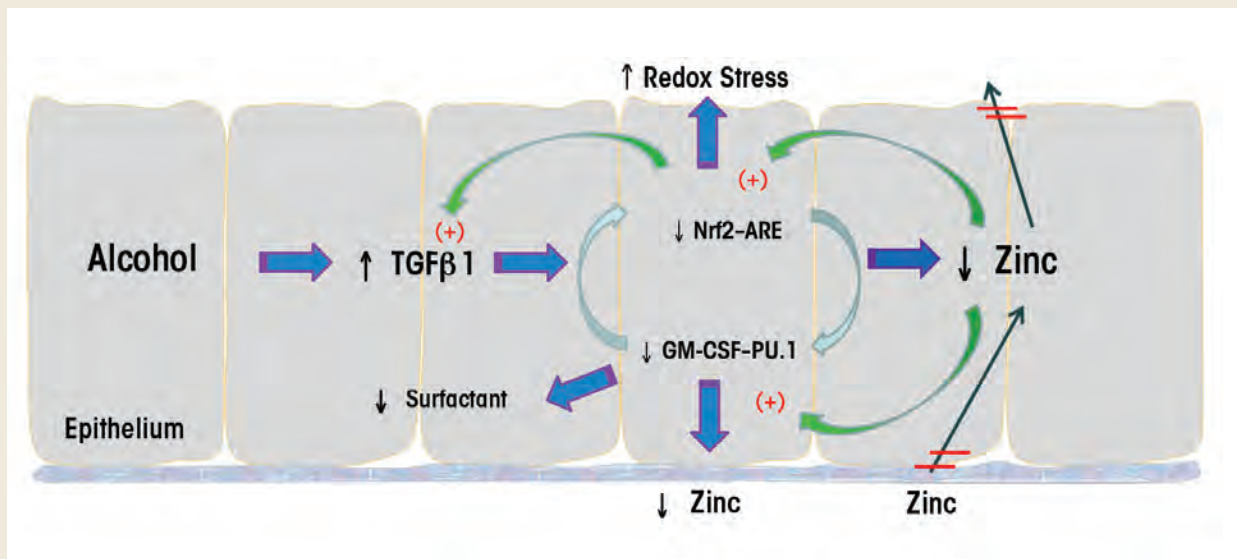


Figure 2 Hypothetical scheme of alcohol's effects on the alveolar epithelium. Alcohol induces aberrant transforming growth factor beta1 (TGFβ1) expression in the alveolar epithelium and thereby dampens signaling through the granulocyte/macrophage colony-stimulating factor (GM-CSF)–PU.1 and Nrf2–antioxidant responsive element (ARE) signaling pathways. As a consequence, the expression and function of transporters that regulate zinc import and export across the epithelium are disrupted, further inhibiting these zinc-dependent pathways and exacerbating TGFβ1 expression. This results in an increase in redox stress, reduced surfactant levels, and damage to the tight junctions between cells, with severe ramifications for epithelial (and macrophage) function.

characterized by oxidative stress and zinc deficiency. Both clinical and experimental studies have detected increased oxidative stress in the alveolar space after alcohol exposure (Moss et al. 2000; Velasquez et al. 2002). The exact mechanisms responsible for inducing this redox imbalance remain uncertain, but several explanations have been put forth. An experimental rat model of chronic alcohol ingestion identified perturbations in lipid metabolism analogous to what is seen in alcohol-induced fatty liver (Romero et al. 2014). These alterations included suppression of genes responsible for fatty acid metabolism in the lungs of the alcohol-exposed rats, which caused accumulation of triglycerides and free fatty acids in the distal airspaces and resulted in immune dysfunction of the alveolar macrophages. In another model using mice, Yeligar and colleagues (2012) demonstrated that alcohol induced oxidative stress through the upregulation of specific enzymes called NADPH oxidases, which are an important source of oxidants called reactive oxygen species in alveolar macrophages. A similar pattern of NADPH upregulation existed in human alveolar macrophages isolated from people with AUD. Restoring the redox balance in the lung could reverse many of these alcohol-induced defects and improve alveolar macrophage immune function (Brown et al. 2007; Yeligar et al. 2014).

Also, as noted above, chronic alcohol ingestion interferes with Nrf2 signaling in alveolar macrophages (Mehta et al. 2011), thereby disrupting the expression of hundreds of genes that are crucial to combatting oxidative stress. Although the precise role of alcohol-mediated inhibition of the Nrf2–ARE pathway in mediating oxidative stress has not been completely clarified, this pathway represents a strategic target to direct future therapies.

Another important player in alveolar macrophage-mediated immunity is zinc. In experimental models, alveolar macrophages from alcohol-fed animals exhibit zinc deficiency in the fluid of

the epithelial lining and have decreased intracellular zinc levels compared with alveolar macrophages from control-fed animals (Joshi et al. 2009). These findings have been confirmed in alveolar macrophages collected from otherwise-healthy people with underlying AUD, even though these individuals had normal serum levels of zinc (Mehta et al. 2013). Zinc is important for diverse immune functions, and its severe deficiency within the alveolar space may be one mechanism by which alcohol impairs innate immune functions within the lung. This role is further supported by findings that restoration of zinc bioavailability in the alveolar space also restores the phagocytic capacity of alveolar macrophages (Joshi et al. 2009).

As discussed previously, alcohol not only alters the environment of the alveolar space but also directly affects GM-CSF signaling, which regulates the maturation, terminal differentiation, and function of alveolar macrophages. Chronic alcohol ingestion downregulates the expression of GM-CSF receptors on the cell surface of the alveolar macrophages, thereby impairing their immune function (Joshi et al. 2005). Experimental models demonstrate that restoration of GM-CSF signaling reverses this alcohol-induced dysfunction (Joshi et al. 2005), suggesting that this might be a potential therapeutic approach. Also, as mentioned earlier, recent evidence suggests that interactions exist between Nrf2 and the GM-CSF pathway, with Nrf2 regulating the expression and activity of the transcription factor PU.1, which controls GM-CSF expression (Staitieh et al. 2015). Understanding the complex interplay between all of these systems in the alcoholic lung will become exceedingly important in the search for new and effective treatments. For example, zinc supplementation in experimental models of chronic alcohol ingestion improves redox balance, enhances Nrf2 binding in the nucleus, corrects alveolar macrophage immune dysfunction, and restores GM-CSF receptor expression and signaling,

suggesting that one target can interact with several implicated pathways (Joshi et al. 2009; Mehta et al. 2011).

Overall, these alterations in host defense and immune dysfunction explain how chronic excessive alcohol ingestion predisposes to pulmonary infection. It is important to realize, however, that the effects of alcohol on alveolar macrophage innate immune function are just one facet of the complex pathophysiology of alcohol and the lung's immune system. Alcohol also impairs neutrophil migration to the infected lung, and abnormalities in this and other components of the adaptive immune response clearly are involved but are beyond the scope of this brief review.

Potential Therapeutic Strategies for the Alcoholic Lung

Currently there are no specific therapies that can modify the alcoholic lung in the clinical setting. Clearly, as with all alcohol-related health issues, the ideal treatment would be abstinence in people with underlying AUD and/or a safe level of consumption in people who choose to drink for social reasons. However, this ideal will be impossible to achieve in any meaningful time-frame and it therefore is critical to identify, test, and validate therapeutic strategies that can limit the morbidity and mortality of alcohol-related diseases, including acute lung injury and pneumonia.

For identifying candidate approaches, it is important to recognize that a large percentage of people with AUD are otherwise healthy and can be identified by relatively simple health-screening questionnaires well before they develop serious organ dysfunction (de Oliveira et al. 2014; Spithoff and Kahan 2015). Also, many people with AUD seek treatment, and structured alcohol treatment programs offer an opportunity to initiate adjunctive therapies designed to enhance lung health. The experimental results reviewed in this article provide some suggestions

Glossary

Adduct: A product of a direct addition of two or more distinct molecules, resulting in a single reaction product.

Alveolar: Pertaining to the *alveoli*.

Alveoli: The small sac-like structures at the end of the airways where gas exchange occurs.

Antioxidant: A substance (e.g., glutathione and vitamins A and E) that inhibits oxidation, serving as a defense against harmful free radicals and *oxidative stress*.

Aspiration: Entry of material (e.g., food, drink, saliva) from the digestive tract into the airways.

Bronchoalveolar Lavage: Procedure where fluid is injected through a tube into a small airway of the lung and then collected and tested for the presence of bacteria and other compounds; the procedure is used to diagnose infections and other lung diseases.

Bronchodilating: Causing widening of the airways.

Cilia: Fine, hair-like structures on the surface of *epithelial cells* lining the airways; their coordinated movement helps clear microorganisms and other particles as well as mucus from the airways.

Edema: Abnormal accumulation of fluid in the tissues and body cavities; typically causes swelling of the tissues.

Empyema: Collection of pus in a cavity of the body; if pus accumulation occurs in the pleural cavity, it

is considered a subtype of *parapneumonic effusion*.

Endothelial Cell: Type of cell lining the interior of body cavities and blood vessels; endothelial cells control the passage of materials and the transit of white blood cells into and out of the bloodstream.

Epithelial Cell: Type of cell lining the tissues exposed to the outside of the body, such as the skin, but also the lining of the intestine and airways.

Gingivitis: A common gum disease that causes irritation and inflammation of the gums, characterized by redness and swelling of the gums.

Granulocyte/Macrophage Colony-Stimulating Factor (GM-CSF):

Cytokine released by various white blood cells and lung *epithelial cells* that stimulates the growth of additional white blood cells; it is produced as part of the immune response so that activation of a small number of immune cells, particularly macrophages, leads to a rapid increase in the number of these cells.

Leukopenia: Abnormal reduction in the number of white blood cells.

Mitochondria: Structures within cells that generate most of cell's energy through the production of adenosine triphosphate (ATP).

Mucociliary: Pertaining to the mucus and the *cilia* of the *epithelial cells* lining the airways.

Mucociliary Apparatus: The layer of cells lining the surface of the respiratory tract that are covered

by a thin layer of mucus and which carry *cilia*, whose rhythmic beating helps clear the airways.

Oxidative Stress: An imbalance between oxidants (e.g., free radicals) and *antioxidants* that can lead to excessive oxidation and cell damage.

Parapneumonic Effusion: Type of fluid accumulation between the two membranes surrounding the lungs (i.e., in the pleural cavity) that results from pneumonia and other lung disorders.

Permeability: Capacity of membranes (e.g., the walls of the blood vessels) to allow the passage of fluids, small molecules, or even whole cells from one side of the membrane to the other.

Phagocytosis: Process by which certain cells (phagocytes) engulf foreign particles in special small vesicles where they can be destroyed by enzymes.

Promoter: A DNA segment located at the start of a gene's coding sequence that provides a binding site for the enzymes that initiate transcription.

Reactive Oxygen Species (ROS): Highly reactive oxygen-containing free radicals that are generated during oxidative metabolism. ROS can react with and damage lipids, proteins, and DNA in cells, causing *oxidative stress*. Common ROS include hydrogen peroxide, superoxide radicals, and hydroxyl radicals.

Sepsis: Inflammatory response throughout the body in response to

Continued on next page

Glossary (continued)

an infection; can lead to multiple organ failure and death.

Surfactant: Lipoprotein complex formed by *alveolar* cells that helps ensure the lungs' ability to expand during respiration, regulate the size of the *alveoli*, and prevent fluid accumulation in the lungs;

also plays a role in innate immunity of the lungs.

Transforming Growth Factor Beta 1 (TGFβ1): Cytokine that helps control the growth, proliferation, differentiation, and cell death of different types of immune cells;

it thereby helps control the activity of the immune system.

Transcription Factor: A protein that regulates gene expression by binding to *promoters* on the DNA near the start of the gene, thereby activating other proteins involved in transcription.

for promising approaches that could be used in such settings. For example, as discussed previously, clinical studies have shown that even otherwise-healthy people with AUD have glutathione and zinc deficiency within the alveolar space (Mehta et al. 2013; Moss et al. 2000). Moreover, animal studies found that dietary supplementation with zinc and/or a glutathione precursor such as SAME can enhance lung health even in the context of chronic alcohol ingestion (Guidot et al. 2000; Holguin et al. 1998; Joshi et al. 2009; Mehta et al. 2011; Velasquez et al. 2002; also see the article by Barve and colleagues). Accordingly, researchers at the Atlanta VA Medical Center initiated a randomized, placebo-controlled trial of dietary zinc and/or SAME in otherwise-healthy individuals with AUD enrolled in the center's Substance Abuse Treatment Program (available at clinicaltrials.gov, trial NCT01899521). This trial currently is in progress with the goal of determining whether these supplements, alone or in combination, can enhance glutathione and zinc bioavailability in the alveolar space and improve alveolar macrophage immune function.

Another potential therapeutic target is Nrf2, which can be activated by plant-derived compounds (i.e., phytochemicals), such as sulforaphane (Hybertson et al. 2011; Jensen et al. 2013). One clinical study (Burnham et al. 2012) evaluating the effects of 7-day treatment with the Nrf2 activator

Protandim® in patients with AUD did not identify any significant improvement in glutathione levels or epithelial function. However, it is possible that combination therapy with an Nrf2 activator plus zinc and/or SAME may be more effective than zinc and/or SAME alone, and clinical trials in the near future hopefully will be able to answer that question.

The goal of these treatments clearly would not be to make it safe(r) to consume excessive amounts of alcohol. However, just as clinicians try to mitigate the health effects of metabolic syndrome in obese patients using medications that target diabetes, hypertension, or dyslipidemia while the patients struggle with weight loss, it is imperative to decrease the risk of pneumonia, acute lung injury, and other life-threatening complications while people with AUD work to achieve abstinence. There also may be some concerns about alcoholic patients' compliance with chronic oral treatments, such as zinc and SAME supplements. However, many patients with AUD seek care for their addiction precisely because they are motivated to become or remain healthy and, consequently, are likely to adhere to their treatment regimen. Moreover, inadequate adherence to medical regimens is not a concern unique to this patient population but occurs in patients with many chronic medical conditions; examples include the low use of continuous positive airway pressure therapy for obstructive sleep apnea (Russell

2014) and poor adherence with anti-diabetic medications in adults with type 2 diabetes (Sapkota et al. 2015). Even if patients seeking treatment for AUD have equally low adherence rates, tens of thousands of individuals could benefit from these relatively simple and inexpensive treatments every year in the United States alone. Researchers and clinicians are just beginning to scratch the surface of this challenging problem, but the rapid pace of experimental and clinical research in the past two decades offers hope that in the relatively near future the devastating effects of AUD on lung health can be ameliorated.

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Alcohol's Effects on the Cardiovascular System

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Alcohol use has complex effects on cardiovascular (CV) health. The associations between drinking and CV diseases such as hypertension, coronary heart disease, stroke, peripheral arterial disease, and cardiomyopathy have been studied extensively and are outlined in this review. Although many behavioral, genetic, and biologic variants influence the interconnection between alcohol use and CV disease, dose and pattern of alcohol consumption seem to modulate this most. Low-to-moderate alcohol use may mitigate certain mechanisms such as risk and hemostatic factors affecting atherosclerosis and inflammation, pathophysiologic processes integral to most CV disease. But any positive aspects of drinking must be weighed against serious physiological effects, including mitochondrial dysfunction and changes in circulation, inflammatory response, oxidative stress, and programmed cell death, as well as anatomical damage to the CV system, especially the heart itself. Both the negative and positive effects of alcohol use on particular CV conditions are presented here. The review concludes by suggesting several promising avenues for future research related to alcohol use and CV disease. These include using direct biomarkers of alcohol to confirm self-report of alcohol consumption levels; studying potential mediation of various genetic, socioeconomic, and racial and ethnic factors that may affect alcohol use and CV disease; reviewing alcohol–medication interactions in cardiac patients; and examining CV effects of alcohol use in young adults and in older adults.

Key words: Alcohol consumption; alcohol use patterns; alcohol effects and consequences; cardiovascular system; heart; hypertension; coronary heart disease; stroke; peripheral arterial disease; cardiomyopathy; atherosclerosis; inflammation; alcohol-related research

Data from numerous epidemiologic studies over the last two decades have revealed complex associations between alcohol use and cardiovascular (CV) conditions such as hypertension (HTN), coronary heart disease (CHD), stroke, peripheral arterial disease (PAD), and cardiomyopathy. In particular, these associations are strongly modulated by the dose and pattern of alcohol consumption. Low-to-moderate daily alcohol consumption (i.e., <15 to 20 g/day, 1 to 2 standard drinks) is associated with a reduced risk of CV disease and mortality, whereas greater amounts of alcohol consumption and a binge pattern of drinking (see definition in “Alcohol Consumption: Categories, Measurement, and Patterns”) have been linked to an increased risk. Consequently, the effects of alcohol

consumption can be a double-edged sword. This article reviews these effects of alcohol consumption on CV conditions, such as HTN, CHD, stroke, PAD, and alcoholic cardiomyopathy, as well as mechanisms that may mediate the positive and the adverse effects of alcohol.

Alcohol Consumption: Categories, Measurement, and Patterns

There are certain factors that are critically important to understanding and interpreting the data related to the health consequences of alcohol consumption. For example, how was alcohol consumption measured? What were the drink sizes and alcohol concentrations? How often did the

subjects drink alcohol? What was the pattern of drinking? And was the study prospective (following subjects over time) or aggregate (pulling together data from several different studies to look for common trends)?

The way in which alcohol consumption has been measured and categorized varies, sometimes making it challenging to compare data among studies. More studies today report alcohol consumption in terms of either “drinks” or grams/units of ethanol per day or week, and alcohol consumption is measured by self-report. Most investigators also define the amount of alcohol that constitutes a “standard” drink as 12 to 15 g (with only slight variation).

Despite the progress in standardizing measurement of alcohol, studies still vary in how they define the different

levels of drinking, such as low-risk or moderate and heavy drinking. Most often, low-risk or moderate drinking has been defined as 1 to 2 standard drinks per day and heavy alcohol consumption as 4 or more standard drinks per day. However, ascertaining the exact alcohol consumption threshold for determining both the benefit and risk has been challenging, and threshold levels continue to differ across studies. Additional factors make it difficult to interpret the results of these studies, including underreporting of alcohol consumption, study design characteristics (case-control studies), and unaccounted confounding variables such as socioeconomic or lifestyle characteristics that may inadvertently affect results (Emberson and Bennett 2006).

Advances are being made to address these factors. For example, alcohol consumption typically has been measured through self-report. Future studies would benefit from using direct biomarkers of alcohol consumption, such as phosphatidylethanol (PEth), to corroborate self-report of alcohol consumption and distinguish among low, moderate, and heavy alcohol consumption (Kechagias et al. 2015; Piano et al. 2015). With this in mind, the National Institute on Alcohol Abuse and Alcoholism (NIAAA) sponsored a biomarker research challenge to discover and develop biomarkers of alcohol consumption (NIAAA 2015a).¹ Such a biomarker would corroborate self-reported consumption and bring more uniformity of reporting within and across studies.

Another trend in recent studies of alcohol and CV risk and disease is to include a measurement for binge drinking. In most investigations, this means consuming more than 5 standard drinks on a single occasion for men and more than 4 standard drinks for women. NIAAA defines binge drinking as a pattern of drinking alcohol that brings the blood

alcohol concentration to 0.08 percent or above. A typical adult consuming the defined number of standard drinks for binge drinking would reach a blood alcohol concentration of 0.08 in about 2 hours (NIAAA 2015b).

Alcohol's Effects on Blood Pressure and Incident Hypertension

In healthy adults, consuming low-to-moderate amounts of alcohol each day typically has no short-term (i.e., acute) or substantial impact on hemodynamics or blood pressure (BP). However, data suggest that binge drinking (more than 5 standard drinks in a single sitting) is associated with transient increases in BP that range from 4 to 7 mmHg for systolic BP and 4 to 6 mmHg for diastolic BP (Potter et al. 1986; Rosito et al. 1999; Seppä and Sillanaukee 1999).

Nearly four decades ago, using data from the Kaiser Permanente Multiphasic Health Study, Klatsky and colleagues (1977) reported that HTN prevalence (BP $\geq 160/95$ mmHg) in White and Black subjects (men and women) consuming more than 6 drinks per day was about twice as much as in nondrinkers. Using a lower systolic BP cutoff value for the diagnosis of HTN (systolic BP >140 mmHg), data from many studies generally have reaffirmed that high daily levels of alcohol consumption are associated with increased risk for HTN and overall incident HTN. However, data from current meta-analyses indicate that the risk-threshold effect or amount of daily alcohol intake associated with HTN is much lower than originally reported in the Klatsky study.

Halanych and colleagues (2010) examined the relationship between different categories of alcohol consumption and incident HTN (systolic BP ≥ 140 mmHg/diastolic BP ≥ 90 mmHg or antihypertensive medication use) among participants in the CARDIA study. The alcohol consumption categories included never-drinkers (never

drank alcohol at baseline), former drinkers (no alcohol in previous year), light drinkers (<7 drinks/week for men and <4 drinks/week for women), moderate drinkers (7 to 14 drinks/week for men and 4 to 7 drinks/week for women), and at-risk drinkers (>14 drinks/week for men and >7 drinks/week for women). After adjusting for age, gender, body mass index (BMI), smoking status, family history of HTN, and numerous socioeconomic variables, alcohol use generally was not associated with 20-year incidence of HTN. The exception was in European-American women, for whom the risk of incident HTN was lower in those with any current alcohol consumption (Halanych et al. 2010). These CARDIA results differ from meta-analyses and other large prospective studies, such as the Nurses' Health Study II (Thadhani et al. 2002) and the Physicians' Health Study (Camargo et al. 1997; Sesso et al. 2008), which show a relationship between consuming greater levels of alcohol and incident HTN.

In a systematic review and meta-analysis that included 16 prospective studies on the effects of alcohol consumption on the risk of HTN (systolic BP >140 mmHg/diastolic BP >90 mmHg), Briassoulis and colleagues (2012) found that consuming more than 20 g ethanol/day (~ 1 to 2 drinks/day) significantly increased risk of HTN in women, and higher amounts (31 to 40 g/day) increased risk of HTN in men. In women, there was a J-shaped relationship between alcohol consumption and HTN, where consumption of <10 g/day was associated with a reduced risk of HTN, whereas in men the alcohol-risk relationship was more linear (figure 1).

Results from another meta-analysis of 12 cohort studies found a similar dose-response relationship between alcohol consumption and HTN for males. A J-shaped relationship for females showed protective effects at or below consumption levels of 15 g/day (Taylor et al. 2009). These data highlight how gender may be an important modifier of the alcohol threshold level

¹ The results of the NIAAA Wearable Alcohol Biosensor Challenge were announced May 19, 2016, at <https://www.niaaa.nih.gov/research/challenge-prize>.

and can shape the alcohol benefit–risk relationship.

The discrepancy in findings across studies suggests that other characteristics differ among the study subjects. However (and importantly), the meta-analysis by Briasoulis and colleagues (2012) included all of the former studies and found that in the pooled analysis, for both men and women, consuming >20 g ethanol/day (~1 to 2 drinks/day) was associated with a higher risk of developing HTN.

Mori and colleagues (2015) examined the dose-dependent effects of drinking on BP measured at regular intervals in healthy premenopausal women ages 20–45. These repeated measurements allowed comparison of BP among 24 participants at 3 drinking levels, each for a 4-week consumption interval. The study included periods of low-volume and higher-volume alcohol consumption as well as of drinking alcohol-free red wine for each participant, whether or not she initially had been a lower-level or higher-level drinker as defined by the study. Awake

systolic BP and diastolic BP were 2.3 mmHg/1.3 mmHg higher in women who consumed greater amounts of alcohol (146 to 218 g/week, ~2 to 3 standard drinks/day) than in those who drank less (42 to 73 g/week, ~0.5 to 1 standard drink/day) or none at all. There was no BP-lowering effect with lower alcohol amounts. In women, these findings support the data from meta-analyses and prospective studies, suggesting that greater amounts of alcohol consumption may increase BP and contribute to the development of HTN. However, findings from this study do not support a BP-lowering effect at the lower level of alcohol consumption (42 to 73 g alcohol/week, or ~3 to 5 standard drinks). Interestingly, in the Mori study, higher alcohol consumption was associated with a 10 percent increase in high-density lipoproteins (HDLs, which remove cholesterol from the blood and are associated with reduced risk of atherosclerosis and heart disease) and a 14 percent reduction in levels

of fibrinogen (a glycoprotein that helps form blood clots).

Most of the studies included in the Briasoulis meta-analysis examined the effects of alcohol consumption on subjects with Stage I HTN (BP >140/90 mmHg). Fan and colleagues (2014) examined the prevalence of prehypertension (systolic BP/diastolic BP 120 to 139 mmHg/80 to 89 mmHg) and found 52 percent of male current drinkers and 29 percent of female current drinkers had pre-HTN.

To summarize, in both men and women, alcohol consumption at levels above about 1 to 2 drinks per day is associated with HTN. The alcohol–risk relationship tends to be J shaped in women and linear in men. More research is needed to determine if certain ethnic or socioeconomic groups are more vulnerable to alcohol-induced HTN. The American Society of Hypertension and the International Society of Hypertension recommended that men limit their alcohol consumption to no more than 2 drinks a day, and women to no more than 1 drink a

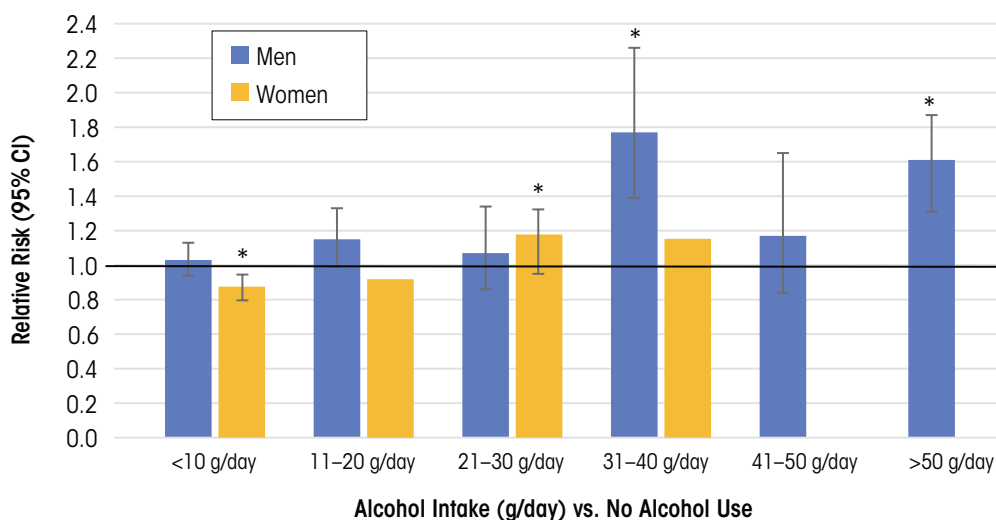


Figure 1 Incidence of hypertension in men and women.

NOTE: * Indicates data significantly different from nondrinkers. For females, data at higher alcohol consumption levels (>40 g/day) were not analyzed.

SOURCE: Data from Briasoulis et al. 2012.

day (Weber et al. 2014). To put the importance of BP control into perspective, at a population level, a 2-mmHg increase in BP increases mortality from stroke by 10 percent and from coronary artery disease (CAD) by 7 percent (Lewington et al. 2002; Mori et al. 2015).

Potential Biologic Mechanisms Underlying Alcohol-Induced BP Effects

Several mechanisms may underlie alcohol's effects on blood pressure. These include impairments in cells that lead to buildup of plaque in arteries (i.e., through alterations in endothelial cell function and nitric oxide availability), and disruptions in arterial-vascular function (i.e., through myogenic mechanisms and changes in baroreceptor function), and hormonal imbalances that control the body's fluid and BP regulation (through the renin-angiotensin-aldosterone system [RAAS]). Some adverse BP-related mechanisms that may be triggered by alcohol include changes in intracellular calcium levels, baroreflex control, and heart rate and activation of other neurohormonal systems besides the RAAS, such as the sympathetic nervous system (Marchi et al. 2014).

Several reports indicate that alcohol first exerts a seemingly positive effect, followed by a more negative impact (i.e., it is biphasic) on the endothelial-nitric oxide-generating system. The endothelium is a key regulator of vascular function. Endothelial dysfunction is an early indicator of blood vessel damage and atherosclerosis, as well as a strong prognostic factor for future CV events (Deanfield et al. 2007; Ras et al. 2013). Low-to-moderate levels of alcohol consumption may initially improve endothelial function, whereas high daily levels and binge drinking may impair it.

Other studies have shown that low-to-moderate concentrations of ethanol (20 mM) increase endogenous nitric oxide synthase (eNOS) expression in certain cells (i.e., human umbilical-

vein endothelial cells) (Liu et al. 2002). Low-to-moderate ethanol consumption in rats (36 percent of caloric intake) for 6 weeks increased nitric oxide production and eNOS expression in the aortic vascular wall (Kleinhenz et al. 2008). Nitric oxide helps regulate vascular tone. eNOS has a protective function in the cardiovascular system, which is attributed to nitric oxide production. However, higher daily ethanol (blood alcohol levels >29 mM) for 6 weeks in another animal model was associated with decreased eNOS expression, increased release of endothelial-derived vasoconstrictor prostanoids, and greater responsiveness of mesenteric arterioles to phenylephrine (Tirapelli et al. 2008). Taken together, these findings show lower amounts of alcohol may have a positive effect on nitric oxide signaling, but higher amounts alter this system and change arteriolar reactivity, which may lead to an increased risk for HTN.

In humans, endothelial function is assessed by measuring the widening (i.e., dilation) of the brachial artery under different conditions. Some research noted that endothelial function is impaired in abstinent individuals with a long-term history of alcohol abuse or alcoholism (Di Gennaro et al. 2007, 2012; Maiorano et al. 1999). Other studies have examined the effect of a single binge-drinking episode and found impairment in brachial artery endothelial-dependent and -independent vasodilation (Bau et al. 2005; Hashimoto et al. 2001; Hijmering et al. 2007). Therefore, as in animal studies, the effects of ethanol on endothelial function in humans likely depend on the dose and duration of ethanol consumption.

Vascular wall oxidative stress also is a key mechanism in ethanol-induced HTN. Oxidative stress is an imbalance between production of free radicals and the body's ability to detoxify or fight off their harmful effects through neutralization by antioxidants. Various studies with animals and humans indicate that ethanol can increase the development of reactive oxygen species (ROS), leading to increases in redox-

signaling pathways and decreases in protective antioxidant levels. Alcohol also can increase levels of co-enzymes or reducing equivalents (e.g., reduced nicotinamide adenine dinucleotide phosphate [NADPH]), which lead to increases in ROS formation and decreases in eNOS activity (Ceron et al. 2014). Several excellent reviews offer more detailed assessments of vascular cellular mechanisms (Cahill and Redmond 2012; Husain et al. 2014; Marchi et al. 2014; Toda and Ayajiki 2010).

Alcohol, CHD, and Stroke

The relationship between and among alcohol consumption, CHD, and stroke has been extensively investigated. Many of these studies have been conducted in middle-aged and older people and across populations. These studies have used ecologic designs (in which at least one risk-modifying health factor is measured at the group level); case-control designs (which compare clinical cases with control subjects to determine if an exposure is associated with an outcome); and longitudinal research designs (which gather data from the same subjects repeatedly over a length of time). Examples include the Health Professionals Follow-up Study (Harvard School of Public Health 2016a), the Nurses' Health Study (Harvard School of Public Health 2016b), the Framingham Heart Study (National Heart, Lung, and Blood Institute and Boston University 2016), the British Doctors Study, the Physicians' Health Study,² the Copenhagen City Heart Study (Schnohr et al. 2002), and INTERHEART (Leong et al. 2014). The availability of these diverse datasets has allowed for completion of several comprehensive systematic reviews and meta-analyses of alcohol, CHD, and stroke relationships. This section summarizes data from meta-analyses, along

² *British Doctors Study*. Available at: <http://www.epi.umn.edu/cvdepi/study-synopsis/british-doctors-study/>. Accessed December 13, 2016. *Physicians' Health Study*. Available at: <http://pshs.bwh.harvard.edu/>. Accessed December 13, 2016.

with data from large international studies such as INTERHEART (Leong et al. 2014) and other recent studies using new methodologies such as Mendelian randomization (reviewed below in “Alcohol Consumption and Total Stroke Incidence and Prevalence”).

Alcohol Consumption and CHD

In a comprehensive systemic review and meta-analysis, Ronksley and colleagues (2011) analyzed data from several prospective studies ($n = 84$), of which 40 percent reported on all-male cohorts, 7 percent reported on women only, and 53 percent included men and women. The most data-adjusted analysis, which included both men and women, noted that various alcohol consumption levels (g/day) among active drinkers compared with non-drinkers were associated with a reduced relative risk for CV mortality, incident CHD, and CHD mortality. Alcohol consumption levels between 2.5 g/day and 30 to 60 g/day (<1 standard

drink/day to ~5 drinks/day) were cardioprotective for both CV mortality and CHD mortality (figure 2). However, the association between alcohol consumption and CV mortality was insignificant when alcohol consumption was >60 g/day, but remained significantly reduced for CHD mortality.

Findings from INTERHEART, a 52-country case-control study of individuals with first myocardial infarction (MI), also supported the fact that “alcohol use” was associated with a reduction in the odds ratio for first-time MI (table 1) (Leong et al. 2014). In addition, in the 24 hours after alcohol use, there was no effect of “alcohol use” on risk of MI.

It is important to note that, unlike other studies with more discrete alcohol consumption categories, alcohol use was nonspecifically defined in INTERHEART as the consumption of at least 1 alcoholic beverage within the previous 12 months (Leong et al. 2014). Interestingly, the strength of this association was not consistent across different geographic regions. Alcohol use was

protective against CHD for subjects in most countries, except for people of South Asian ethnicity living in South Asia (India, Bangladesh, Nepal, Pakistan, and Sri Lanka). INTERHEART results also suggested that the protective effect of any alcohol use against MI was greater in women and those over age 45. Finally, data from INTERHEART support the finding that the risk of MI is increased in the 24 hours after consumption of 6 or more drinks, suggesting that binge drinking increases MI risk (table 1).

Mostofsky and colleagues (2016) conducted a systematic review and meta-analysis ($n = 23$ studies) to examine the effects of alcohol consumed in the 24 hours before MI onset. These investigators found a U-shaped relationship between alcohol intake and MI risk, with the greatest benefit occurring after ~28 g of alcohol (~2 drinks, or moderate consumption) in 1 day and a higher risk after ~108 g (~9 drinks, or heavy consumption) in 1 day. Within a week after alcohol consumption, there was a lower risk of

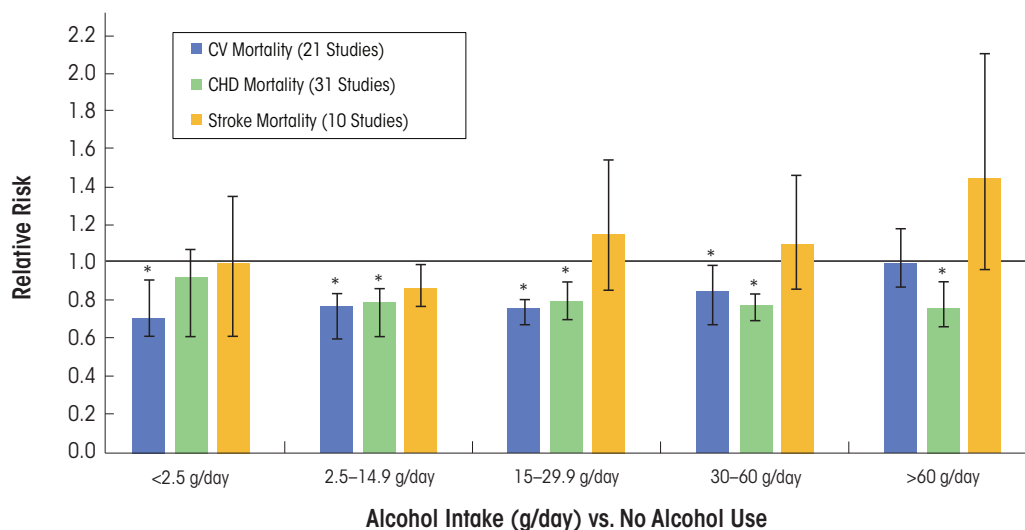


Figure 2 Relative risks (95% confidence intervals) for cardiovascular (CV), coronary heart disease (CHD), and stroke outcomes.

SOURCE: Data used from Ronksley et al. 2011.

MI with moderate alcohol consumption but a greater risk with heavy alcohol consumption (Mostofsky et al. 2016).

Alcohol Consumption and Total Stroke Incidence and Prevalence

Many epidemiologic studies also have been conducted to evaluate the association between alcohol consumption and total stroke incidence and prevalence, as well as the separate effects on specific stroke subtypes (e.g., ischemic and hemorrhagic). In the same systematic review and meta-analysis noted above, Ronksley and colleagues (2011) systematically examined the relationships between and among different levels of alcohol consumption and incident stroke and stroke mortality. They found a decrease or no effect on relative risk for incident stroke and stroke mortality, respectively, at <2.5 g and 2.5 to 14.9 g of alcohol/day, and almost no overall associations of alcohol consumption with levels between 15 to 29.9 g and 30 to 60 g of alcohol/day (figure 2). For heavier drinkers (60 g/day) the risk for incident stroke was greater compared with abstainers, and the risk for stroke mortality was about one and a half times greater (figure 2). A subanalysis of stroke subtypes revealed that when pooling the risk among current alcohol drinkers compared with nondrinkers, the risk was actually higher for incident hemorrhagic stroke than for ischemic stroke ($n = 12$ studies) (Ronksley et al. 2011).

In another analysis of these same studies, Zhang and colleagues (2014) found a J-shaped relationship between alcohol intake and stroke. Compared with no alcohol intake, consumption of 0 to 20 g/day (or <2 drinks) was associated with a reduced risk of total stroke, ischemic and hemorrhagic stroke, and stroke mortality. However, alcohol levels >30 g/day (>2 drinks), and in particular >45 g/day (>3 drinks), were associated with increased risk of all stroke outcomes. It is important to note that in this meta-analysis, “low-alcohol intake” also included

“no alcohol intake” or 0 g/day (Zhang et al. 2014).

New Methods for Analyzing Alcohol Consumption and Stroke-Related Outcomes

Investigators are using new methods to examine the relationship between alcohol consumption and CV outcomes. One such method includes Mendelian randomization, an epidemiologic study design that incorporates genetic information into traditional epidemiologic methods. Mendelian randomization offers an opportunity to test the relationship between a causal factor (e.g., alcohol consumption) and a specific outcome (e.g., CV disease). Holmes and colleagues (2014) used Mendelian randomization to determine if a relationship exists between drinkers with a certain variant in the alcohol dehydrogenase *ADH1B* gene (rs1229984), which is associated with reduced alcohol consumption, and the likelihood of having fatal or nonfatal

CHD and stroke. The investigators found that individuals with the A allele variant *ADH1B*rs1229984 consumed less alcohol and had a reduced risk of CHD and ischemic stroke compared with noncarriers. This implies that lower alcohol consumption, even in light-to-moderate drinkers, was beneficial for CV health. These results challenge the findings from several of the studies mentioned earlier that support a cardioprotective effect of low-to-moderate alcohol consumption. They also suggest that traditional epidemiologic studies may not capture important nuances related to selection bias or other errors that can affect study results (Chikritzhs et al. 2015; Holmes et al. 2014).

Conclusions About Alcohol Consumption, CHD, and Stroke

Based on these findings in both men and women, alcohol consumption of about 1 to 2 drinks per day is associated with a decrease in CHD.

Table 1 INTERHEART Data, Patterns of Alcohol Use, and Odds Ratio (OR) for Myocardial Infarction (MI).

Pattern of Alcohol Use	OR (95%) for MI	p value
Any alcohol use within 12 months		
Unadjusted	0.92 (0.87–0.96)	<0.001
Adjusted (model 1)	0.81 (0.76–0.87)	<0.001
Adjusted (model 2)	0.87 (0.80–0.94)	0.001
Pooled*	0.84 (0.71–0.99)	0.04
Any alcohol use and risk of MI in subsequent 24 hours		
≥ 6 Drinks and risk of MI in subsequent 24 hours	1.0 (0.9–1.2)	0.70
≥ 6 Drinks and risk of MI in subsequent 24 hours	1.4 (1.1–1.9)	0.01

NOTE: Model 1 was adjusted for age (categorized as <45, 45–65, and >65 years), gender, geographic region, Dietary Risk Score, exercise, smoking, marital status, employment, education level, depression, stress at work or at home, financial stress, body mass index (BMI), and waist-to-hip ratio. Model 2 was adjusted as for Model 1 and for serum ratio of apolipoprotein B to apolipoprotein; total cholesterol, high-density lipoprotein, low-density lipoprotein, and triglyceride concentrations; and history of hypertension or diabetes mellitus. CI = confidence interval; MI = myocardial infarction; OR = odds ratio.

*Pooled effect estimates from conditional logistic regression were stratified by geographic region and adjusted for Dietary Risk score, exercise, smoking, marital status, employment, education level, depression, stress at work or at home, financial stress, BMI, and waist-to-hip ratio.

SOURCE: Used with permission from Leong et al. 2014.

However, alcohol consumption may not have cardioprotective effects in certain racial or ethnic groups, such as in people of South Asian ethnicity living in South Asia (Leong et al. 2014).

Although results related to levels of alcohol consumption and stroke events are less clear, some conclusions can be drawn. Approximately 1 to 2 drinks per day may have no effect on or lead to a slight reduction in stroke events; however, greater daily alcohol levels increase the risk for all stroke events and incident stroke types. In terms of stroke subtypes, compared with nondrinkers, current alcohol drinkers have an increased risk (~14 percent) for hemorrhagic stroke (Ronksley et al. 2011).

Alcohol and Heart Failure

Several studies and meta-analyses have been conducted to determine the relationship between alcohol consumption and the risk of developing heart failure in healthy subjects, as well as in those with a history of MI or CHD. Heart failure is a syndrome that often results from an MI or CHD. Studies also have examined the “safety” of alcoholic beverage consumption in subjects with heart failure.

In a meta-analysis of prospective studies ($n = 8$) of healthy people ages 21–81, Larsson and colleagues (2015) reported that, compared with nondrinkers, the risk for heart failure across different levels of alcohol consumption was greatest for those consuming 12 drinks per week, intermediate for those consuming 3 drinks/week as well as for those consuming 14 drinks/week, and least for those consuming 7 drinks/week. Based on dose–response analysis, consumption of 7 drinks/week was associated with a 17 percent lower risk of developing heart failure.

In contrast, Wannamethee and colleagues (2015) recently examined different levels of alcohol consumption and risk for heart failure in an older population (mean age ~68) and found no evidence that light-to-moderate

drinking had a protective effect on incident heart failure in this age group. On the other hand, drinking ≥ 5 drinks/day (or ≥ 35 drinks/week) was associated with a significant risk of heart failure. In subjects with reduced ejection fraction–related heart failure (with the fraction of outbound blood pumped from the heart with each heartbeat, or ejection fraction, at < 35 percent) and a history of ischemic heart disease or CAD (mean age 59), Cooper and colleagues (2000) found that light-to-moderate drinking (1 to 14 drinks/week) was associated with a significant reduction in progressive heart failure and hospitalization. However, there were no positive effects in subjects with mechanical or electrical dysfunction of heart muscle, or non-ischemic heart disease, and although not significant, there was a slight risk for hospitalization for heart failure.

More recently, Cosmi and colleagues (2015) examined the effects of daily wine consumption in subjects enrolled in an Italian trial of heart failure patients (mean age ~67), most of whom had reduced ejection-fraction heart failure. Different levels of daily wine consumption (i.e., sometimes, 1 to 2 glasses/day, and ≥ 3 glasses/day) had no effect on fatal or nonfatal outcomes (e.g., hospitalization for a CV event). Subjects who drank wine more often, however, were less likely to have symptoms of depression and more likely to have a better perception of health status. They also had lower levels of circulating inflammatory markers, such as C-terminal proendothelin-1 and pentraxin-3 (Cosmi et al. 2015).

Thus, low levels of alcohol consumption (1 to 2 drinks, but not every day) in patients with heart failure may not exacerbate the condition, especially in those with heart failure attributable to ischemic CHD. Because heart failure patients usually are older (over age 65) and often are prescribed numerous medications, both the effects of age and of medication use should be carefully considered by patients, clinicians, and researchers.

Alcohol and PAD

Compared with CHD and stroke, the relationship between alcohol consumption and PAD has been examined less often, and to date there are no meta-analyses or systematic reviews. PAD is used broadly to refer to pathophysiologic conditions affecting the arterial system. Fifteen years ago, two large prospective studies, one from the United States (the Strong Heart Study) (Fabsitz et al. 1999) and one from Europe (the Rotterdam Study) (Vliegenthart et al. 2002), examined the effects of alcohol consumption on PAD. Both studies used the ankle-to-brachial index (< 0.90), which compares BP measured at the ankle with BP measured at the upper arm, as a measure of PAD.

The Strong Heart Study enrolled only American Indian subjects. After controlling for other factors, “current alcohol drinking” in this cohort was inversely associated with PAD prevalence in men and women (Fabsitz et al. 1999). Because more specific information was not available about levels or amounts of alcohol consumption associated with what the researchers called “current alcohol drinking” and only American Indians were included, it is difficult to generalize these findings.

The Rotterdam Study was designed to prospectively evaluate the occurrence of chronic diseases in an aging population (Vliegenthart et al. 2002). Their findings suggest that moderate alcohol consumption had no effect on PAD in nonsmoking men (Vliegenthart et al. 2002). In contrast, nonsmoking women had a significantly lower risk of PAD compared with nondrinking women for all levels of alcohol consumption, with the lowest risks found in women consuming 20 g/day of alcohol, or < 2 drinks (odds ratio 0.32 [95% CI 0.11–0.91]).

Evaluating results from the Cardiovascular Health Study, Mukamal and colleagues (2008) found that drinking 1 to 13 drinks/week was associated with a lower risk of hospitalization related to lower-extremity arterial disease

(defined as the diagnosis of atherosclerosis of native arteries of the extremities or peripheral vascular disease) in older adults (mean age >70) compared with former and nondrinkers. However, older adults who consumed >14 drinks/week did not experience the same reductions in PAD risk. In the latter study, analyses were adjusted for gender and many other confounding variables, but men and women were not analyzed separately.

Xie and colleagues (2010) conducted a large cross-sectional study of Chinese men ages ≥ 35 ($n = 14,618$). They found an inverse association between a key indicator of heart disease (i.e., ankle-to-brachial artery index >0.9) and alcohol consumption of <60 g/day, or about 4 drinks. Drinking ≥ 60 g/day was associated with a decrease in the ankle-to-brachial artery index, indicating greater risk for PAD. In that same study, no effect or relationship was found between any level of alcohol consumption and the ankle-to-brachial artery index in women.

Garcia-Diaz and colleagues (2011) examined the effects of alcohol consumption among PAD patients ($n = 1,073$). The PAD subjects chosen had either cramping pain in the leg brought on by exertion (typically caused by obstruction of the arteries) and known as intermittent claudication, with an ankle-to-brachial index <0.9 ; or they had previous vascular intervention or limb amputation for PAD. These subjects were enrolled in the Factores de Riesgo y Enfermedad Arterial (FRENA) registry, which was designed to examine the natural history of PAD in subjects (men and women) with a mean age >62 . Over an average followup of 13 months, there were no differences between alcohol consumers and nonconsumers in PAD outcomes. However, mortality rates were greater in nonconsumers compared with alcohol consumers. The FRENA registry found mortality benefits across all different levels of alcohol consumption measured (e.g., <20 g/day, 21 to 61 g/day, and >60 g/day). However, as the authors

note, only 19 percent of the subjects in the FRENA registry reported consuming >60 g/day of alcohol, limiting the generalizability of these findings.

As a result, existing data in this area suggest either a weak positive or small inverse relationship between low-to-moderate alcohol consumption (e.g., 1 to 13 drinks/week) and PAD prevalence in men. Compared with other studies, Xie and colleagues (2010) reported a greater “protective” threshold of alcohol consumption (<60 g/day) for PAD in men. However, in that study, the mean age of both male and female participants was ~ 50 years, nearly 15 to 20 years younger than in other studies (Mukamal et al. 2008; Vliegthart et al. 2002). Findings are less clear for women, with some studies reporting a moderate inverse effect (Vliegthart et al. 2002) and others detecting none at all (Xie et al. 2010).

In terms of specific PAD complications, Garcia-Diaz and colleagues (2011) found no differences in PAD outcomes between alcohol consumers and nonconsumers who had PAD. However, varying levels of daily alcohol consumption were associated with lower CV mortality and overall mortality rate (Garcia-Diaz et al. 2011). Larger prospective studies are required to define the association between dose, frequency, duration, and pattern of alcohol use and peripheral vascular disorders more precisely, so that researchers may formulate specific recommendations for men and women with PAD across populations.

Mechanisms Related to Alcohol's Positive and Adverse Effects on CV Conditions

Many of the CV conditions outlined above share the pathophysiologic process of atherosclerosis and inflammation. Therefore, alcohol may exert its protective or enhancing effects on these conditions by modifying three broad categories of mechanisms: risk factors (e.g., lipid profiles, carotid intima-medial thickness [cIMT], and insulin

sensitivity), hemostatic factors (e.g., fibrinogen levels and platelet reactivity), and inflammation. In addition, and specific to CHD, alcohol consumption may modulate ischemia-reperfusion mechanisms as blood flow is restored to tissues after oxygen deprivation. Several of these potential mechanisms are briefly reviewed below.

Risk Factors

One common risk factor for CV disease is the composition of the lipids found in the blood, and the effects of alcohol consumption on lipid profiles have been extensively studied. Many researchers have found that alcohol intake increases HDL cholesterol (HDL-c) levels, HDL (“good cholesterol”) particle concentration, apolipoprotein A-I, and HDL-c subfractions (Gardner et al. 2000; Muth et al. 2010; Vu et al. 2016). Findings have been equivocal for other lipids, such as low-density lipoprotein cholesterol (LDL-c) (the estimated amount of cholesterol within LDL particles, or “bad cholesterol”) and triglyceride levels (Rimm et al. 1999; Volcik et al. 2008; Waskiewicz and Sygnowska 2013). High triglyceride levels in the blood stream have been linked to atherosclerosis and, by extension, increased risk of CHD and stroke. However, in a recently conducted Mendelian randomization study, Vu and colleagues (2016) reported that low-to-moderate alcohol consumption reduced triglyceride and LDL-c and increased HDL-c, in particular the HDL2-c subfraction. Interestingly, the researchers found a nonlinear effect of alcohol consumption on HDL2-c levels. This supports the findings from other studies that the alcohol-induced changes in HDL-c do not fully account for the lower risk of CHD in moderate alcohol drinkers (Mukamal 2012).

Other risk factors that are surrogate markers of atherosclerosis and future CHD events, such as cIMT, also have been examined. The relationship between alcohol consumption and cIMT was

inconsistent. Mukamal and colleagues (2003b) reported that older adults (age >70) consuming 1 to 6 drinks/week had lower cIMT compared with abstainers and those having ≥14 drinks/week. This is in contrast to results from other large population-based studies of older (age >70) (Zureik et al. 2004) or middle-aged (ages 45–64) (Demirovic et al. 1993; Djousse et al. 2002) adults, which did not find a relationship between level of alcohol intake and cIMT.

Some reports suggest that low-to-moderate alcohol consumption is associated with favorable effects in insulin sensitivity and glucose metabolism, key risk factors in the development of diabetes (Greenfield et al. 2005).³ Randomized placebo-controlled trials conducted with nondiabetic postmenopausal women showed that 2 drinks per day significantly lowered insulin levels during fasting and after meals and increased insulin sensitivity (Davies et al. 2002). Increased insulin sensitivity, which is the opposite of insulin resistance, is associated with a reduced risk for the development of type 2 diabetes and CHD.

Hemostatic Factors

Alcohol consumption can be associated with both a favorable hemostatis/coagulation profile as well as an adverse one (Salem and Laposata 2005). Several epidemiologic and randomized controlled studies have found alcohol consumption decreases coagulation factors such as fibrinogen, which is a CV risk marker at elevated levels (Mori et al. 2015; Rimm et al. 1999). In addition to being essential to the coagulation

cascade, fibrinogen also may play a proinflammatory role in the development of certain CV diseases, including vascular wall disease and atherosclerosis (Davalos and Akassoglou 2012). Findings from a meta-analysis of 42 studies by Rimm and colleagues (1999) suggested that 30 g of alcohol/day (2 standard drinks) was associated with a 7.5 mg/dl (–17.7 to 32.7) decrease in fibrinogen concentration. Similarly, the results from the small randomized crossover trial by Mori and colleagues (2015) found that women consuming alcohol (146 to 218 g/week, ~2 to 3 standard drinks/day) for 4 weeks showed a 14 percent reduction in fibrinogen levels.

Platelets and their role in clotting also affect CV disease. Altered platelet responses (e.g., increased platelet activation/aggregation) leads to blood-clot formation (or thrombosis) in certain CV conditions. Anticlotting therapies are therefore the cornerstone of managing acute coronary syndromes. Not surprisingly, alcohol consumption has complex and varying effects on platelet function. Studies using different methodologies have shown that low-to-moderate alcohol consumption decreases platelet activation and aggregation in certain cases—for example, in response to certain physiologic stimuli such as adenosine 5′-diphosphate (Salem and Laposata 2005). On the other hand, significant daily alcohol consumption increases platelet aggregation and reactivity. Infection or other stressful events also can lead to immune-triggered platelet production, a condition called rebound thrombocytosis, which may occur immediately after withdrawal from both heavy and one-time heavy (binge) drinking (Numminen et al. 1996). Although highly individualized and dose dependent, alcohol use also can increase bleeding time (i.e., taking longer to develop a clot) (Salem and Laposata 2005).

Inflammation

The effects of alcohol consumption on inflammation are twofold. Lower doses

are associated with reduced inflammation, as indicated by markers such as C-reactive protein and certain interleukins. Conversely, higher levels induce oxidative stress and a wide variety of inflammatory markers. As reviewed below, oxidative stress in particular is likely a key event in the development of alcoholic cardiomyopathy (discussed in “Acute and Long-term Effects of Alcohol on the Myocardium”). Data from numerous types of research studies show that alcohol may alter levels of antioxidant enzymes and stimulate oxidative damage, and it may therefore be involved in the pathogenesis of many types of alcohol-induced diseases (Ceni et al. 2014; Piano and Phillips 2014).

Ischemic Preconditioning

Another mechanism underlying the cardioprotective effects of low-to-moderate alcohol consumption and CHD in particular may be related to a phenomenon known as ischemic preconditioning, which produces resistance to the loss of blood supply (and oxygen) to organs or tissues. If the blood supply is impaired briefly (usually for <5 minutes) and then restored so that blood flow resumes, and the process is repeated two or more times, the cells downstream of the tissue or organ are protected from a final ischemic insult, when the blood supply is cut off. In the heart, this would protect the heart muscle (myocardium) from subsequent, more prolonged episodes of restricted blood flow (ischemia) followed by injury when that blood flow returns to the heart (called reperfusion injury or ischemia-reperfusion injury; Veighey and Macallister 2012). Ischemic preconditioning results in smaller infarct sizes, fewer and less severe arrhythmias, and prevention of endothelial cell dysfunction (Veighey and Macallister 2012). During the ischemic phase, the flow of oxygen and nutrients to the tissues is reduced, most significantly to the heart, brain, and kidneys. In contrast, during the reperfusion phase, despite

³ Greenfield and colleagues (2005) studied the effects of alcohol at meal time in a group of nonsmoking, healthy postmenopausal women. Each woman was given either no alcohol or 15 g of alcohol (1 standard drink) with either a low-carbohydrate or a high-carbohydrate, high-fat meal. The women's metabolic measurements were then taken over the next 6 hours. The researchers found that the alcohol-drinking subjects (particularly those who were insulin sensitive) had higher insulin levels and a slower rise in glucose levels after a low-carb meal. They recommended confirming these results in younger women and in men, particularly since their subjects had been older women, who have more significant cardiovascular risk.

restoration of blood flow, a series of dysfunctional biochemical and metabolic changes are initiated that lead to extensive accumulation of ROS. ROS induce a number of changes. One is the opening of the mitochondrial permeability transition pore, which is formed in the mitochondria during ischemic incidents, contributing to reperfusion injury and cell death. Others include recruitment of neutrophils (white blood cells that are among the first inflammatory cells to respond during inflammation) and dysfunction of the sarcoplasmic reticulum, which can affect calcium ion storage and release into muscle fibers.

Alcohol may affect various mechanisms implicated in ischemic preconditioning. Among these is the activation of mitogen-activated protein kinases (MAPK) signaling cascades. MAPKs are activated in response to stressful stimuli and help regulate apoptosis. There also is desensitization of the

mitochondrial permeability transition pore, which can mitigate ischemia-reperfusion injury (Walker et al. 2013). In addition, alcohol may attenuate ischemia-reperfusion injury by activating protein kinase C epsilon (PKCε) (Walker et al. 2013). Activation of PKCε may protect the myocardium against ischemia-reperfusion injury by stimulating the opening of mitochondrial ATP-sensitive potassium channels. This in turn prevents the opening of the mitochondrial permeability transition pore (Walker et al. 2013).

Figure 3 summarizes the potential mechanisms underlying the cardioprotective and adverse effects of alcohol consumption. One or more mechanisms may be in effect and/or may cancel out another. This area of research was briefly outlined here; more comprehensive reviews on these mechanisms are available (Krenz and Korthuis 2012; Mathews et al. 2015).

Impact of Drinking Patterns and Types of Alcoholic Beverages on Risk

Drinking patterns, and in particular a binge pattern of drinking and higher frequency of binge drinking, are associated with a heightened risk of CV conditions such as HTN, stroke, and MI, as well as sudden death or increased mortality after MI (Leong et al. 2014; Marques-Vidal et al. 2001; Mukamal et al. 2005; Sundell et al. 2008; Wannamethee and Shaper 1992). In a systematic review ($n = 37$ studies), Feigin and colleagues (2005) found that excessive ethanol intake (>150 g ethanol/week) was associated with a doubled risk of subarachnoid hemorrhage. The latter findings may relate to the overall large quantity of alcohol consumed (~ 12 standard drinks/week) rather than a binge pattern.

Binge drinking in younger individuals also may increase the risk of stroke. Haapaniemi and colleagues (1996)

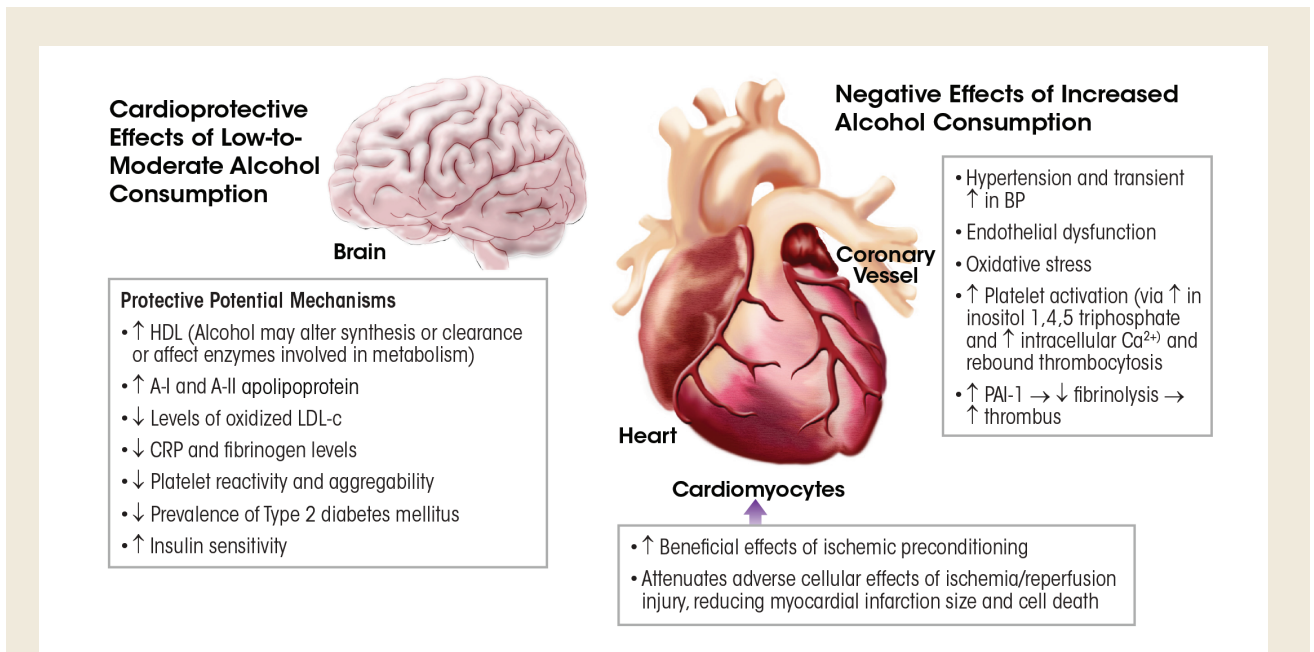


Figure 3 Mechanisms related to the positive and adverse effects of alcohol on cardiovascular conditions, such as coronary heart disease and stroke as well as cardiomyopathy. Different mechanisms may be in effect depending on the dose, duration, and pattern of alcohol consumption.

NOTE: BP = blood pressure, Ca^{2+} = calcium, CRP = C-reactive protein, DM = diabetes mellitus, HDL = high-density lipoprotein, LDL = low-density lipoprotein, PAI-1 = plasminogen activator inhibitor-1. SOURCE: Adapted from Krenz and Korthuis 2012.

found that acute intake on weekends and holidays of >40 g ethanol was significantly associated with cerebral infarction within 24 hours in young (ages 16–40) and middle-aged (ages 41–60) subjects. One possible mechanism for the binge-associated increased stroke risk is HTN. However, at least in younger people, HTN prevalence is low, suggesting that other mechanisms may be involved.

It has been debated whether beverage type has differential effects. Some investigators have suggested that drinking wine may offer more protection against CV disease because it contains polyphenols, such as resveratrol and flavonoids, which are micronutrients with antioxidant activity (Tangney and Rasmussen 2013). However, among studies designed to examine the influence of beverage type, no differences have been found in CV disease outcomes or biologic markers, such as HDL-c (Mukamal et al. 2003a; Volcik et al. 2008). Differential associations of CV risk with certain beverage types such as wine instead have been attributable to other lifestyle factors (e.g., increased physical activity) or drinking with meals (Malarcher et al. 2001). The findings from INTERHEART, in which “any alcohol use” had no cardioprotective effects in certain populations, such as in people of South Asian ethnicity who live in South Asia (e.g., India, Bangladesh, Nepal), led to speculation about beverage type, beverage quality, and drinking pattern as important mediators (Leong et al. 2014).

Finally, in studies of people from certain Eastern European countries, investigators have failed to find a cardioprotective effect with any level of ethanol consumption (Britton and McKee 2000). This suggests that alcoholic beverage type may be an important mediator, because in countries such as Russia, spirits are the alcoholic beverage of choice. However, the negative associations between alcohol consumption and CV outcomes in these countries also may relate to pervasive patterns of binge drinking (Leon et al. 2009).

Acute and Long-term Effects of Alcohol on the Myocardium

Acute Effects

The acute effects of alcohol on the myocardium include a weakening of the heart’s ability to contract (negative inotropic effect). Data from isolated papillary and heart muscle cell (myocyte) experiments demonstrate that acute physiologic intoxicating doses of alcohol (80 mg% to 250 mg%) can have a negative inotropic effect (Danziger et al. 1991; Guarnieri and Lakatta 1990). These effects also may involve an irregular and often very fast heart rate (arrhythmia) during which the heart’s upper chambers (atria) contract chaotically out of coordination with its lower chambers (ventricles), known as atrial fibrillation, or (rarely) sudden cardiac death.

Investigators have used a variety of noninvasive tests to evaluate the acute effects of alcohol consumption on myocardial function and hemodynamics in healthy humans. As with isolated animal heart experiments, some investigators have found that acute alcohol exposure (blood alcohol levels 40 to 110 mg%) depresses myocardial systolic function in humans (Delgado et al. 1975; Lang et al. 1985; Timmis et al. 1975). However, these changes were transient, with small changes from baseline. For example, in one study, the ejection fraction decreased by 4 percent after alcohol consumption (Delgado et al. 1975). In another study by Lang and colleagues (1985), however, the researchers noted a decrease in the maximum pressure developed by a ventricle at any given left ventricular volume, plotted as the end-systolic pressure dimension slope, as well as a decrease in the rate-corrected velocity of left-ventricular fiber shortening—and cardiac output was increased. Most likely, the decrease in contractility was offset by corresponding decreases in afterload (end-systolic wall stress), systemic vascular resistance,

and aortic peak pressure, which maintained cardiac output.

Other researchers have reported that acute alcohol consumption resulting in blood alcohol levels of 100 to 120 mg% exerted no effect on cardiac performance (Blomqvist et al. 1970; Child et al. 1979; Kupari et al. 1983a,b). It is important to note that most studies were performed >30 years ago with young subjects (mean age 23–35) and with small sample sizes ($n = 4–12$). As a result, whether or how these findings generalize to older healthy people and those with CV disease is unknown. However, in an elderly community-based population (i.e., the Atherosclerosis Risk in Communities Study, mean age at time of study 74–76 years), Gonçalves and colleagues (2015) examined the effects of different levels of weekly alcohol consumption on alterations in cardiac structure and function. These investigators found increasing amounts of alcohol were associated with mild alterations in cardiac structure and function, which were greater in women. In the United States, it is estimated that by 2060 there will be 98 million adults age >65, more than twice the number in 2014 (Administration on Aging 2014). In addition, recent research indicates that this generation will potentially consume alcohol at higher rates than previous generations (Barry and Blow 2016). Consequently, more research may be necessary to better understand the effects of alcohol consumption on the CV systems of older adults.

Certain arrhythmias, such as atrial fibrillation, may be the most serious consequence of consuming large amounts of alcohol, and in particular binge drinking. Larsson and colleagues (2014) have reported that binge drinking (defined by these researchers as having more than 5 drinks on a single occasion) was associated with an increased risk of new-onset atrial fibrillation. Atrial fibrillation is one of the most common arrhythmias and is strongly associated with adverse CV events, such as stroke (Conen et al. 2011). Results from retrospective studies

enrolling adults ages 40–60 also have linked binge drinking to a heightened risk of sudden death (Wannamethee and Shaper 1992).

Long-term Effects

Alcoholic cardiomyopathy (ACM) is a heart-muscle disease found in individuals with a history of long-term heavy alcohol consumption. It is characterized by a dilated left ventricle (LV), normal or reduced LV wall thickness, increased LV mass, and (in advanced stages) a reduced LV ejection fraction (<40 percent) (Piano and Phillips 2014). There are no specific immunohistochemical or immunological biomarkers or other criteria for an ACM diagnosis (Piano and Phillips 2014). Therefore, a key factor in diagnosing ACM is a long-term history of heavy alcohol abuse without CHD or other cardiac conditions such as inflammation of and damage to the myocardium, known as myocarditis.

ACM's exact prevalence remains elusive. The proportion of cardiomyopathy cases attributable to alcohol abuse has ranged from 23 to 40 percent (Piano and Phillips 2014). Recently, Guzzo-Merello and colleagues (2015) reported that, among 282 patients with a dilated cardiomyopathy phenotype, 33 percent had ACM. Both men and women can develop ACM. However, some reports indicate that alcohol-dependent women develop ACM after consuming less alcohol over a shorter period than do age-matched alcohol-dependent men (Fernández-Solà et al. 1997; Urbano-Marquez et al. 1989).

In humans, the exact amount and duration of alcohol consumption associated with development of ACM remains unknown. The point at which alcohol-induced abnormalities appear over the course of a person's lifetime drinking also is not well established and is highly individualized. This suggests either protective or adverse interaction effects of genetic or lifestyle factors (Piano and Phillips 2014). Among ACM patients ($n = 94$) referred to a heart failure and heart transplant unit, Guzzo-Merello and colleagues (2015)

found the mean alcohol consumption was ~11 drinks/day for at least 20 years, with most patients consuming slightly less (6 to 8 drinks/day). The researchers reported a mean age for these ACM patients of ~50, and more than half were current cigarette smokers.

ACM patients can present with either diastolic or systolic dysfunction and may or may not have symptoms of heart failure. When these patients are treated with standard heart failure therapies, they have good clinical outcomes and reduced mortality rates. Factors associated with poor outcomes (e.g., greater mortality or transplantation) included a history of atrial fibrillation; an electrocardiogram QRS width >120 milliseconds (ms), compared with the normal range of 70 to 100 ms; and not being treated with beta-blockers or digoxin (Guzzo-Merello et al. 2015).

Long-term heavy alcohol consumption induces adverse histological, cellular, and structural changes within the myocardium. As with other alcohol-induced pathologies, mechanisms contributing to ACM include oxidative stress, apoptotic (programmed) cell death, impaired mitochondrial bioenergetics and stress, derangements in fatty acid metabolism and transport, and accelerated protein breakdown; these will be discussed in the following sections. These mechanisms contribute to the myocyte cellular changes that lead to intrinsic cell dysfunction, such as sarcoplasmic reticular dysfunction and changes in intracellular calcium handling and myocyte loss. However, modulatory influences related to drinking patterns, genetic susceptibility, nutritional factors, ethnicity, and gender also many play a role (Piano and Phillips 2014) (figure 4).

Oxidative Stress and Apoptosis: Linked Mechanisms

Oxidative Stress

In examining alcohol-induced pathologies, other researchers have suggested

three potential ways in which alcohol exposure can lead to excess free-radical generation and oxidative stress: ethanol metabolism, ethanol effects on antioxidant proteins and antioxidant enzymes, and activation/alteration in neurohormonal systems (table 2) (Fernández-Checa et al. 1998; Piano and Phillips 2014). At least in the myocardium, many adverse cardiac intracellular effects found after chronic alcohol consumption can be attributed to oxidative stress:

- Myocyte loss and disarray;
- Sarcoplasmic reticulum dysfunction, which can lead to systolic dysfunction as well as a thickening of the heart muscle that can make ventricles larger, known as cardiac hypertrophy (Bing et al. 1974; Segel et al. 1981);
- Changes in handling of intracellular calcium ions (Zhang et al. 2014);
- Depressed or disturbed mitochondrial function (Pachinger et al. 1973; Segel et al. 1981; Weishaar et al. 1977);
- Decreased myofibrillar ATPase activity, which affects muscle contraction (Hastillo et al. 1980; Segel et al. 1975);
- Decreased myofibrillar calcium sensitivity, which affects contractile force generation in the heart (Piano et al. 1999);
- Contractile protein fragmentation and disarray (Jiang et al. 2012; Tsiplenkova et al. 1986; Urbano-Marquez et al. 1989); and
- Fatty acid accumulation within intracellular organelles, which can include atypical storage of fat in heart tissue that can lead to dysfunction (Beckemeier and Bora 1998; Hu et al. 2013).

In various biologic systems, oxidative stress can be measured or inferred by several biologic indexes. These can include measurement of antioxidant enzymes (e.g., catalase or glutathione peroxidase) or scavenging proteins (e.g., glutathione), oxidative damage (e.g., increase or presence of protein carbonyl and conjugated diene levels), or measurement of circulating oxidative products (e.g., isoprostanes) (Dalle-Donne et al. 2006; Montuschi et al. 2004). Collectively, data from human and animal models suggest that alcohol may alter important components of the antioxidant defense system, such as

levels of antioxidant substrates (e.g., decreased glutathione levels) or levels or activity of antioxidant enzymes (e.g., decreases in catalase or superoxide dismutase) (Edes et al. 1986, 1987; Ribiere et al. 1992; Vendemiale et al. 2001) and/or lead to increased levels or accumulation of other markers of oxidative stress (such as conjugated dienes, protein carbonyls, or 3-nitrotyrosine) (Edes et al. 1986, 1987; Ribiere et al. 1992; Tan et al. 2012; Vendemiale et al. 2001; Zhang et al. 2014).

Evidence of oxidative stress is found after short periods of alcohol consumption (2 to 18 weeks), at least in

animal models. These data suggest that antioxidant defense mechanisms that attempt to protect the heart against oxidative damage appear to be initiated soon after drinking alcohol. Also, as noted below, data from other studies demonstrate the protective role of administered antioxidants, such as a synthetic compound that mimics the native superoxide dismutase enzyme, called a superoxide dismutase mimetic. This suggests a direct or indirect role for ethanol-mediated oxidative stress in the heart (Jiang et al. 2012; Tan et al. 2012).

Data from transgenic animal models and pharmacologic approaches strongly support a role for ethanol-induced oxidative stress in CV disease. Using both pharmacologic and transgenic approaches, Tan and colleagues (2012) showed that administering an ROS scavenger (a superoxide dismutase mimetic) to mice receiving a diet high in ethanol for 2 months significantly reduced nitritative damage (i.e., decreased 3-nitrotyrosine accumulation, a marker of decreased cell damage and inflammation). In addition, there was no evidence of nitritative damage in mice bred to disrupt (i.e., knock out) the gene for angiotensin I receptor (AT1-KO) that had been given ethanol for a similar length of time (Tan et al. 2012). Both experimental approaches also prevented accumulation of ethanol-induced scarring (collagen and fibronectin); apoptotic cell death; and changes in the size, shape, and function of the heart after injury to heart muscle (ventricular remodeling).

Other researchers have used genetic approaches (i.e., transgenic animals) to prevent ethanol-induced oxidative stress. One approach included overexpression of proteins such as insulin-like growth factor (IGF-1), which stimulates growth and cell proliferation and has antiapoptotic effects (see Zhang et al. 2014). In contrast to control mice, the IGF-1-expressing animals exhibited no evidence of changes in expression of antioxidant enzymes (i.e., superoxide dismutase-1) or any decreases in contractile function after 16 weeks of ethanol consumption. The findings

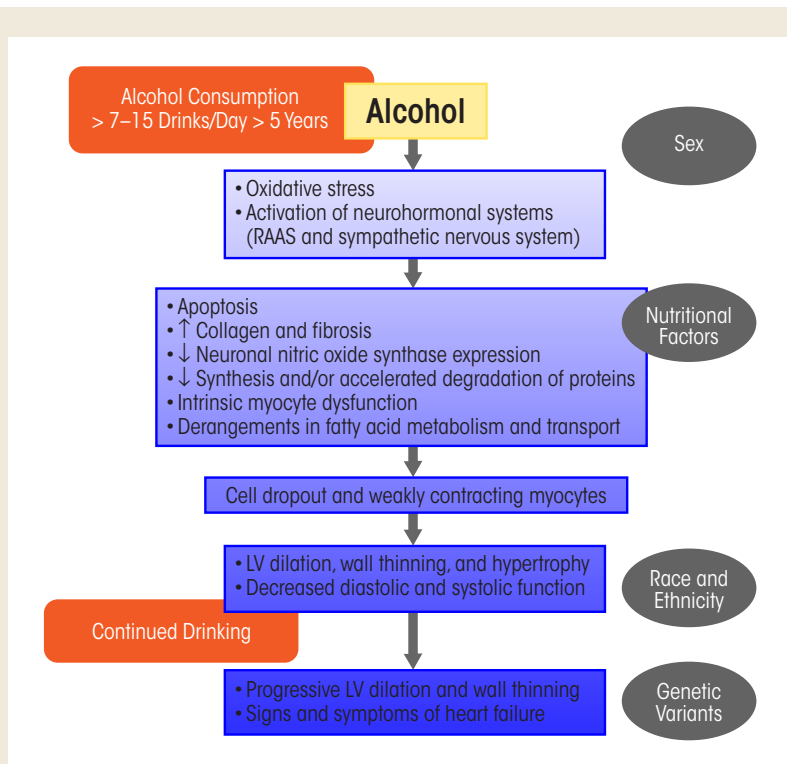


Figure 4 Pathophysiologic schema for the development of alcoholic cardiomyopathy (ACM). As noted in the text, the exact amount and duration of alcohol consumption that results in ACM in human beings varies. The exact sequence of the development of ACM remains incompletely understood. Data from animal models and human beings with a history of long-term drinking suggest that oxidative stress may be an early and initiating mechanism. Many cellular events, such as intrinsic myocyte dysfunction, characterized by changes in calcium homeostasis and regulation and decreased myofilament sensitivity, can come about due to oxidative stress. Variables in gray ovals represent potential mediating factors.

NOTE: LV = left ventricle, RAAS = renin-angiotensin-aldosterone system.
SOURCE: Adapted from Piano and Phillips 2014.

suggest a protective effect of overexpression of IGF-1 in the transgenic animals (Zhang et al. 2014).

Apoptosis

Apoptosis also may be an important mechanism in ACM and a consequence of oxidative stress. Studies have found evidence of apoptosis in humans with ACM and in animal ACM models. Fernández-Solà and colleagues (2006) evaluated apoptosis in the hearts of adults with long-term alcoholism ($n = 19$, drinking for 26 years), adults with long-standing hypertension ($n = 20$), and those with no known disease (control subjects, $n = 7$). Apoptosis as evidenced by increased protein expression of two key proteins—one that promotes apoptotic cell death (i.e., BAX) and one that inhibits it (i.e., BCL-2)—was significantly higher in both the alcoholic subjects and in the hypertensive subjects, compared with control subjects (Fernández-Solà et al. 2006). Moreover, apoptosis was of a similar magnitude in the alcoholic and the hypertensive subjects. More recent findings from this research group corroborate that apoptosis occurs in humans who have a long history of heavy alcohol consumption (Fernández-Solà et al. 2011).

Mitochondrial Dysfunction and Changes in Mitochondrial Bioenergetics

Researchers have found evidence of mitochondrial dysfunction or impaired bioenergetics related to alcohol consumption. This is not surprising, because mitochondria are a major target for free-radical injury. Dysfunctional mitochondria are less efficient, can become a source of ROS, and are more likely to initiate apoptosis (Marzetti et al. 2013).

Histological studies published several decades ago reported evidence of mitochondrial injury, such as mitochondrial enlargement and disorganization,

Table 2 Potential Ethanol-Induced Sources of Reactive Oxygen Species.

Ethanol Metabolism

- An \uparrow in the flux of reducing equivalents into the electron transport chain due to an \uparrow in nicotinamide adenine dinucleotide production related to ethanol metabolism (\uparrow NADH/NAD⁺ ratio).
- An \uparrow in cytochrome P450 2E1 metabolism of ethanol.
- An \uparrow in alcohol dehydrogenase metabolism of ethanol and accumulation of acetaldehyde (leading to ROS formation and acetaldehyde adduct formation).
- Nonoxidative metabolism by fatty acid ethyl ester synthase and/or phospholipase D.

Ethanol Effects on Antioxidant Proteins and Antioxidant Enzymes

- Alcohol-induced inhibition of transport proteins responsible for transporting glutathione from cytosol into the mitochondria (e.g., glutathione transport from cytosol into the mitochondria) and \downarrow antioxidant enzyme levels and activity (e.g., superoxide dismutase).

Activation/Alteration in Neurohormonal Systems

- Increased autooxidation of catecholamines.
- An \uparrow in angiotensin II and norepinephrine levels.

NOTE: NAD = nicotinamide adenine dinucleotide, NADPH = nicotinamide adenine dinucleotide diphosphate, ROS = reactive oxygen species.
SOURCE: Used with permission from Plano and Phillips 2014.

increased number of mitochondria, mitochondriosis (small mitochondria closely packed together), and an increase in lysosome-like structures that break down biomolecules in myocardial post-mortem biopsy samples from people with a long-term history of heavy alcohol consumption (Hibbs et al. 1965; Tsiplenkova et al. 1986; Vikhert et al. 1986).

More contemporary studies have not found evidence of mitochondrial injury in biopsy samples from long-term alcohol drinkers (Miró et al. 2000). Differences among results from human studies may relate to small sample sizes, duration of drinking, and degree of myocardial dysfunction. In the Miró study, alcohol drinkers also had been receiving pharmacologic treatments such as beta-adrenergic blocking agents that reduce blood pressure and also may have antioxidant effects.

Changes in mitochondrial function have been reported from a number of animal studies in different species, under various alcohol consumption paradigms (ethanol in water or liquid diet), and after variable durations of chronic ethanol consumption (6 weeks to 6 months). Through the process of oxidative phosphorylation, the mitochondria generate ~90 percent of cellular

ATP. Common findings in alcohol studies from the 1970s and early 1980s included decreases in mitochondrial indices that reflected mitochondrial state III respiration, or ADP-stimulated respiration (Pachinger et al. 1973; Segel et al. 1981; Williams and Li 1977). The latter changes in these indices could be brought about by ethanol-induced imbalances in the reducing equivalents nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide hydrogen (NADH), an important chemical pathway involved in oxidative stress. In cardiomyocyte mitochondria as well as other mitochondrial types, such imbalances could lead to further decreases in cellular respiration and oxidative phosphorylation.

More recent studies have confirmed that 4 to 16 weeks of ethanol consumption was associated with mitochondrial dysfunction. This was evidenced by decreased myocardial ATP content levels, changes in the mitochondrial membrane potential, and decreases in cytochrome oxidase activity in conjunction with decreased myocardial contractility (e.g., decreased ejection fraction and fractional shortening) (Hu et al. 2013; Laurent et al. 2014). Although the connection is

still speculative, this reduction in ATP synthesis may be enough to depress important intracellular functions that support heart health, such as sarcoplasmic reticulum uptake of calcium ions and cross-bridge cycling in muscle contraction. Prolonged ethanol consumption also may decrease expression of several types of mitochondrial proteins, such as NADH dehydrogenase, isocitrate dehydrogenase, and long-chain-specific acyl-CoA dehydrogenase, as well as proteins within the citric acid cycle (Fogle et al. 2010). This kind of mitochondrial dysfunction, including decreased expression of some of these proteins, is integral to cardiac ischemic-reperfusion injury, which occur routinely with MI—the most common incident of CV disease, itself the number-one cause of death (Tompkins et al. 2006).

Derangements in Fatty Acid Metabolism and Transport

Derangements in fatty acid metabolism and transport and formation of fatty acid ethyl esters (FAEEs) also have been implicated in ethanol-induced cell injury. FAEEs can be formed in the body during ethanol metabolism, when ethanol reacts with fatty acids or triglycerides. FAEEs can attach to mitochondria and disrupt mitochondrial function. Lange and Sobel (1983) were the first to identify an increase in FAEE content in postmortem myocardium samples obtained from those subjects who routinely had used large amounts of alcohol ($n = 2$) and who had a history of recent alcohol intoxication ($n = 4$).

The idea that FAEEs are involved in ACM pathogenesis and are cytotoxic is supported by the fact that increased tissue levels of FAEE are considered the mechanism underlying cell death induced using a procedure to control and prevent recurrence of cardiac arrhythmias (i.e., septal ablation) (Yoerger et al. 2006).

Other researchers have confirmed in animal models that long-term ethanol

consumption can also affect long-chain fatty acid (LCFA) uptake, as well as increased expression of the genes encoding for proteins involved in the formation of triglycerides from free fatty acids and glycerol, or triglyceride esterification, and in LCFA transporters (Hu et al. 2013).⁴

Accelerated Protein Degradation

Long-term alcohol use decreases myocardial protein expression and synthesis and accelerates protein degradation in the myocardium (Lang et al. 2005). This in turn disrupts myocardium function, including contraction and relaxation of the cardiac walls, impairing the heart's ability to pump blood. Using mass spectrometric-based proteomic analysis in an animal model, Fogle and colleagues (2010) found that long-term alcohol consumption was associated with decreases of 30 to 54 percent in cell-scaffolding proteins (myofibrillar α -myosin and actin) and mitochondrial proteins (mitochondrial dehydrogenases and electron transport proteins), glycolytic enzymes (glycogen phosphorylase and alpha-enolase), and fatty acid metabolism proteins (fatty acid transport protein and LCFA acyl-CoA ligase). These investigators also found decreases in peroxiredoxin 5, antioxidant protein 2, and glutathione transferase 5—important antioxidant enzymes whose cardiovascular protective functions still are not fully understood. For example, some findings suggest an inverse role between peroxiredoxin 5 and stroke severity. During a severe stroke, peroxiredoxin 5 is consumed and its production impaired (Kunze et al. 2014).

Other ethanol-induced changes may be related to enzymes that modulate protein synthesis and/or breakdown (e.g., ubiquitin-ligases). Several reports

⁴ Triglycerides are the main component of body fat in humans. They do not pass readily through cell membranes, and they are major components of very-low-density lipoproteins (VLDLs), which are converted in the blood to LDLs. High levels of triglycerides in the blood have therefore been linked to atherosclerosis, heart disease, and stroke.

suggest that ethanol-induced decreases in myocardial protein synthesis may be mediated in part by decreased activity of an enzyme called mammalian (or mechanistic) target of rapamycin (mTOR) (Lang and Korzick 2014; Vary and Deiter 2005; Vary et al. 2008). mTOR regulates cell growth, proliferation, motility, and survival; protein synthesis; and transcription (Donohue 2009). Decreases in mTOR activation may play a role in reduced myocardial protein synthesis, ventricular wall thinning, and dilation.

Alterations in protein physiology and content also can result from accelerated protein degradation. The normal destruction of molecules and cell organelles (called autophagy) may be especially important in triggering ACM. Autophagy is performed by lysosomes and involves a breakdown (catabolism) of unnecessary or damaged proteins in the cell. This mechanism also is essential to cell and organism survival during stress and nutrient deprivation. Under the latter conditions, autophagy helps generate and recycle carbons and amino acids through degradation of large (macromolecular) cellular constituents. However, as with other CV pathological conditions, such as heart failure and cardiac hypotrophy, there is evidence of increased autophagy with chronic alcohol consumption. Guo and colleagues (2012) studied autophagy in mice with and without an overexpression of the enzyme alcohol dehydrogenase to show that 8 weeks of ethanol consumption was associated with increased myocardial markers of autophagy, such as autophagy-related 7 protein.

Increased autophagy as a possible mechanism underlying the adverse myocardial effects of ethanol is intriguing. This is especially true in light of the relationship between a sensor of stress (mTOR) and nutrient deprivation and how essential autophagy is to cell survival. Activation of mTOR leads to inhibition of autophagy. As noted above, chronic alcohol exposure leads to a decrease in mTOR activity, which corresponds to increased markers of autophagy (Lang and Korzick 2014).

The autophagy pathway also is rapidly upregulated during ATP depletion, mitochondrial dysfunction, and oxidative stress. Ethanol-mediated increases in autophagy therefore may be an important mechanism underlying the adverse myocardial effects of ethanol.

Some of the potential cellular changes related to ethanol consumption reviewed above are illustrated in figure 5. More than one cellular event may be happening at the same time, and, as with other chronic health conditions, the relevant mechanisms may be synergistic and interrelated.

Summary and Future Directions for Research

Alcohol consumption remains a major risk factor for global burden of disease

(Rehm et al. 2009). Nearly all the data on humans exploring the relationship between alcohol consumption and CV risk—including some indications of potential CV benefits associated with low-to-moderate alcohol consumption—are derived from epidemiologic studies. Therefore, because there are no randomized controlled trials, health care professionals should not recommend alcohol consumption as a primary or secondary lifestyle intervention. Instead, clinicians should continue to recommend strategies such as a healthy diet and exercise. Adults who choose to drink can be encouraged to follow the alcohol consumption recommendations from NIAAA (table 3).

Data derived from systematic reviews and meta-analyses suggest that alcohol-dose and CV-health relationships differ for various CV conditions. For

example, certain levels of alcohol consumption that lower risk for CHD may increase it for other CV conditions, such as stroke. In addition, data from studies using new research methods, including Mendelian randomization, suggest that the relationship between low-to-moderate alcohol consumption and cardioprotection merits more critical appraisal (Holmes et al. 2014).

Alcohol use remains, as Saitz (2015) has memorably noted, “no ordinary health risk.” Heavy daily alcohol consumption and binge drinking increase the risk of developing CV disease. The growing rate of binge drinking in the United States is a serious concern (Kanny et al. 2013). ACM, though not a leading cause of heart failure nationwide, can be associated with marked changes in cardiac function, symptoms, and poor quality of life.

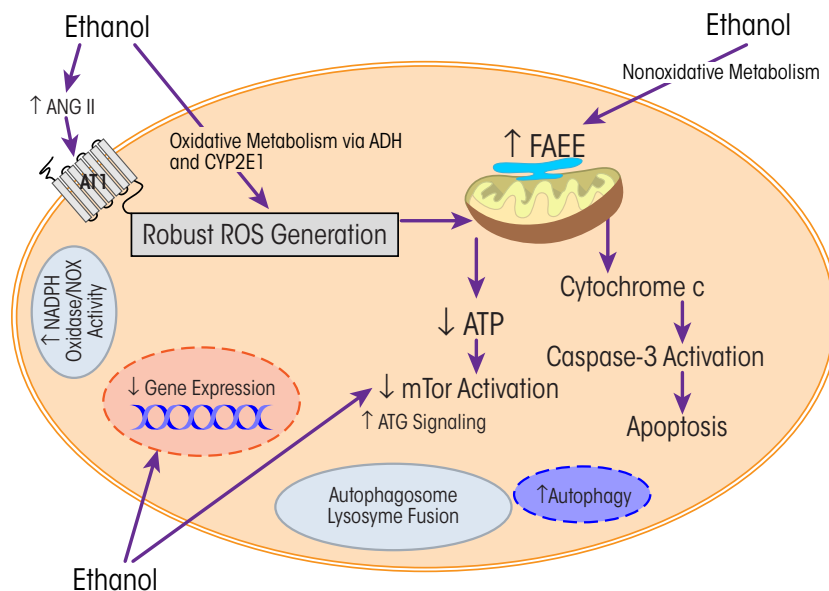


Figure 5 Summary of potential cellular changes related to ethanol. Ethanol-induced changes may be related to oxidative or nonoxidative pathways of ethanol metabolism. More than one mechanism may be activated and may lead to the multitude of ethanol-induced changes in cellular proteins and cell function. As reviewed in the text, data from pharmacologic and transgenic approaches revealed an important role for oxidative stress and the hormone angiotensin II.

NOTE: Ang II = angiotensin II, ATG = atrogen, ATI = angiotensin I receptor, ATP = adenosine triphosphate, CYP2E1 = cytochrome P450 2E1, FAEE = fatty ethyl esters, mTOR = mammalian (or mechanistic) target of rapamycin, NADPH oxidase/NOX = nicotinamide adenine dinucleotide phosphate-oxidase, ROS = reactive oxygen species.
SOURCE: Adapted from Piano and Phillips 2014.

Table 3 Drinking Levels Defined

The National Institute on Alcohol Abuse and Alcoholism defines low risk drinking for developing alcohol use disorder as:

- No more than 4 drinks on any single day and no more than 14 drinks per week for men age 65 or younger.
- No more than 3 drinks on any single day and no more than 7 drinks per week for women and men over the age of 65.

Susceptibility factors, such as gender, race/ethnicity, genetics, and socioeconomic factors, may influence alcohol's positive and adverse health effects. When clinicians are counseling patients about alcohol consumption, they should consider all these factors, as well as any history of alcohol dependence. Of course, any advice about alcohol consumption and related health issues needs to be targeted for each patient.

This review suggests several recommendations for future research:

- Using direct biomarkers of alcohol, such as PEth, to corroborate self-report of alcohol consumption and distinguish between and among low, moderate, and heavy alcohol consumption.
- Examining the potential mediation of genetic, socioeconomic, and racial and ethnic factors within the alcohol–CV disease relationship, specifically regarding development of ACM.
- As suggested by Klatsky (2015), reviewing alcohol–medication interactions, considering the large number of antiplatelet agents, lipid-lowering, and antihypertensive therapies prescribed to people with CV conditions.
- Examining the CV effects related to alcohol use in young adults (ages 18–30), a group that consumes the most alcohol and binge drinks the most.

- Considering the growing number of older adults, more research is needed to better understand the effects of alcohol consumption on the CV systems of older populations.

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Because of space limitations, not all of the excellent scientific work on alcohol and the cardiovascular system could be assessed in this review. Some of the information presented here was previously published. For further detail, please see Piano 2002 or Piano and Phillips 2014.

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Glossary

Actin: A family of globular multifunctional proteins, each of which forms (together with myosin) the contractile filaments of muscle cells, and is also involved in motion in other types of cells.

Afterload: The pressure in the left ventricle wall during ejection of blood; the end load against which the heart contracts to eject blood.

Alpha-Enolase: Also known as enolase 1 (ENO1). A glycolytic enzyme expressed in most tissues, one of the isoenzymes of enolase. Functions as a glycolytic enzyme and as a structural lens protein, with a shorter isoform functioning as a tumor marker.

Alpha [α]-Myosin: A protein of the myofibril (elongated contractile thread in striated muscle cells); with actin, forms actomyosin, responsible for the contractile properties of muscle.

Angiotensin I Receptor: A cellular receptor responsible for signal transduction of the vasoconstricting stimulus of angiotensin II, the main effector hormone; important in the *renin-angiotensin-aldosterone system (RAAS)*, which regulates plasma sodium concentration and arterial blood pressure.

Antioxidant Protein 2: Antioxidant that affects certain disease processes, including *atherosclerosis*.

Apolipoprotein A-I: The major protein component of high-density lipoprotein (HDL) in plasma, or “good cholesterol”; plays a specific role in lipid metabolism.

Atherosclerosis: A disease of the arteries characterized by deposition of plaques (made up of fats, cholesterol, cellular waste products, calcium, and fibrin) on arterial inner walls, which can restrict blood flow; commonly called hardening of the arteries.

Atrogin: Protein expressed during muscle atrophy; atrogin-1 is expressed in cardiac and skeletal muscle and directs muscle protein degradation after muscle atrophy response has been triggered.

Autophagy-Related 7 Protein: Myocardial marker of autophagy; also thought to modulate tumor-suppression protein-dependent cell cycle pathways during prolonged metabolic stress.

Baroreflex: Also known as baroreceptor reflex; one of the body’s homeostatic mechanisms that helps to maintain blood pressure at nearly constant levels; part of a rapid negative-feedback loop that can begin to act in less than the duration of a cardiac cycle (fractions of a second), and thus a key factor in dealing with postural hypotension, the tendency for blood pressure to decrease on standing due to gravity.

BAX Protein: Apoptosis-regulator protein that promotes apoptotic cell death by interacting with and increasing the opening of the mitochondrial voltage-dependent anion channel (VDAC), which leads to loss in membrane potential and release of cytochrome c and other pro-apoptotic factors from the mitochondria.

BCL-2 Protein: Apoptosis-regulator protein that inhibits apoptotic cell death.

Body Mass Index (BMI): Quantifies the amount of tissue mass (muscle, fat, and bone) in an individual based on mass (weight) and height, with categories labeled underweight, normal weight, overweight, or obese; commonly accepted BMI ranges are underweight: under 18.5, normal weight: 18.5 to 25, overweight: 25 to 30, obese: over 30.

Carotid Intima-Medial Thickness (cIMT): Measurement of the thickness of the carotid arteries, which supply the brain, used to assess cardiovascular disease risk, since increased thickness is a marker of early stages of heart disease; a cIMT test is done with ultrasound, takes 10 minutes, is painless, and involves no radiation exposure.

C-Reactive Protein: Acute-phase reactant protein in blood plasma, secreted by the liver, whose level increases in response to inflammation; can help predict risk of heart disease or stroke.

Glossary (*continued*)

C-terminal Proendothelin-1: Circulating inflammatory marker, elevated during heart failure and acute myocardial infarction.

Cross-Bridge Cycling: Four-step cycle of muscle contraction in skeletal and cardiac muscle; named for myosin protein heads (cross-bridges) of thick filaments in a sarcomere, the functional unit of a myofibril or contractile protein, which bind to and move along actin in the sarcomere's thin filament, an interaction that is the molecular basis for force generation and movement in muscle cells.

Cytochrome P450 2E1: Membrane protein expressed in high levels in the liver, responsible for fatty acid oxidation and conversion of ethanol to acetaldehyde and to acetate in humans; also metabolizes foreign chemical substances in the body, including toxic environmental chemicals and carcinogens.

Endogenous Nitric Oxide Synthase (eNOS): Protective enzyme that produces most of the *nitric oxide* in the vascular system and helps prevent platelet aggregation and adhesion.

Fatty Acid Ethyl Ester (FAEE): Nonoxidative metabolite of ethanol, sometimes used as a biomarker of alcohol consumption; intoxicated humans have high levels of FAEE in blood, pancreas, liver, and hair.

Fibronectin: Glycoprotein that in soluble plasma form is a major protein component of blood plasma and plays a major role in cell adhesion, growth, migration, and differentiation and is important in wound healing and formation of blood clots.

Glutathione: Antioxidant capable of preventing damage to important cellular components caused by *reactive oxygen species* such as free radicals, peroxides, lipid peroxides, and heavy metals.

Glutathione Transferase 5: Metabolic isoenzyme that helps in detoxification by catalyzing the binding of the

reduced form of *glutathione* to certain foreign chemicals, which makes these molecules less toxic.

Glycogen Phosphorylase: Glycolytic enzyme that catalyzes the rate-limiting step in the breakdown of glycogen, the main storage form of glucose in the body.

Glycoproteins: Proteins important to various cell–cell interactions, including white blood cell recognition; found on surface membranes of platelets and integral to bleeding cessation, formation of blood clots, and normal platelet aggregation and adherence to the endothelium.

Interleukin: *Glycoprotein* produced by white blood cells for regulating immune response, including three dozen currently identified types.

Isocitrate Dehydrogenase: Enzyme that catalyzes the third step of the citric acid cycle while converting NAD⁺ to NADH in the mitochondria.

Isoprostane: Inflammatory mediator that augments the perception of pain and is a biomarker of oxidative stress; elevated levels may contribute to increased risk of heart attack in people taking certain kinds of nonsteroidal anti-inflammatory drugs.

Long-Chain Fatty Acids (LCFAs): Chief components of dietary fats; turned into triglycerides and taken up into cells, where they are metabolized by the mitochondria and yield large quantities of ATP; ingestion through certain foods promotes lipid accumulation and insulin resistance.

Long-Chain-Specific Acyl-CoA Dehydrogenase: Mitochondrial enzyme that participates in fatty acid metabolism; mitochondrial mutations in it may be associated with some forms of dilated cardiomyopathy, which enlarges and weakens the left ventricle, making it harder for the heart to pump blood.

Lysosome: Membrane-bound organelle in most animal cells that contains hydrolytic enzymes that can break down almost all biomolecules; involved in cellular secretion, plasma membrane repair, cell signaling, energy metabolism, and waste disposal.

Glossary (*continued*)

Mechanistic (or Mammalian) Target of Rapamycin (mTOR): Kinase that regulates cellular metabolism, growth, and proliferation; inhibits T-cell proliferation and proliferative responses induced by certain cytokines.

Mendelian Randomization: A epidemiologic method of using measured variation in genes of known function to examine the causal effect of a modifiable exposure on disease in nonexperimental studies.

NADH Dehydrogenase: Flavoprotein that reversibly oxidizes NADH to NAD⁺ in mitochondria, in the metabolic pathway that cells use to oxidize nutrients, thereby releasing energy to reform ATP.

Nicotinamide Adenine Dinucleotide Phosphate-Oxidase (NADPH oxidase): Membrane-bound enzyme complex found in plasma membrane that faces the extracellular space; major cause of *atherosclerosis* from its production of *reactive oxygen species* and resulting accumulation of cholesterol-containing macrophages in arterial walls.

Nitric Oxide: Important cellular signaling molecule involved in many physiological and pathological processes; integral to vasodilation and increased blood flow in blood vessels; acts as powerful vasodilator with a short half-life of a few seconds in the blood; can contribute to reperfusion injury after ischemia.

Pentraxin-3: Circulating inflammatory marker integral to protection against pathogens and to control of autoimmunity; facilitates pathogen recognition by macrophages and dendritic cells; appears to be primarily protective in both acute infections and acute coronary syndromes.

Peroxioredoxin 5: Protein that acts protectively as an antioxidant in different tissues under normal conditions and during inflammatory processes; cardioprotective functions still to be determined.

Phenylephrine: Selective α_1 -adrenergic receptor used as a decongestant and also as a vasopressor to increase blood pressure in patients with reduced blood pressure, especially from septic shock.

Phosphatidylethanol: Phospholipid formed only in the presence of ethanol, used as a direct biomarker of previous alcohol consumption.

Prostanoid: Signaling molecules that include prostaglandins, which mediate inflammatory and anaphylactic reactions; thromboxanes, which mediate vasoconstriction and help form blood clots (*thrombosis*); and prostacyclins, which help resolve inflammation.

Reactive Oxygen Species (ROS): Chemically reactive molecules containing oxygen, formed as a natural byproduct of normal oxygen metabolism and integral to cell signaling and homeostasis; in times of environmental stress or with ionizing radiation, may result in significant damage to cell structures, known as oxidative stress.

Renin–Angiotensin–Aldosterone System (RAAS): Neurohormonal system involved in regulation of plasma sodium concentration and arterial blood pressure; can be activated by loss of blood volume or drop in blood pressure; angiotensin-converting enzyme (ACE) inhibitors reduce formation of angiotensin II, a strong vasoconstrictor.

Sarcoplasmic Reticulum: Smooth endoplasmic reticulum found in muscle cells that regulates the calcium ion concentration in the cytoplasm of striated muscle cells; stores calcium ions and pumps them into sarcoplasm when the muscle fiber is stimulated, thereby playing a major role in muscle contraction.

Subarachnoid Hemorrhage: Bleeding into the subarachnoid space of the brain, the area between the arachnoid membrane and the pia mater, the brain's innermost membrane; a form of stroke that can lead to severe disability or death.

Thrombocytosis: Excessive production of platelets (thrombocytes) in the body; often without symptoms, but can cause *thrombosis* or formation of blood clots.

Glossary (*continued*)

Thrombosis: Formation of a blood clot inside a blood vessel that obstructs blood flow; a clot that breaks free (embolus) can result in hypoxia, anoxia, and tissue death.

Triglyceride Esterification: Formation of triglycerides (which have three fatty acids and a glycerol molecule)

from free fatty acids and glycerol by linking the acid group of a fatty acid and the alcohol group of glycerol to form an ester; high levels of triglycerides in the blood have been linked to *atherosclerosis* and increased risk of heart disease and stroke.

Alcoholic Myopathy: Pathophysiologic Mechanisms and Clinical Implications

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Skeletal muscle dysfunction (i.e., myopathy) is common in patients with alcohol use disorder. However, few clinical studies have elucidated the significance, mechanisms, and therapeutic options of alcohol-related myopathy. Preclinical studies indicate that alcohol adversely affects both anabolic and catabolic pathways of muscle-mass maintenance and that an increased proinflammatory and oxidative milieu in the skeletal muscle is the primary contributing factor leading to alcohol-related skeletal muscle dysfunction. Decreased regenerative capacity of muscle progenitor cells is emerging as an additional mechanism that contributes to alcohol-induced loss in muscle mass and impairment in muscle growth. This review details the epidemiology of alcoholic myopathy, potential contributing pathophysiologic mechanisms, and emerging literature on novel therapeutic options.

Key words: Alcohol consumption; alcohol use disorder; alcohol effects and consequences; alcoholic myopathy; skeletal muscle; skeletal muscle dysfunction; myopathy; muscle

Alcohol use disorder (AUD) affects approximately 15 to 20 million individuals in the United States (Center for Behavioral Health Statistics and Quality 2016), and excessive alcohol consumption is associated with \$249 billion in economic costs (Sacks et al. 2015). Each year, alcohol consumption is linked to 2.3 million years of potential life lost, with over \$150 billion attributable to effects of physical inactivity (Bouchery et al. 2011). Skeletal muscle dysfunction (i.e., myopathy) is common in patients with AUD, and alcoholic myopathy occurs in 40 to 60 percent of chronic alcoholics (Fernández-Solà et al. 2007; Urbano-Marquez and Fernández-Solà 2004). Although alcohol-related muscle disease is nearly 5 times more common than liver cirrhosis (which is present in 10 to 15 percent

of people with AUD), data are lacking on its contribution to long-term health and disease in patients with AUD (Estruch et al. 1993). This review explores the epidemiology of alcohol-related myopathy, highlights the emerging literature on pathophysiologic factors associated with its development, and reviews novel targets for treatment.

Epidemiology of Alcohol-Related Myopathy

Alcoholic myopathy is common among people with AUD and may manifest as an acute or chronic condition. Acute alcoholic myopathy is present in 0.5 to 2.0 percent of alcoholics, with an estimated overall prevalence of 20 cases per 100,000 people in the Western

Hemisphere (Preedy et al. 2003). Chronic alcoholic myopathy is one of the most common types of myopathy, with an overall prevalence of 2,000 cases per 100,000 people. Based on these prevalence estimates, chronic alcohol-related myopathy is 10 times more common than the most common inherited myopathy (i.e., nemaline myopathy), which has a prevalence of 200 cases per 100,000 individuals, and 67 to 1,000 times more common than Duchenne's muscular dystrophy with an estimated prevalence of 2 to 30 per 100,000 people (Preedy et al. 2003). However, it is difficult to ascertain the exact prevalence, because the spectrum of clinical disease in alcohol-related myopathy varies (Estruch et al. 1993). In a study of alcoholics without a known diagnosis of myopathy, up to

46 percent exhibited myopathic changes on muscle biopsies and presented with demonstrable reductions in strength compared with healthy control subjects (Urbano-Marquez et al. 1995). The role of this subclinical disease in the development of future clinically evident symptoms remains poorly understood.

The presence of liver cirrhosis also may influence the development of myopathy in people with AUD, because patients with cirrhosis secondary to chronic alcohol consumption commonly manifest muscle wasting. In a study of chronic alcoholic men, lean muscle mass was significantly lower in those with cirrhosis than in those without cirrhosis (Estruch et al. 1993). Lifetime ethanol consumption was an independent predictor of greater muscle loss among this population (Nicolas et al. 1993). Recent studies also suggest that the loss of muscle mass and strength associated with aging (i.e., sarcopenia) is more prevalent with advancing stages of cirrhosis and frequently occurs even in the absence of concomitant alcohol use (Hanai et al. 2016). The mechanisms involved in development and propagation of cirrhosis-related muscle disease are not completely understood and warrant further study; a further discussion is beyond the scope of this review.

Clinical Manifestations

Clinically, acute alcoholic myopathy is characterized by weakness, pain, tenderness, and swelling of affected muscles. It often occurs after an alcohol binge characterized by consumption of 4 to 5 alcoholic drinks during a single episode, resulting in blood alcohol levels of 0.08 g/dL or above, and resolves within 1 to 2 weeks of abstinence from alcohol (Perkoff 1971). A common manifestation of acute alcoholic myopathy is a breakdown of muscle tissue and release of muscle-fiber content into the blood (i.e., rhabdomyolysis). It most severely affects muscles close to the body's midline (i.e., proximal muscles), primarily the pelvic and shoulder girdles, in a focal and asymmetric fashion.

Clinical evidence of this type of myopathy may be associated with laboratory evidence of muscle injury, accompanied by elevations in the enzyme creatinine kinase and the protein myoglobin that is found in heart and skeletal muscle. This so-called rhabdomyolytic variant of acute alcoholic myopathy, which in severe cases may precipitate acute renal failure, represents the most common nontraumatic cause of rhabdomyolysis in hospitalized patients (Urbano-Marquez and Fernández-Solà 2004).

Conversely, chronic alcoholic myopathy—the most frequent presentation of alcohol-related myopathy—presents with progressive proximal muscle weakness over weeks to months. Infrequently, patients experience pain, local muscle atrophy, muscle twitching, and/or muscle tightness (i.e., myotonia) (Urbano-Marquez and Fernández-Solà 2004). Chronic alcoholic myopathy is uncommon in patients under the age of 30 (Urbano-Marquez and Fernández-Solà 2004). Evidence of myopathy is associated with cumulative lifetime consumption of alcohol, with changes most evident with long-term, high-dose consumption (>10 kg of pure alcohol/kg of body weight) (Preedy et al. 2003; Urbano-Marquez and Fernández-Solà 2004). Thus, chronic alcohol-related myopathy occurs most commonly among people ages 40 to 60, with equal distribution between men and women. Chronic alcoholic myopathy has a higher incidence in patients with evidence of other alcohol-related organ dysfunction, occurring in 50 percent of patients with liver cirrhosis and 82 percent of those with alcohol-related heart muscle disease (i.e., cardiomyopathy) (Urbano-Marquez and Fernández-Solà 2004). Clinical series also indicate that patients with chronic alcoholic myopathy may be predisposed to presenting with episodes of acute alcoholic skeletal myopathy. Up to 30 to 46 percent of patients with a history of chronic alcohol abuse report episodic muscle pain (i.e., myalgia), weakness, and darkening of urine

following an alcohol binge (Urbano-Marquez and Fernández-Solà 2004).

Alcohol's Effects on Mechanisms Controlling Muscle Mass and Function

Altered Nutritional Status

Chronic heavy alcohol consumption can lead to protein calorie malnutrition, which frequently is related to the severity of alcoholic liver disease. One of the hallmarks of malnutrition resulting from chronic heavy alcohol consumption is a negative nitrogen balance, which can result from decreased nutrient intake and/or malabsorption of nutrients. As a result, micronutrient availability as well as circulating and tissue levels of growth factors are markedly altered following chronic alcohol consumption in animal models (Lang et al. 1998; Soszynski and Frohman 1992) and in humans (Röjdmarm and Brismar 2001). Among the most frequently reported deficiencies in people with AUD are folate, thiamine, vitamin B6, zinc, and iron (Halsted 2004). Moreover, the incidence of vitamin D deficiency reportedly is greater in people with AUD than in healthy control subjects (Anty et al. 2015), and this has been suggested as a possible contributor to alcoholic myopathy (Wijnia et al. 2013). Although nutritional deficits likely are factors in alcoholic myopathy, a wealth of literature describes additional specific biochemical, metabolic, and epigenetic alterations that play important roles in the underlying pathophysiology of alcoholic myopathy. A brief summary of the most relevant mechanisms is provided below.

Reduced Protein Synthesis

A major mechanism contributing to decreased muscle mass in alcohol-related myopathy is the imbalance between protein synthesis (i.e., anabolic reactions) and protein breakdown (i.e., catabolic

reactions). In particular, alcoholic myopathy is characterized by decreased protein synthesis (Steiner and Lang 2015) of both myofibrillar and sarco-plasmic proteins (Preedy and Peters 1988). Preclinical studies have identified several specific sites of alcohol-induced impairment in protein metabolism (figure 1). One of these is a protein called mammalian target of rapamycin (mTOR), which plays a central role in protein synthesis and is important for controlling skeletal muscle mass. It integrates signals from nutrients, growth factors, energy status, and stress and regulates cell size. mTOR is a subunit of two different protein complexes: mTOR complex 1 (mTORC1), which

also contains a protein called Raptor and can be inhibited by a compound called rapamycin; and mTORC2, which also contains a protein called Rictor and is rapamycin insensitive. mTORC1 is the master regulator of protein synthesis. On stimulation, mTORC1 activates two parallel signaling pathways:

- It phosphorylates, and thereby activates, S6 kinase 1 (S6K1), which leads to activation of the ribosomal protein S6 that is required for protein synthesis (i.e., translation).
- It phosphorylates and activates eukaryotic initiation factor 4E

(eIF4E)-binding protein (4EBP1). eIF4E is necessary for translation initiation; this process is inhibited by 4EBP1, which therefore acts as a translational repressor. Phosphorylation of 4EBP1 by mTORC1 releases this inhibition of eIF4E.

Chronic alcohol consumption has been shown to decrease activation of S6K1/S6 pathway and ribosomal protein S6 (rpS6) phosphorylation in skeletal muscle and in cultured muscle cells (i.e., myocytes) (Korzick et al. 2013). Alcohol also decreases the phosphorylation of 4EBP1 under both in vivo and in vitro conditions. This is associated with a redistribution of eIF4E from an active complex (i.e., eIF4E–eIF4G) to an inactive complex (i.e., eIF4E–4EBP1), thereby preventing mRNA translation. Finally, alcohol decreases the phosphorylation of mTOR itself (Lang et al. 2003) (figure 1). All of these effects contribute to decreased protein synthesis.

In addition to direct effects on the mTOR pathway, alcohol consumption significantly decreases levels of the insulin-like growth factor-1 (IGF-1) in both plasma and muscle, which is correlated with decreased muscle protein synthesis (Lang et al. 1998). Myocytes incubated with ethanol or its main metabolic products (i.e., acetaldehyde or acetate) show decreased IGF-1 and insulin-stimulated protein synthesis without significantly altering protein degradation (Steiner and Lang 2015).

An additional and alternative pathway controlling muscle mass involves the Smad family of proteins that act as transcription factors regulating the expression of several genes. These proteins are activated in response to transforming growth factor-beta (TGF-β), activin, and myostatin (figure 2). Smad signaling is required for myostatin-mediated inhibition of Akt/mTORC1 signaling, myotube atrophy, and TGF-β–induced fiber atrophy (Goodman and Hornberger 2014; McFarlane et al. 2006), all of which lead to a reduction of muscle

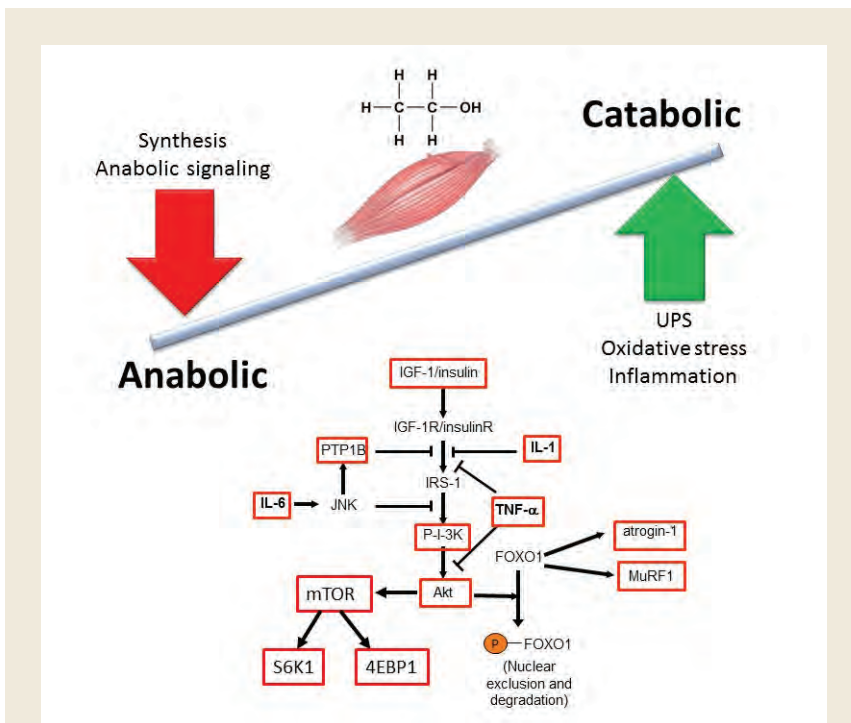


Figure 1 Principal effects of chronic alcohol abuse on anabolic and catabolic mechanisms that maintain skeletal muscle mass. Protein synthesis and breakdown are regulated by multiple factors, including anabolic hormones, nutrients, and myokines. Alcohol, depicted here by its chemical formulation, influences multiple aspects of both the anabolic and catabolic arms of the pathway. Numerous regulatory components of these pathways are altered by chronic alcohol exposure (see red boxes).

NOTE: 4EBP1 = eukaryotic initiation factor 4E binding protein; FOXO1 = Forkhead box protein O1; IGF-1 = insulin-like growth factor; IL-1 = interleukin 1; IL-6 = interleukin 6; IRS-1 = insulin receptor substrate 1; JNK = c-Jun N-terminal kinase; mTOR = mammalian target of rapamycin; MuRF1 = muscle RING-finger protein-1; P-I-3K = phosphatidylinositol 3-kinase; PTP1B = protein tyrosine phosphatase 1B; S6K1 = S6 kinase 1; TNF-α = tumor necrosis factor alpha; UPS = ubiquitin proteasome system.

mass. Alcohol exposure increases expression of insulin-like growth factor binding protein-1 (IGFBP-1) and myostatin, resulting in decreased skeletal muscle protein synthesis (Steiner and Lang 2015). Studies in macaques infected with the simian immunodeficiency virus (SIV) have demonstrated that chronic binge alcohol administration (CBA) increased myostatin mRNA levels, TGF- β levels, and fibrosis-promoting (i.e., profibrotic) gene expression in skeletal muscle at end-stage disease compared with sucrose-fed, SIV-infected macaques (Dodd et al. 2014; Molina et al. 2008). The increased expression of TGF- β and associated receptors and downstream signaling components could potentially decrease protein synthesis in addition to modulating extracellular matrix remodeling and promoting a profibrotic phenotype (Hong-Brown et al. 2015).

Increased Protein Degradation

Protein degradation in skeletal muscle is directed primarily by two pathways, the ubiquitin proteasome pathway (UPP) and the autophagic-lysosomal system (Steiner and Lang 2015). Ubiquitin is a small protein found in almost all tissues of the organism. In the UPP, three enzymatic components are responsible for linking chains of ubiquitin to proteins destined for degradation. They include ubiquitin-activating enzymes (E1 enzymes), ubiquitin carrier or conjugating proteins (E2 proteins), and ubiquitin ligases (E3 ligases), which are also known as atrogens. Two of these E3 ligases—atrogen-1 (also known as MAFbx) and MuRF1—are specific to the muscle. Proteins linked to the ubiquitin chains are recognized by a large protein complex called the 26S proteasome that is responsible for the degradation of intracellular proteins. This proteasome is composed of a 20S catalytic core, where actual protein degradation occurs, and two 19S polar caps that have regulatory functions.

Alcohol can interfere with normal functioning of the UPP in several ways, including both the proteasome itself and the ubiquitin binding to intracellular proteins. In the SIV-infected macaque model, CBA increased the expression of several proteins of the 19S caps, including S5A, Rpn6, and Rpn12 (LeCapitaine et al. 2011). Of these, Rpn6 is critical for assembly of the 19S cap and proper functioning of the 26S proteasome (Fernández-Solà et al. 2007). In rodents, both acute and chronic alcohol administration was associated with increased

expression of the two E3 ligases atrogen-1 and MuRF1 (Korzick et al. 2013; Vary et al. 2008). Elevated expression of MuRF1 and atrogen-1 also has been found in a number of catabolic conditions affecting muscle and is thought to reflect increased UPP activity. Similarly, atrogen-1 expression was significantly increased in skeletal muscle at end-stage disease in CBA-administered, SIV-infected macaques (Molina et al. 2008). Moreover, the molecular changes in the UPP system protein expression were associated with increased proteosomal activity

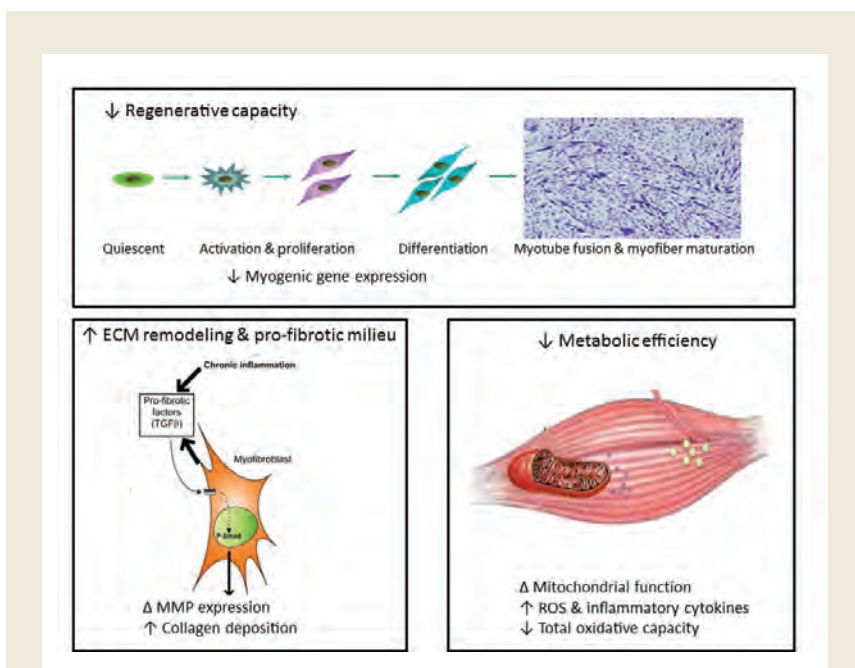


Figure 2 Mechanisms contributing to alcohol-induced loss of muscle mass and impairment in muscle growth. Decreased skeletal-muscle regenerative capacity is reflected as decreased myogenic gene expression, which prevents satellite-cell differentiation and myotube fusion and myofiber maturation. Chronic heavy alcohol consumption leads to skeletal-muscle inflammation, which favors expression of profibrotic factors such as transforming growth factor β (TGF- β), stimulating an increase in the expression and activation (phosphorylation) of transcription factors such as Smad (P-Smad). This in turn results in altered gene expression of matrix metalloproteinases (MMPs) and increased collagen deposition in the extracellular matrix (ECM) of skeletal muscle, which can prevent adequate satellite-cell activation, proliferation, and differentiation. Direct and indirect evidence indicates that alcoholic myopathy is associated with decreased mitochondrial function, enhanced reactive oxygen species (ROS) generation, and decreased total oxidative capacity, particularly in type 2 fibers. An increased proinflammatory and oxidative milieu in skeletal muscle likely is the underlying mechanism leading to the decreased regenerative capacity, development of a profibrotic milieu, and diminished metabolic efficiency.

in skeletal muscle from end-stage, alcohol-treated, SIV-infected macaques (LeCapitaine et al. 2011). The findings are not universal, however, because studies in mice fed a liquid alcohol diet for about a month failed to show changes in the UPP pathway (Thapaliya et al. 2014).

The autophagic-lysosomal system is a protein degradation system activated by cellular stress that mediates breakdown of misfolded proteins. During this process, a double-membrane structure (i.e., phagophore) engulfs the proteins destined for degradation, as well as a portion of the cell's cytoplasm, and then fuses to form an autophagosome. The mature autophagosome fuses with other vesicles (i.e., lysosomes) that contain enzymes which degrade the autophagosome contents (Shibutani and Yoshimori 2014); this is known as autophagy. There are contradicting data on the contribution or role of autophagy in alcohol-mediated muscle protein degradation. Thapaliya and colleagues (2014) have demonstrated increased expression of autophagy markers in the skeletal muscle of patients with alcoholic cirrhosis and in alcohol-fed mice. The study also demonstrated that *in vitro* treatment of C2C12 myotubes with 100 mmol/L alcohol (a concentration that exceeds physiological levels of alcohol) increased autophagic gene expression within 6 hours. Other investigators, however, did not observe a difference in autophagic gene or protein expression between chronic alcohol-fed mice and time-matched control animals (Steiner and Lang 2015; Steiner et al. 2015). Similarly, precursors of muscle cells (i.e., myoblasts) derived from CBA-treated macaques did not show changes in autophagic markers compared with myoblasts derived from sucrose-treated macaques (Simon et al. 2014). Thus, although decreased protein synthesis has been shown by several studies to play a role in alcohol-induced muscle loss, the contribution of catabolic pathways, particularly the UPP, also cannot be ignored.

Mechanisms Implicated in Alcohol's Effects on Factors Controlling Muscle Mass

Alcohol exposure seems to influence a variety of processes in the cells that may contribute to the altered protein synthesis and degradation levels described above. These include inflammatory reactions, oxidative stress, mitochondrial dysfunction, impaired muscle regeneration, as well as epigenetic and microRNA (miRNA)-related mechanisms (see figure 2).

Inflammation

Acute alcohol intoxication reduces inflammation in response to infectious challenges; however, chronic alcohol consumption or administration promotes an inflammatory milieu, which may contribute to tissue injury (Molina et al. 2014). Alcohol-mediated increases in inflammation have been linked to oxidative stress as well as to organ damage or impaired function in muscle, brain, and cardiovascular and immune systems (Molina et al. 2014). Chronic inflammation also has been implicated as an underlying mechanism for loss of muscle mass. Proinflammatory cytokines such as tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6) may play a role in these processes (see figure 1). In chronic alcoholics, TNF- α was inversely related to lean mass, especially in the legs (Gonzalez-Reimers et al. 2011). Moreover, rodent studies demonstrated that chronic alcohol feeding led to a sustained increase in TNF- α and IL-6 mRNA as well as activation of a regulatory enzyme called JNK (Lang et al. 2014). Similarly, SIV-infected macaques that had received CBA developed a proinflammatory milieu in skeletal muscle with increased expression of TNF- α and IL-6 (Molina et al. 2006, 2008). Increased TNF- α expression increases protein degradation by the UPP in skeletal and cardiac muscle (Karlstad et al. 2000), supporting alcohol-induced chronic inflammation as an underlying

mechanism that promotes skeletal muscle protein degradation and, consequently, loss of muscle mass.

Oxidative Stress

Alcohol is primarily metabolized to acetaldehyde by alcohol dehydrogenase and cytochrome p450 2E1 (CYP2E1) in the liver. Alcohol oxidation by CYP2E1 is upregulated with chronic alcohol abuse and has been shown to produce a large amount of reactive oxygen species (ROS) (Cederbaum 2001). Alcohol metabolism, production of ROS, impaired antioxidant mechanisms, and changes in the cellular redox state all are well-known mediators of tissue injury in several organ systems (Fernández-Solà et al. 2007; Molina et al. 2014). Chronic alcohol-fed rats show reductions in several antioxidant systems, including total and free glutathione levels, glutathione reductase activity, glutathione peroxidase activity, and superoxide dismutase 2 activity (Otis et al. 2007). In addition, skeletal muscle exhibits increased protein carbonylation (Dekeyser et al. 2013) as well as elevated cholesterol hydroperoxide and malondialdehyde content (Fernández-Solà et al. 2007), all of which reflect oxidative injury. This increase in oxidative stress promotes protein degradation, including increased expression of the UPP system in myotubes (Gomes-Marcondes et al. 2002), and increases the expression of atrogin-1 and TGF- β 1 (Otis et al. 2007). As discussed earlier, these factors work together to promote protein degradation and most likely impaired regeneration, resulting in muscle loss.

Mitochondrial Dysfunction

Mitochondria are critical not only for providing the energy necessary for muscle contraction but also because they are involved in the regulation of redox homeostasis and integration of cell-death signaling (Marzetti et al. 2010). Most of the ROS formed in the

cells are a byproduct of biochemical reactions (i.e., oxidative phosphorylation) in the mitochondria. At the same time, multiple defense mechanisms, including detoxifying enzymes and nonenzymatic antioxidant networks, are located in the mitochondria to cope with this physiological ROS production. However, excessive ROS generation, defective oxidant scavenging, or both have been implicated in mitochondrial dysfunction in sarcopenia and the pathogenesis of different myopathologies (Calvani et al. 2013; Lightfoot et al. 2015). Oxidative stress increases the incidence of mutations in the mitochondrial DNA (mtDNA), which is more susceptible to this damage than the DNA in the cell nucleus for several reasons. First, the mtDNA is located in close proximity to the site of ROS production, namely the electron transport chain (ETC). Second, the mtDNA's repair system is less efficient compared with that of the nuclear DNA. Finally, mtDNA lacks noncoding sequences (i.e., introns) where mutations would have no effect on the final protein product; therefore, any damage to the mtDNA immediately affects the proteins encoded by that DNA. Oxidative-stress-induced mutations in the mtDNA, in turn, lead to defective ETC components, decreased ATP production, and more ROS generation (Calvani et al. 2013).

However, ROS damage not only the mtDNA but also lipids, including cardiolipin (DiMauro 2006), which is an integral part of the inner mitochondrial membrane and, among other functions, can trap protons generated in the ETC as well as can trigger apoptosis. Similarly, ROS may damage proteins that reduce ETC activity and promote apoptosis (Gonzalez and Gottlieb 2007). In addition, oxidative stress activates transcription factors that mediate catabolic processes (McClung et al. 2010) and modify contractile elements, making them targets for protein breakdown (i.e., proteolysis) (Grune et al. 2003). All of these aspects of mitochondrial dysfunction can contribute to myopa-

thies. However, mitochondrial myopathies are considered to be genetic defects that impair the synthesis, assembly, or maintenance of ETC components and involve primary mtDNA mutations as well as nuclear mutations that disrupt the replication of mtDNA, synthesis of ETC components, or mitochondrial protein synthesis (Sharp and Haller 2014).

The adverse effects of chronic alcohol abuse on mitochondrial function in skeletal muscle are unclear. In a study of chronic alcoholics, Cardellach and colleagues (1992) demonstrated that alcoholic myopathy is not associated with impaired mitochondrial energy supply. However, chronic alcohol ingestion leads to increased glycogen and lipid storage, enlarged and distorted mitochondria, and a dilated sarcoplasmic reticulum (Rubin et al. 1976), strongly suggesting mitochondrial dysfunction. Moreover, mitochondrial fusion is inhibited in skeletal muscle from alcohol-fed rats. This is indicated by a reduction in the outer mitochondrial membrane fusion protein mitofusin-1 (Eisner et al. 2014), a decline in mitochondrial integrity primarily resulting from dysfunction of the enzyme mitochondrial topoisomerase (Laurent et al. 2014), and a significant decrease in glycolytic enzymes and mitochondrial respiration rates (Trounce et al. 1990). Other studies found evidence that chronic alcoholics with clinical neurological manifestations exhibit lower levels of aerobic metabolic reactions (which require mitochondrial functions such as the ETC) but greater levels of anaerobic reactions (which do not require mitochondrial activity) (Haida et al. 1998), further indicating that chronic heavy alcohol consumption is associated with mitochondrial dysfunction. Additional mechanistic studies are warranted to determine whether this is caused by abnormal aerobic enzyme activity or other mitochondrial abnormalities. Regardless of the underlying mechanisms, mitochondrial abnormalities may ultimately result in impaired bioenergetics and disturbances in the process through

which a nerve signal to the muscle leads to the muscle's contraction (i.e., in excitation–contraction coupling of the muscle).

Impaired Muscle Regeneration

Skeletal muscle injury triggers a well-defined healing or regenerative process involving inflammation, necrosis and degeneration of the affected tissue, activation of precursor cells (i.e., satellite cells), and subsequent regeneration of the muscle. Alcoholics are at an increased risk of several types of injuries, including those resulting from nerve damage to the limbs (i.e., peripheral neuropathies), falls caused by incoordination and imbalance, motor accidents, or muscle atrophy (Dekeyser et al. 2013). These injuries necessitate the activation of quiescent satellite cells, inducing them to proliferate and differentiate into myotubes to compensate for the enhanced skeletal muscle proteolysis and loss (Yin et al. 2013). Normally, during the initial inflammatory phase following skeletal muscle injury, two distinct subpopulations of immune cells called macrophages invade the injured muscle. The first population secretes inflammatory cytokines, such as TNF- α and IL-1. Subsequently, the proliferation and differentiation of satellite cells are facilitated by a second set of macrophages that secrete anti-inflammatory cytokines (Yin et al. 2013). Studies in SIV-infected male macaques have demonstrated that CBA results in accentuated upregulation of proinflammatory cytokine expression and depletion of antioxidant capacity in skeletal muscle, whereas administration of sucrose produces an overall skeletal muscle milieu that should trigger repair and regeneration capacity of satellite cells (LeCapitaine et al. 2011; Molina et al. 2008). Moreover, myoblasts isolated from skeletal muscle samples obtained from CBA-treated macaques show a marked reduction in differentiation potential, which translates into decreased myotube formation, accompanied by

decreased myogenic gene expression (Simon et al. 2014). These reports, as well as others in the literature, suggest that chronic alcohol administration or consumption may cause altered patterns of growth-factor and fibrotic gene expression in skeletal muscle (Dodd et al. 2014; Eisner et al. 2014), which may contribute to impaired regenerative capacity of muscle stem cells.

Epigenetic and microRNA Alterations

In vivo and in vitro exposure to alcohol can modify gene expression through epigenetic mechanisms in several tissues, including the liver, brain, and immune system (Shukla and Lim 2013; Zakhari 2013). Emerging evidence also suggests that epigenetic modulation may mediate fetal alcohol spectrum disorders (Zakhari 2013). Epigenetic modulation involves chemical modifications of the DNA, such as methylation; histone modifications, such as methylation, acetylation and deacetylation, phosphorylation, addition of ubiquitin molecules (i.e., ubiquitinylation), addition of adenosine-diphosphate ribose (i.e., ADP-ribosylation), and addition of small ubiquitin-like molecules (i.e., sumoylation); as well as the actions of noncoding microRNAs (miRNAs). Unlike genetic alterations (i.e., mutations) or defects, epigenetic alterations do not alter the DNA sequence itself and can be reversed by therapy. Thus, elucidating alcohol-induced epigenetic changes opens new avenues for therapy of alcohol abuse and the resulting organ damage, such as the use of compounds that prevent histone deacetylation (i.e., histone deacetylase inhibitors), miRNA modulation, and similar approaches.

Alcohol exposure can induce epigenetic changes through several mechanisms. For example, alcohol metabolism in the liver, but also in other tissues (e.g., skeletal muscle), produces oxidative metabolites such as acetaldehyde, acetate, acetyl-CoA, and ROS, as well

as nonoxidative products such as phosphatidylethanol and fatty acid ethyl ester (Curtis et al. 2013; Molina et al. 2014). Many of these products can induce tissue-specific epigenetic changes. For example, both in vivo and in vitro experiments have demonstrated that alcohol and its metabolites cause selective acetylation of histone H3 at a specific amino acid (i.e., the amino acid lysine at position 9 in the H3 molecule) (Kim and Shukla 2006).

Epigenetic mechanisms underlying alcohol-induced end-organ damage have been studied extensively in alcoholic liver disease. However, few studies to date have assessed alcohol-mediated epigenetic modulation specifically in the skeletal muscle. Ongoing studies are focusing on elucidating chronic alcohol-induced epigenetic and miRNA modifications that may contribute to impaired regeneration, metabolic dysregulation, and skeletal muscle wasting in people living with HIV/AIDS.

Implications of Alcohol's Effects on Muscle Mass and Function for Health and Disease

Human studies have demonstrated significant reductions in muscle mass associated with chronic alcohol consumption. Computerized tomography imaging of a region of the lower back (i.e., the L4 vertebrae) in a small cohort of chronic alcoholic subjects demonstrated a significantly reduced muscle area compared with healthy control subjects (Thapaliya et al. 2014). Similar results were found by Kvist and colleagues (1993) who noted significantly reduced femoral and gluteal muscle areas in chronic alcoholics, even though total lean body mass as determined from total potassium content did not differ significantly.

In contrast to the findings on muscle-mass changes in chronic alcoholics, analyses of the impact of alcohol consumption and abuse on exercise capacity have yielded conflicting results. Exposure to low doses of

alcohol produced no effect on peak exercise capacity in healthy participants undergoing cycle ergometry or treadmill testing (Bond et al. 1984; Houmard et al. 1987). Similarly, reductions in peak strength measured by dynamometry were evident with moderate alcohol consumption, but not in individuals reporting lower alcohol consumption. However, consumption of higher doses of alcohol before exercise resulted in prolonged exercise times and failure to reach maximum oxygen consumption in healthy subjects (Lecoultre and Schutz 2009; McNaughton and Preece 1986). Thus, alcohol may have a dose-dependent effect on exercise-induced muscle changes (Barnes et al. 2010). Finally, studies of postexercise muscle function suggest that alcohol consumption may impair normal muscle remodeling after exercise-induced injury (Barnes et al. 2010).

Although an increasing number of studies are investigating the effects of alcohol use in healthy athletes, few studies have evaluated the long-term impact of chronic heavy alcohol consumption on muscle function. In survey studies, up to 15 percent of patients with AUD reported significant mobility impairments that occurred more frequently with greater alcohol disease severity and with the presence of alcohol-related comorbidities (Gossop et al. 2007; Gunther et al. 2007; Kim and Kim 2015). Studies of male patients enrolled in alcohol treatment programs also showed alterations in exercise capacity. For example, compared with age-adjusted control subjects, detoxified alcoholics demonstrated significant reductions in isokinetic torque, work, and power as well as isometric and isotonic muscle loading (Pendergast et al. 1990; York et al. 1999). Reductions in maximal isometric voluntary force measured by knee extension also were more pronounced in older recovering alcoholics (Pendergast et al. 1990; York et al. 1999).

Therapeutic Options for Alcohol-Related Myopathy

Currently, the only known effective treatment for alcoholic myopathy is complete abstinence from alcohol (see figure 3). Fortunately, up to 85 percent of patients with biopsy-proven alcoholic myopathy demonstrate objective functional improvement in muscle strength within the first year of alcohol-drinking cessation and complete normalization of strength by the fifth year of abstinence (Estruch et al. 1998; Fernández-Solà et al. 2000). Even for patients unable to completely abstain from alcohol, reduced cumulative alcohol consumption results in improvements in muscle strength over time (Estruch et al. 1998; Fernández-Solà et al. 2000). Acute alcoholic myopathy usually reverses within days or weeks of abstinence, whereas chronic myopathic changes usually resolve within 2 to 12 months (Peters et al. 1985). Moreover, nutritional optimization, including correction of vitamin and electrolyte deficiencies, is associated with greater improvement of muscle health (Urbano-Marquez and Fernández-Solà 2004).

Physiotherapy often is recommended in patients with acute or chronic alcoholic myopathy, although its benefit on myopathy resolution has not been studied rigorously. Use of physical-activity interventions, primarily aerobic exercise and/or resistance-training activities, has been shown to improve exercise capacity in AUD patients (Brown et al. 2014; Capodaglio et al. 2003). Although previous randomized controlled trials of exercise were not limited to patients with alcohol-related myopathy, they demonstrated significant improvements in maximal oxygen consumption and baseline heart rate in individuals with AUD subjected to standardized exercise interventions compared with control subjects (Brown et al. 2014; Capodaglio et al. 2003). Additional studies are needed to understand whether exercise interventions are particularly beneficial to patients with AUD and alcohol-related

myopathy and to further elucidate the effects of exercise on alcohol-related muscle changes, particularly at the cellular and molecular levels.

Novel therapeutic agents increasingly are being explored for treatment of myopathic disease. Although to date many of these have not been studied in alcohol-related myopathy, they present exciting targets for potentially ameliorating the substantial burden of alcoholic myopathy. Agents targeting hormonal pathways, muscle-injury pathways, and vitamin deficiencies related to muscle disease are being

actively investigated. Studies stimulating the growth hormone axis using injection of IGF-1 and IGF-1 binding complex in alcohol-fed rats have achieved restoration of muscle protein synthesis to basal control values (Lang et al. 2004). Other studies found that oral or intravenous administration of ghrelin, an upstream regulator of the growth hormone/mTOR axis, can help maintain lean muscle mass in patients with wasting (i.e., cachexia) related to cancer or chronic lung disease (García et al. 2013; Miki et al. 2012). Treatment with agents that

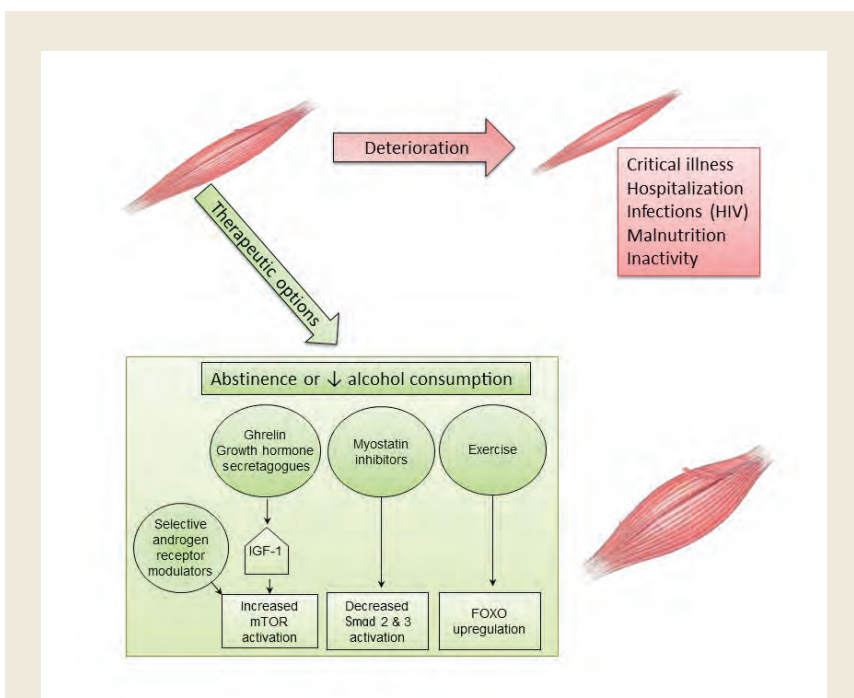


Figure 3 Aggravating conditions of, and therapeutic options for, alcoholic myopathy. Alcoholic myopathy may be further exacerbated by critical illness, prolonged hospitalization, chronic infection (e.g., HIV), malnutrition, and inactivity. Therapeutic options to achieve muscle mass accretion and restoration of skeletal muscle function include complete abstinence or at least decreased alcohol consumption, as well as aerobic exercise and/or resistance training. Other approaches currently being tested in myopathies of different etiologies also could prove effective for alcoholic myopathy. These include manipulation of the growth-hormone axis through administration of either insulin-like growth factor-1 (IGF-1), the principal mediator of growth-hormone action, or ghrelin, an upstream regulator of the growth hormone/mammalian target of rapamycin (mTOR) axis. Inhibition of myostatin, a negative regulator of muscle growth, may reduce Smad signaling, thereby preventing loss of muscle mass. Finally, exercise may lead to upregulation of Forkhead box protein O1 (FOXO1). Further studies are needed to determine the efficacy of these therapies for amelioration of alcoholic myopathy.

can inhibit myostatin function (i.e., myostatin antagonists) in heart failure and sarcopenia models resulted in a reduction in Smad signaling, preventing loss of muscle mass (Heineke et al. 2010; Murphy et al. 2011). Active trials of pharmacologic myostatin antagonists are in early-stage clinical investigations. Finally, there are conflicting data regarding the use of selective androgen receptor modulators to maintain muscle mass. Early (i.e., Phase 1 and 2) clinical studies of these agents in patients with cancer and age-related sarcopenia showed increased lean muscle mass; however, these findings could not be replicated in larger Phase 3 trials (Dalton et al. 2011; Dobs et al. 2013; Stewart Coats et al. 2011). Additional studies are needed to understand the role of selective androgen receptor modulators as

single-drug or combination therapy for muscle wasting.

Summary

Alcohol-related muscle disease is the most common clinical manifestation of AUD. Despite the high prevalence of disease, alcohol-related myopathy frequently is unrecognized. Most importantly, myopathy significantly contributes to long-term impairments in physical function and diminishes health-related quality of life for people with AUD. Therefore, research on the mechanisms underlying alcoholic myopathy and potential therapeutic approaches to ameliorate the disease, particularly in individuals with comorbid conditions, is extremely relevant. Researchers increasingly are recognizing the molecular pathways contributing

to alcohol-induced muscle wasting, including reductions in mTOR-mediated protein synthesis and excessive protein degradation by activation of the UPP and autophagic-lysosomal system. In the settings of acute inflammation, oxidative stress, and/or mitochondrial dysfunction, exacerbation of these pathways is associated with accelerated muscle wasting. Clinical manifestations of muscle wasting include impairments in muscle dynamics (i.e., strength, power, and force) and loss of mechanical unloading, which also promote alcohol-related bone loss. Identification of these key pathways offers novel targets for therapeutics aimed at reducing the burden of alcohol-related muscle disease. Further studies are needed to understand the role of exercise and drug interventions, such as growth hormone regulators,

Glossary

Creatinine Kinase (CK): CK is the most widely used enzyme for diagnosing and monitoring muscle injury. It is located on the inner mitochondrial membrane of myofibrils and in muscle cytoplasm. CK catalyzes the transfer of a phosphate from phosphocreatinine to adenosine diphosphate (ADP), producing creatinine and adenosine triphosphate (ATP).

Cytokine: Small proteins released from cells that have a specific effect on the interaction between cells, on the communication between cells, or on the behavior of cells.

Electron Transport Chain (ETC): Series of compounds that transfer electrons from electron donors to electron acceptors via reduction and oxidation. The ETC serves as the most productive pathway of cellular respiration in humans.

Epigenetic: Modifications in gene function that do not involve changes in the DNA sequence.

Extracellular Matrix: The collection of extracellular molecules (e.g., collagen) that are secreted by cells and provide structural and biochemical support to the surrounding cells.

Histone: Any of a group of five small proteins occurring in the nucleus of eukaryotic cells that organize DNA

strands into nucleosomes by forming molecular complexes around which DNA winds.

microRNA (miRNA): A short, noncoding segment of RNA that suppresses gene expression by binding to complementary segments of messenger RNA and interfering with protein formation during translation.

Mitochondria: Cellular organelles found outside the nucleus that produce energy for the cell via cellular respiration.

Myostatin: A skeletal-muscle protein that acts as a transforming growth factor to restrain muscle growth.

Myotonia: Tonic spasm of one or more muscles.

Necrosis: Localized death of living tissue.

Redox State: Ratio of the concentration of oxidized species to the concentration of reduced species.

Sarcoplasmic Reticulum: Specialized endoplasmic reticulum of cardiac and striated skeletal muscle that functions as a storage and release area for calcium.

Satellite Cell: A stem cell that lies adjacent to a skeletal muscle fiber and plays a role in muscle growth, repair, and regeneration.

myostatin antagonists, and androgen modulation, on alcohol-related muscle wasting.

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Uniting Epidemiology and Experimental Disease Models for Alcohol-Related Pancreatic Disease

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Findings from epidemiologic studies and research with experimental animal models provide insights into alcohol-related disease pathogenesis. Epidemiologic data indicate that heavy drinking and smoking are associated with high rates of pancreatic disease. Less clear is the association between lower levels of drinking and pancreatitis. Intriguingly, a very low percentage of drinkers develop clinical pancreatitis. Experimental models demonstrate that alcohol administration alone does not initiate pancreatitis but does sensitize the pancreas to disease. Understanding the effects of alcohol use on the pancreas may prove beneficial in the prevention of both pancreatitis and pancreatic cancer.

Key words: Alcohol-related disease; alcohol-related pancreatic disease; pancreas; pancreatitis; pancreatic cancer; epidemiology; smoking; animal models; experimental disease models

Inflammation of the pancreas, or pancreatitis, can occur suddenly (i.e., acute pancreatitis) or after a long period of damage (i.e., chronic pancreatitis). Chronic pancreatitis is characterized by inflammation that does not improve, and becomes worse over time. Gallstones are a common cause of acute pancreatitis, which is usually resolved with adequate treatments in a few days. Heavy alcohol use over many years is the most common cause of chronic pancreatitis (Yadav and Lowenfels 2013), but cystic fibrosis, tobacco smoking, autoimmune conditions, high levels of calcium or fat in the blood, and certain medications can also cause chronic pancreatitis (National Institute on Diabetes and Digestive and Kidney Diseases 2016). Chronic pancreatitis can lead to diabetes and pancreatic cancer (Yadav and Lowenfels 2013). Since there are no current methods for

treating pancreatitis or preventing recurrent episodes of nongallstone-related pancreatitis, understanding the risk factors for this condition is critical to prevention.

Following a review of the epidemiology of both acute and chronic pancreatitis, and pancreatic cancer and the influence of alcohol use and other risk factors, this article examines current experimental models that explore alcohol's role in pancreatic disease and the cellular mechanisms at work. It focuses on the currently accepted view of alcohol-related pancreatic disease, which holds that alcohol mediates the progression from acute to chronic disease through a number of mechanisms. Following recurrent acute attacks, alcohol may trigger changes leading to chronic pancreatitis and pancreatic cancer. This can happen through alterations in cell signaling pathways; the toxic effects of

alcohol's metabolites on pancreatic cells; oxidative stress; and by promoting activation of pancreatic stellate cells (PSCs), which play an important role in the development of scarring (i.e., fibrosis), inflammation, and tissue damage.

The Burden of Pancreatic Diseases

Acute pancreatitis is among the most common gastrointestinal causes of inpatient admission to U.S. hospitals. The annual incidence of acute pancreatitis ranges from 13 to 45 per 100,000 people, and chronic pancreatitis from 2 to 14 per 100,000 (Machicado et al. 2016; Yadav and Lowenfels 2013). The incidence of chronic pancreatitis in European countries varies from 1.8 cases per 100,000 people in the Netherlands (Spanier et al. 2013) to 13.4 cases per 100,000 in Finland (Jaakkola and Nordback 1993). A population-based U.S. study noted little change in the incidence of chronic pancreatitis between two time periods (from 3.3 in 1940–1969 to 4.0 per 100,000 in 1977–2006). In Japan, however, a progressive increase in incidence from 5.4 in 1994 to 11.9 in 2007 and 14.0 in 2014 has been noted (Machicado et al. 2016).

Prevalence estimates for chronic pancreatitis are limited to only a few countries (Machicado et al. 2016). Although these rates vary widely, from 13.5 per 100,000 in China to 126 per 100,000 in India, estimates show less variability in the United States, France, Spain, and Japan, ranging from 25 to 50 per 100,000. Similar to incidence, prevalence estimates from Japan increased from 28.5 per 100,000 people in 1994 to 52.4 per 100,000 people in 2014 (Machicado et al. 2016). A 10-year study of patients at 22 hospitals in China also found an increasing prevalence (from 3.08 cases per 100,000 people in 1996 to 13.52 per 100,000 in 2003) (Wang et al. 2009). Although acute pancreatitis affects men and women equally, chronic

pancreatitis, especially alcohol-related cases, is more common among men (Yadav and Lowenfels 2013).

Pancreatic cancer accounts for about 3 percent of all cancers in the United States and about 7 percent of cancer deaths (American Cancer Society 2016). Worldwide, the annual incidence rate for pancreatic cancer is about 8 per 100,000 people (Yadav and Lowenfels 2013). Both pancreatitis and pancreatic cancer affect Blacks more than Caucasians, although the reasons for this racial disparity are unclear (Wilcox et al. 2016; Yadav and Lowenfels 2013).

Progression from Acute to Chronic Pancreatitis

The risk of progression from acute to chronic pancreatitis is higher among alcoholics and smokers, and higher in men than in women. A meta-analysis of 14 studies on this progression concluded that 10 percent of patients with a first episode of acute pancreatitis and 36 percent of patients with recurrent acute pancreatitis develop chronic pancreatitis (Sankaran et al. 2015). Other research found that, following an episode of alcohol-related acute pancreatitis, the risk of progression to chronic pancreatitis was approximately 14 percent with complete abstinence or only occasional drinking, 23 percent with decreased but daily drinking, and 41 percent with drinking at the same level as before the acute episode (Takeyama 2009).

Morphological Changes in the Pancreas from Acute to Chronic Pancreatitis

Nikkola and colleagues (2014) used imaging technology (secretin-stimulated magnetic resonance cholangiopancreatography) to examine the morphological changes induced by an initial episode of alcoholic pancreatitis. The researchers followed 44 patients after their first episode of alcohol-associated pancreatitis for up to 9 years. They found

that whereas a single episode of acute pancreatitis could induce chronic changes, morphological progression (i.e., pancreatic cysts, parenchymal changes, and atrophy) was more frequent in patients with moderate or severe first attacks and in those who had recurrent attacks of pancreatitis.

Risk Factors for Alcohol-Related Pancreatic Disease

A meta-analysis of 51 international population-based studies concluded that heavy alcohol use was an important risk factor for pancreatic disease (Alsamarrai et al. 2014). Overall, the studies demonstrated an estimated 40 percent increased risk of pancreatic disease in heavy drinkers (i.e., those reporting more than 20 drinks per week). The prevalence of pancreatitis is approximately four times higher among people with a history of alcoholism (Yadav et al. 2007). Historically, an estimated 60 to 90 percent of chronic pancreatitis cases were attributed to alcohol use (Coté et al. 2011). However, more recent research suggests a lower prevalence of heavy drinking among chronic pancreatitis patients than previously estimated (Frulloni et al. 2009; Yadav et al. 2009). One recent study estimating the prevalence of alcohol-related pancreatitis used data from 539 patients and 695 unaffected study participants enrolled in a study of pancreatic disease at U.S. treatment centers (Coté et al. 2011). An estimated 44.5 percent of chronic pancreatitis cases were classified as alcohol related, based on physician assessment. The authors acknowledge that the lower-than-expected rate of alcohol-related disease may be due to the specialized nature of the treatment centers, the fact that alcohol users may be less likely to seek care, or because physicians who attribute a patient's disease to alcohol use would be less likely to refer them to a specialist's care. In Japan, a questionnaire to assess alcohol use among patients with alcoholic pancreatitis

found that women developed pancreatitis at a younger age, with shorter duration of alcohol use, and after smaller cumulative amounts of alcohol consumption compared with male patients (Masamune et al. 2013). In this study, continued drinking led to the recurrence of pancreatitis.

Some studies have suggested a threshold of alcohol use above which there is an increased risk for pancreatitis. Yadav and colleagues (2009) found the threshold to be 5 drinks per day for chronic pancreatitis. The relationship between lower levels of alcohol consumption and pancreas disorders is less well defined. In one recent meta-analysis of seven published studies, researchers noted a dose-dependent relationship between alcohol use and chronic pancreatitis in both sexes and for acute pancreatitis among men (Samokhvalov et al. 2015). Interestingly, a J-shaped relationship for the association with acute pancreatitis was noted among women, with a protective effect at less than 40 grams of ethanol per day (2 to 3 drinks) (Samokhvalov et al. 2015). Another recent study across a large diverse population not included in the meta-analysis observed a protective effect of moderate drinking on recurrent acute or chronic pancreatitis in men, and for all pancreatitis in women (Setiawan et al. 2016). Suggested explanations for this observation are a decreased risk of gallstone formation with moderate drinking, characteristics of the study population (older cohort), difficulty in assessing accurate exposure information, and possible contamination of the control group with former drinkers (Yadav 2016). Biological plausibility for how moderate drinking may have a protective effect is discussed later in this review. Data on the role of type and pattern of alcohol consumption and risk of pancreatitis are too limited to make definitive conclusions.

For pancreatic cancer, results from meta-analyses estimate a 20-percent increased risk from consuming 3 drinks per day (Maisonneuve and Lowenfels 2015; Tramacere et al.

2010). Another meta-analysis of individual participant data for more than 800,000 people found 22 percent increased risk of pancreatic cancer among people who consumed more than 3 drinks per day, although the association was only significant in women (Genkinger et al. 2009). A meta-analysis of alcohol's impact on risk for 23 types of cancer that included 572 studies found that heavy drinkers had a significantly higher risk of pancreatic cancer (relative risk of 1.19) compared with nondrinkers and occasional drinkers (Bagnardi et al. 2015).

Alcohol and Smoking Interactions

Cigarette smoking and heavy alcohol use, commonly co-occurring behaviors, increase risk for pancreatitis and pancreatic cancer (Yadav and Whitcomb 2010). A study of 108 smokers with alcohol-related chronic pancreatitis examined disease outcomes in relation to tobacco dose. The researchers concluded that smoking accelerates the course of pancreatic disease in a dose-dependent fashion, separate from the level of alcohol consumption (Rebours et al. 2012). A meta-analysis of 12 studies reported that while smoking increases the risk of chronic pancreatitis independently from alcohol, the effects of smoking are stronger for alcohol-related pancreatitis (Andriulli et al. 2010). In a recent study, Setiawan and colleagues (2016) found that smoking was significantly associated with nongallstone acute and chronic pancreatitis. The risk associated with current smoking was highest among men who consumed more than 4 drinks per day. For pancreatic cancer, among current smokers, heavy alcohol consumption was associated with a significantly increased pancreatic cancer risk. Risk was increased insignificantly among light and moderate drinkers who were smokers (Rahman et al. 2015).

Research comparing pancreatic duct-cell function in current and former smokers with never-smokers found that smoking was an independent predictor of cell dysfunction, after controlling for age, gender, and alcohol intake. The study also found no interaction between smoking status and alcohol consumption in predicting duct-cell dysfunction (Kadiyala et al. 2013).

Alcohol and Genetic Interactions

Although alcohol abuse and smoking are major environmental risk factors for pancreatic disease, only a small percentage of drinkers and smokers develop pancreatic disease (Yadav and Lowenfels 2013). This has led to a search for a role of genetic differences that could explain the susceptibility of some individuals to the effects of alcohol on the pancreas. Whitcomb and colleagues (2012) identified an association between genetic variants of Claudin-2 (*CLDN2*) and the risk of alcoholic pancreatitis. *CLDN2* is an X-linked gene involved in tight junction permeability and is expressed by pancreatic acinar cells. Alterations in the function of tight junctions in the pancreas or possibly in the intestinal epithelium could inappropriately expose the pancreas to toxins that could interact with the direct effects of alcohol in the pancreas. A recent study (Koziel et al. 2015) concluded that genetic mutations in SPINK1, a protein that inhibits activation of trypsinogens within the pancreas, may predispose individuals to severe acute pancreatitis, especially in patients that abuse alcohol.

As described in these epidemiologic studies (Yadav and Lowenfels 2013), pancreatic disease appears to be triggered by repeated acute attacks in combination with heavy alcohol use and other factors such as smoking and genetic factors.

Molecular Mechanisms of Alcohol-Related Acute and Chronic Pancreatitis

The general concepts that have been followed in developing animal models for alcohol research are based on observations originally described by Comfort and colleagues (1946). They found histological changes consistent with acute pancreatitis in patients with chronic pancreatitis. When followed longitudinally, these patients had greater amounts of necrosis indicative of acute pancreatitis early in the disease course and fibrosis in later stages, suggesting that chronic pancreatitis developed from repeated attacks of acute pancreatitis.

Studies using animal models of pancreatitis have supported the idea that alcohol-related exocrine pancreatic disease is induced by the combination of ethanol and other factors. For example, cholecystokinin (CCK) analogues cause pancreatitis in rodents in the absence of alcohol treatments only at doses much greater than those needed to activate known physiologic responses such as pancreatic enzyme secretion and gallbladder contraction (Lam et al. 2007). However, in ethanol-fed animals, CCK causes acute pancreatitis when given at more physiologic doses (Pandolfi et al. 1999). In other examples, ethanol feeding exacerbates pancreatitis due to hyperlipidemia and pancreatic-duct obstruction (Grauvsogel et al. 2010). Ethanol-feeding models have also been used to show that alcohol impedes recovery from acute pancreatitis, resulting in promotion of chronic-pancreatitis features of chronic inflammation and fibrosis (Gukovskiy et al. 2008).

Other animal models are based on previous observation of the increased susceptibility of people with compromised immunity (a common consequence of alcohol abuse) to viral pancreatitis. Using a mouse model, Jerrells and colleagues (2007) found that ethanol consumption alone does not produce pancreatic damage but causes viral pancreatitis to be more

severe and prolonged. Similarly, others have shown that alcohol feeding and lipopolysaccharide (LPS) administration, to mimic the effects of alcohol on increased circulating LPS in humans, promotes pathologic features of chronic pancreatitis (Fortunato et al. 2006; Nakayama et al. 2014; Vonlaufen et al. 2007, 2011). Importantly, Vonlaufen and colleagues (2011) showed in the LPS-alcohol model that alcohol withdrawal causes regression of the features of chronic pancreatitis, indicating the importance of alcohol in promoting disease progression as originally described in humans (Comfort et al. 1946).

To emphasize, alcohol feeding alone had minimal pathologic effects in these models. Furthermore, the initiating agents for causing pancreatitis (i.e., CCK, LPS, duct obstruction, or viral infection) at the doses used in the corresponding models have minimal effects on pancreatitis responses in the absence of alcohol treatments.

Role of Pancreatic Acinar Cells and Ductal Cells

Research into the molecular mechanisms of alcohol-related pancreatitis has largely focused on the pancreatic acinar cell, the component of the pancreas devoted to synthesis, storage, and secretion of digestive enzymes. These studies suggest that alcohol does not directly damage acinar cells but may make cells susceptible to other factors that trigger cell damage. For example, *in vitro* and *in vivo* studies that focus on the effects of CCK on the transcription factor NF- κ B, an intracellular signaling pathway involved in the inflammatory response of pancreatitis, show that alcohol treatments augment CCK-induced NF- κ B activation (Pandolfi et al. 1999). Another study suggested that alcohol activates a specific isoform of the signaling molecule known as protein kinase C (i.e., protein kinase C epsilon, PKC ϵ), which, in turn, is involved in NF- κ B activation and the initiation of pancreatitis

(Satoh et al. 2006). Further research using experimental models of acute pancreatitis examined the mechanisms through which PKC ϵ regulates cell death. The researchers found that PKC ϵ knockout mice (in which PKC ϵ is genetically deleted) had decreased inflammation and necrosis and less severe acute pancreatitis in response to high doses of CCK analogues (Liu et al. 2014). In addition, alcohol has been found to promote secretion of digestive enzymes from the basolateral aspect of the acinar cell via mechanisms involving protein kinase C (Cosen-Binker 2007). Basolateral enzyme secretion would inject the digestive enzymes into the tissue of the pancreas where they can cause injury to the pancreas and pancreatitis.

More recently, studies have turned to determining effects of alcohol on the pancreatic duct cell, which is important for producing fluid secretion and carrying digestive enzymes secreted by the acinar cell into the gut lumen, where they are needed for meal digestion. These studies show that excessive alcohol drinking can cause inhibition of the function of the same transporter that is inhibited by mutation in cystic fibrosis (Mal  th et al. 2015). These findings, and the fact that the acinar cells and duct cells must both perform their functions in a coordinated fashion to prevent disease (Hegyi et al. 2011), suggest that alcohol can promote pancreatitis via its actions on one or both of the key cellular components of the pancreas.

Role of Pancreatic Stellate Cells

Alcohol-related pancreatitis has been linked to the activation of pancreatic stellate cells (PaSC) (Apte et al. 1999, 2000; Vonlaufen et al. 2007, 2011). PaSC are normal resident cells in the exocrine pancreas. They are present in the periacinar space and have long cytoplasmic processes that surround the acinar structures and ducts of the exocrine pancreas (Omary et al. 2007).

In their normal state, often referred to as “quiescent,” PaSC provide basement membrane and organization of the pancreatic epithelium. However, in pathologic states such as alcohol-induced pancreatitis, PaSC participate in disease pathogenesis after transforming into an “activated” state (also known as a “myofibroblastic” state) (Omary et al. 2007). These cells target an injured area and play a role in tissue repair (Apte et al. 1999). However, when they develop into a sustained activated state inappropriately, PaSC play a major role in alcohol-related pancreatitis. They mediate both the fibrosis and chronic inflammatory response of chronic alcoholic pancreatitis as well as pancreatic cancer (Apte et al. 2013). Regarding chronic pancreatitis, research suggests that this activation is mediated by alcohol, its toxic metabolite (i.e., acetaldehyde), or oxidative stress. Researchers have sought to identify the intracellular signaling pathways mediating PaSC responses. The goal of such research would be to develop strategies to target specific signaling molecules and interrupt PaSC activation, inhibiting abnormal fibrogenesis.

Recent studies suggest that the mitogen-activated protein kinase (MAPK) pathway, a major intracellular signaling pathway, plays a role in regulating the effects of alcohol and its metabolite acetaldehyde on PaSC (Apte et al. 2007). In addition, alcohol has been shown to activate the membrane-bound enzyme complex nicotinamide adenine dinucleotide phosphate (NADPH) oxidase system, contributing to PaSC proliferation (Hu et al. 2007).

To address the epidemiologic observations of combined effects of alcohol and smoking, Lee and colleagues (2015) showed that cigarette smoking extract as well as nicotine and one of its major metabolites caused activation of PaSC. This activation was mediated via nicotinic acetylcholine receptors they found on the PaSC, and alcohol added to the effects of the smoking molecules.

The following sections summarize other potential co-factors that might

trigger alcohol-related pancreatitis, including the participation of ethanol metabolites in alcohol-induced pancreas pathology.

Ethanol Metabolism in the Exocrine Pancreas

Metabolism of ethanol by the exocrine pancreas occurs by both oxidative and nonoxidative routes (Gukovskaya et al. 2002; Haber et al. 2004). The oxidative pathway is the predominant pathway for ethanol elimination in the body, occurring mostly in the liver. In the oxidative pathway, ethanol is converted to acetaldehyde by alcohol dehydrogenases (ADH), and then acetaldehyde is converted to acetate by mitochondrial aldehyde dehydrogenases (ALDH). Both enzymes are functional and present in the exocrine pancreas. The nonoxidative route of ethanol metabolism involves covalent coupling of ethanol with fatty acids to yield lipophilic fatty acid ethyl esters (FAEEs). This pathway provides the transient storage of ethanol while it awaits oxidative metabolism for removal from the body. The importance of the nonoxidative pathway comes from observations that humans dying from alcohol intoxication have high levels of FAEEs in the pancreas (Laposata and Lange 1986) and the finding that the FAEEs are formed using the enzyme carboxylester lipase, a highly expressed digestive enzyme made in the pancreas and secreted during lipid digestion (Huang et al. 2014).

There has been increasing evidence that the nonoxidative pathway plays an important role in alcohol pathogenesis in the acinar cell. For example, FAEEs were found to cause necrosis in pancreatic acinar cells by inducing sustained increases in free concentrations of Ca^{2+} in the cytoplasm from released intracellular stores, leading to toxicity of mitochondria and failure to produce ATP (Criddle et al. 2004, 2006). In addition, FAEE administration to experimental animals causes pancreas pathology (Lugea et al.

2003). Moreover, studies using pharmacologic and genetic inhibition of ADH caused pancreatitis responses in animal models, while pharmacologic inhibition of carboxylester lipase inhibited pancreatitis responses (Huang et al. 2014; Kaphalia et al. 2010).

Several genetic polymorphisms in the enzymes metabolizing ethanol have been described in humans in the last decade. A recent review by Aghdassi and colleagues (2015) summarizes these polymorphisms and their potential for conferring high susceptibility to alcohol-related pancreatic disorders. The most common polymorphism, an inactive ALDH2 gene, affects 40 to 50 percent of East Asians who exhibit high levels of acetaldehyde in blood after alcohol consumption, and higher susceptibility to acetaldehyde toxicity and certain forms of cancer (Chao et al. 2000; Yokoyama et al. 2010).

However, studies on the relevance of specific genetic polymorphisms of ethanol-metabolizing enzymes on pancreatic disorders have been limited, and the resulting data equivocal. Future studies will help to clarify whether these polymorphisms alone or in combination alter the susceptibility to alcohol-related chronic pancreatitis and pancreatic cancer.

Alcohol and the Cholinergic System

The neurotransmitter acetylcholine may play a role in alcohol-induced pancreatic damage. Lugea and colleagues (2010) found that atropine dramatically reduced cerulein-induced pancreatitis in alcohol-fed rats, indicating that alcohol-ensitizing effects are mediated at least in part through activation of cholinergic pathways. This effect is independent of the effects of smoking on nicotinic receptors present on the PaSC, described below.

Alcohol and Mitochondrial Dysfunction

Mitochondrial membrane permeabilization (MMP) triggers mitochondrial dysfunction and cell death and leads to tissue damage. The mitochondrial permeability transition pore (MPTP) plays a critical role in MMP. Research with pancreatic cells from mice found that oxidative metabolism of ethanol sensitizes mitochondria to activate MPTP, making them more sensitive to the toxicity by low concentrations of Ca^{2+} in the cell. This leads to mitochondrial failure and ATP depletion, making the pancreas susceptible to pancreatitis (Huang et al. 2014; Shalbuva et al. 2013).

Alcohol, Autophagy, and Lysosomes

Autophagy is a natural and regulated process for the cell to disassemble unnecessary or dysfunctional components. This disassembly allows for an orderly recycling of cellular components. The process of autophagy involves isolating targeted cellular constituents within a double-membrane vesicle known as the autophagosome. The autophagosome eventually fuses with the cell's lysosomes to form a compartment where lysosomal enzymes carry out the disassembly. Recent studies have shown the importance of normal autophagy and lysosomal function in the mechanism of pancreatitis (Gukovskaya et al. 2016). That is, animal models created with genetic inhibition of key autophagic mediators (i.e., autophagy protein 5, Atg5, or Atg7) or the glycoprotein required for lysosomal integrity (i.e., lysosomal-associated membrane protein-2, LAMP2) lack normal autophagic processing, resulting in inappropriate processing of digestive enzymes in the acinar cells and spontaneous pancreatitis. Further, in nonalcoholic models of pancreatitis, findings of disordered fusion and function of the lysosomal-autophagic system have been described (Gukovskaya et al. 2016).

Several studies have demonstrated the effects of alcohol on lysosomal and autophagy function. For example, Wilson and colleagues (Haber et al. 1993; Wilson et al. 1990, 1992) demonstrated that an alcohol diet or treatment of isolated lysosomes with FAEEs or cholesteryl esters caused lysosomal fragility and leakage of lysosomal enzymes into the acinar cell cytosol. Furthermore, more recent studies show that alcohol feeding and LPS treatment decrease the expression of LAMP2 in the pancreas of animals (Fortunato et al. 2009; Mareninova et al. 2015). In sum, these studies show that alcohol feeding, FAEE, and LPS cause lysosomal and autophagy dysfunction, which may result in pancreatitis responses.

Dietary Factors

Thiamine Deficiency. Thiamine (vitamin B1) is essential for pancreatic acinar-cell function. Cells obtain thiamine from their surroundings and enzymatically convert it into thiamine pyrophosphate (TPP), which is transported to mitochondria by the mitochondrial TPP transporter (MTPPT). Srinivasan and colleagues (2015) found that, in mice, chronic alcohol exposure significantly inhibited TPP uptake, which was associated with decreased expression of MTPPT protein and activity of the gene for MTPPT in pancreatic acinar cells. The authors suggest that this effect of alcohol could have a negative effect on physiologic function of the mitochondria in the acinar cell and make them susceptible to pathologic responses with stress.

Folate Deficiency. Dietary folate is critical for pancreatic health. A study in rats receiving a chronic alcohol diet found a significant decrease in folate uptake by isolated pancreatic cells compared with rats not receiving alcohol. The alcohol-fed rats also had decreased activity in both of the major folate uptake systems (i.e., reduced folate carrier and proton-coupled folate transporter) (Said et al. 2010).

Fiber. A population-based prospective analysis of dietary factors for pancreatitis in the United States found that the majority of dietary factors were mainly associated with the risk of gallstone-related pancreatitis, with the notable exception of dietary fiber (Setiawan et al. 2017). The investigators found dietary fiber to be inversely associated with both gallstone- and nongallstone-related acute pancreatitis but not suspected chronic pancreatitis. Fiber has been associated with changes in gut microbiota, improvements in gut epithelial tightness, and prevention of endotoxin transit into the system (Blaut 2015; Ghanim et al. 2009). Importantly, experimental animal models of pancreatitis show that endotoxin can promote the development and severity of pancreatitis (Fortunato et al. 2006; Vonlaufen et al. 2007). Insoluble fiber may also have a protective effect by reducing the development of gallstones (Tsai et al. 2004), a major cause of acute pancreatitis. Dietary fiber has also been associated with reduced pancreatic cancer risk (Wang et al. 2015).

Vitamin D. Vitamin D deficiency is associated with several disorders. However, epidemiological data linking vitamin D deficiency to an increased risk for alcoholic and nonalcoholic chronic pancreatitis or pancreatic cancer are scarce and inconsistent (Hoogenboom et al. 2016; Waterhouse et al. 2016).

In experimental settings, a recent study found that a vitamin D agonist decreases features of chronic pancreatitis, including fibrosis and inflammation (Sherman et al. 2014), supporting the participation of vitamin D signaling in the development of pancreas scarring. Further research should clarify the clinical relevance of the experimental data.

Alcohol-Induced Adaptive Systems and Pancreatitis

Despite the increased risk for pancreatic damage among heavy drinkers, the

incidence of clinical pancreatitis in heavy drinkers is low (~5 percent) (Yadav et al. 2007). One potential explanation for the low rate of pancreatitis among heavy drinkers is that alcohol induces adaptive systems that serve to protect the pancreas from the damaging effects of alcohol. This theory holds that disease progresses when the damaging effects are stronger than the protective effects, or when the protective systems are impaired. Thus, the combination of alcohol use and another risk factor could represent an overwhelming burden and therefore lead to disease progression.

Research using animal models has examined the role of a cellular stress response (i.e., the unfolded protein response, UPR) as an adaptive response to heavy alcohol use that may protect the pancreas from alcohol's damaging effects (Lugea et al. 2015; Pandol et al. 2011). The UPR is critical for efficient functioning of the endoplasmic reticulum (ER) in the pancreatic acinar cell, because the ER provides for the synthesis of cellular components necessary for transporting digestive enzymes manufactured in ER to zymogen granules for storage and for secretion.

Lugea and colleagues (2011) examined this protective effect in mice with and without the gene for the X-box binding protein 1 (XBP1), a transcription factor that promotes synthesis of cellular components for protein transport and secretion. XBP1 is a key regulator of the adaptive UPR in the pancreas. The researchers found that ethanol feeding in control mice causes a marked increase in the activated form of XBP1 associated with minor pancreatic damage. But in mice with an inability to increase activated XBP1, ethanol feeding results in pancreatic damage. This protective response stimulated by alcohol may be one reason why so few alcoholics develop pancreatic disease. The results of the experiments suggest that enhancing the protective responses may provide opportunities for prevention and treatment of pancreatic diseases.

Molecular Mechanisms of Alcohol-Related Pancreatic Cancer

Most genetically engineered mouse models of pancreatic cancer are based on genetic mutations in the *Kras* gene. Mice expressing mutant *Kras* develop early and advanced forms of the most common pancreatic cancers in humans. However, *Kras* mutations alone are not sufficient to induce progression to the invasive stage of pancreatic cancer. Rather, different transgenes have been used to create models that progress to invasive cancer. For example, one common model based on *Kras* mutations is the PDX1-Cre;LSL-Kras^{G12D} model. Xu and colleagues (2015) reported using this model in mice exposed to alcohol and given injections of cerulein. The mice developed fibrosis and had an increased level of cancerous lesions. The authors concluded that alcohol independently increased pancreatic-cancer risk associated with fibrosis. Another animal model induces pancreatic cancer through the implantation of dimethylbenzanthracene (DMBA) in the pancreas. Research using this method in mice resulted in the development of both precursor lesions and invasive tumors. There was a higher relative frequency of tumors in mice receiving alcohol compared with the control group (Wendt et al. 2007).

The precise molecular mechanisms by which alcohol use may promote the development and/or progression of pancreatic cancer are not well defined. Although not evaluated in experimental models of pancreatic cancer, the oxidative ethanol metabolite acetaldehyde can act as a carcinogen by forming DNA adducts (Yu et al. 2010). In addition, alcohol might favor cancer development by causing oxidative stress and lipid peroxidation. Alcohol abuse may also accelerate tumor progression by promoting pancreatic inflammation. In this respect, studies using mouse models of pancreatic cancer demonstrated that recurrent pancreatic inflammation is required for the transformation of premalignant

lesions into pancreatic cancer (Guerra et al. 2007), and epidemiologic studies indicate that chronic pancreatitis is a major risk factor for pancreatic cancer in humans (Duell et al. 2012). Finally, recent studies have shown that alcohol use may induce epigenetic changes, mainly histone acetylation and DNA methylation, which affect expression of many genes. However, the full involvement of epigenetic mechanisms in alcohol-related chronic pancreatitis or pancreatic cancer has yet to be investigated.

Conclusions

The combination of epidemiologic and experimental animal-model observations continues to reveal insights into both disease pathogenesis and potential adaptive protective mechanisms of alcohol use. The relationship between heavy alcohol consumption and acute and chronic pancreatitis is well established (Yadav 2016). The highest rates of nongallstone-related pancreatitis are observed in those who drink the greatest amount of alcohol. A recent epidemiological observation of a potential protective effect of moderate alcohol use should be considered preliminary, encourage further research to confirm and determine generalizability of these findings, and elucidate the potential mechanism. Further, smoking is associated with significant risk for nongallstone-related pancreatitis and may add to the risk of pancreatitis with heavy drinking. A very low percentage of drinkers develop pancreatitis. Experimental models demonstrate that alcohol administration alone may not initiate pancreatitis, but it sensitizes the pancreas to pancreatitis by other insults.

Work in these models also reveals that the pancreas adapts to alcohol administration using the endoplasmic reticulum-based UPR to prevent injury. There is increasing interest in the role of carboxyester lipase, a pancreatic digestive enzyme, in forming fatty acid ethyl esters, which exert toxic

effects through sustained increases in intracellular Ca^{2+} concentrations. These in turn cause mitochondrial failure and decreased ATP production necessary to prevent cellular necrosis. The effects of alcohol use on pancreatic-cancer risk are largely through its promotion of repeated episodes of acute inflammatory pancreatitis and chronic pancreatitis. Understanding and preventing the injurious effects of alcohol use on the pancreas resulting in pancreatitis will likely also have a large benefit on prevention of pancreatic cancer. The figure presents a summary of epidemiologic and mechanistic findings in an attempt to provide an impetus for further developments in the field.

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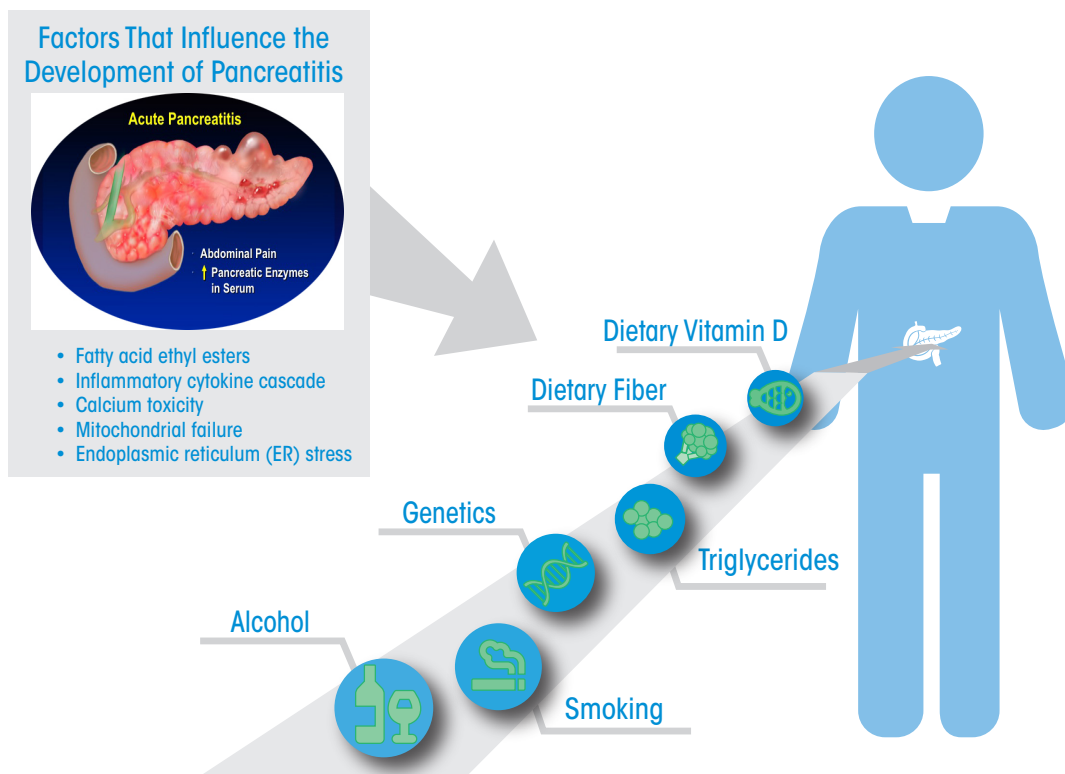


Figure The figure emphasizes the association of alcohol abuse, smoking, high triglycerides, and specific genetic mutations in promoting pancreatic disease. Dietary fiber and vitamin D are associated with protection from pancreatitis. The insert in the upper-left aspect of the figure shows the factors in the pancreatic tissue that are involved in the mechanisms of pancreatitis development.

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Alcohol and Gut-Derived Inflammation

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In large amounts, alcohol and its metabolites can overwhelm the gastrointestinal tract (GI) and liver and lead to damage both within the GI and in other organs. Specifically, alcohol and its metabolites promote intestinal inflammation through multiple pathways. That inflammatory response, in turn, exacerbates alcohol-induced organ damage, creating a vicious cycle and leading to additional deleterious effects of alcohol both locally and systemically. This review summarizes the mechanisms by which chronic alcohol intake leads to intestinal inflammation, including altering intestinal microbiota composition and function, increasing the permeability of the intestinal lining, and affecting the intestinal immune homeostasis. Understanding the mechanisms of alcohol-induced intestinal inflammation can aid in the discovery of therapeutic approaches to mitigate alcohol-induced organ dysfunctions.

Key words: Alcohol consumption; alcohol use, abuse, and dependence; chronic alcohol use; organ damage; gastrointestinal (GI) tract; liver; metabolites; gut-derived inflammation; intestinal inflammation; intestinal microbiota

The gastrointestinal (GI) tract, as the first line of contact with anything ingested into the body, is at particular risk for damage by toxins. And mounting research suggests that poor gastrointestinal health plays a significant role in the body's overall health. Connecting the dots, anything that may cause GI damage, may have consequences far beyond the intestines. In fact, researchers have begun to discover that alcohol, particularly if consumed chronically and in larger amounts, induces a process initiated in the gut that promotes inflammation throughout the body (Patel et al. 2015). This alcohol-induced intestinal inflammation may be at the root of multiple organ dysfunctions and chronic disorders associated with alcohol consumption, including chronic liver disease, neurological disease, GI

cancers, and inflammatory bowel syndrome. This review summarizes the mechanisms by which chronic alcohol intake leads to intestinal inflammation. These mechanisms include alcohol's influences on intestinal microbiota, on the integrity of the barrier between the intestine and the rest of the body, and on immune function within and outside the GI tract. The factors that can modify alcohol-induced gut inflammation and organ damage and the resulting pathologies that are a consequence of gut-derived inflammation are described. Although there may be large gender, racial, and interindividual variations in alcohol's effect on the GI tract, depending on differences in alcohol absorption, distribution, and elimination, they are not the focus of the current review.

Alcohol Metabolism and the Gut

Once consumed, alcohol is absorbed mainly in the upper intestinal tract by diffusion and then enters the liver via the portal vein. Therefore, the effect of alcohol on the distal small intestine and colon should largely come from its circulatory levels. That said, the luminal concentration of alcohol in the latter parts of the small intestine, close to the colon, reaches up to 200 mg/100 ml within an hour of drinking 2 to 2 ½ standard alcoholic drinks (0.8 g/kg) (Halsted et al. 1973).

The majority of alcohol metabolism in humans occurs in the liver, in cells called hepatocytes. During social drinking, defined here as an average of two standard drinks, the body typically processes the ingested alcohol with no

deleterious effects through a process called oxidative conversion, during which the enzyme alcohol dehydrogenase (ADH) converts alcohol into the toxin acetaldehyde. Acetaldehyde dehydrogenase (ALDH) then converts acetaldehyde into acetate. Another alcohol metabolism pathway, the microsomal ethanol-oxidizing system (MEOS), handles a small portion of alcohol metabolism in social drinkers but a significant portion of alcohol metabolism when the body needs to process larger amounts of alcohol. MEOS leads to the production of oxygen free radicals, which can cause cellular damage. Although the majority of alcohol metabolism occurs in hepatocytes, the enzymes involved in the oxidative metabolism of alcohol also are present in the intestinal mucosa (Cederbaum 2012) and intestinal bacteria also produce acetaldehyde in the GI tract. In addition, less commonly, nonoxidative alcohol metabolism occurs in the intestines via reactions with membrane phospholipids and/or free fatty acids. This alternative pathway may become particularly relevant when intestinal injuries occur after chronic alcohol consumption (Elamin et al. 2013a).

Therefore, both the small and large intestine can be affected by alcohol and its metabolites as the result of its oxidative and nonoxidative metabolism. Metabolism of alcohol in the GI tract can then lead to disruption of tissue homeostasis toward a chronic state of intestinal inflammation (Patel et al. 2015; Rao et al. 2004). As will be discussed in this review, mounting evidence shows that alcohol induces intestinal inflammation through various pathways, including changes in intestinal microbiota composition (Engen et al. 2015; Mutlu et al. 2012) and function (Couch et al. 2015), increased permeability of the intestinal mucosa (Tang et al. 2015), and disruptions of the immune system of the intestinal mucosa (Cook 1998).

Underlying Mechanisms for Alcohol and Gut-Derived Inflammation

Alcohol and Intestinal Microbiota

The intestine houses more than 500 bacterial species and achieves bacterial homeostasis when the ratio between “good” bacteria and pathogenic bacteria is appropriately balanced. “Dysbiosis” occurs when disease or environmental factors disrupt the bacterial balance (Belizário and Napolitano 2015). Disruption to the normal gut flora also occurs when there is an overall overgrowth of bacteria. Studies show that alcohol promotes both dysbiosis and bacterial overgrowth (Mutlu et al. 2012; Schnabl and Brenner 2014), which in turn leads to an increase in the release of endotoxins, produced by gram-negative bacteria (Rao et al. 2004). Endotoxins activate proteins and immune cells that promote inflammation (Elamin et al. 2013a; Keshavarzian et al. 2009). This section discusses evidence supporting alcohol’s role in altering intestinal microbiota.

Bacterial Overgrowth

Studies in animals and humans confirm that alcohol increases intestinal bacteria (Canesso et al. 2014). This overgrowth may be stimulated directly by alcohol, but some studies suggest that it also could be an indirect byproduct of poor digestive and intestinal function caused by alcohol consumption. For example, studies of patients with liver cirrhosis (both caused by alcohol and not) found an association between patients with abnormal intestinal motility—the intestine’s ability to move food along—and bacterial overgrowth (Chang et al. 1998). Other studies found a connection between alcohol, bile acid, and bacterial overgrowth. Specifically, alcohol can alter bile-acid metabolism and, in turn, bile acids can affect intestinal bacteria (Schnabl and Brenner 2014). Studies in rats show that alcohol

decreases certain bile acids (Xie et al. 2013) and treating rats with bile acids reversed bacterial overgrowth (Lorenzo-Zúñiga et al. 2003).

Bacterial Dysbiosis

More recent studies use DNA sequencing technology to assess intestinal microbiota populations and indicate a correlation between alcohol and changes in the ratio between beneficial or “good” bacteria, such as strains of *Lactobacillus* and *Bifidobacterium*, and pathogenic bacteria, such as proteobacteria and bacilli (Canesso et al. 2014). For example, mice chronically fed alcohol display a decrease in good bacteria and an increase in bacteria that boost endotoxin production (Bull-Otterson et al. 2013). In another study, researchers found a significant shift in intestinal microbiota composition in rats chronically fed alcohol, but they could prevent the shift by giving the rats *Lactobacillus GG* bacteria and a diet that included probiotic oats (Mutlu et al. 2009). Connecting dysbiosis to alcohol-induced health problems, several studies find that probiotic and synbiotic interventions, which stimulate the growth of beneficial bacteria, attenuate liver injury in rats (Forsyth et al. 2009) and liver dysfunction in cirrhotic patients (Liu et al. 2004). Alcohol-induced bacterial overgrowth also may increase the risk of inflammation because intestinal bacteria can independently metabolize alcohol, producing excess acetaldehyde in the colon, which increases production of proinflammatory alcohol metabolites (Zhong and Zhou 2014).

Alcohol-Induced Intestinal Hyperpermeability

The intestinal barrier regulates the passage of materials between the GI tract and the bloodstream, allowing for the absorption by the blood of key nutrients and preventing the absorption of noxious substances. It is made up of a layer of water, mucous gel, and epithelial and connective tissue. The epithelial

layer can become leaky or “permeable,” allowing pathogens and other deleterious substances into the bloodstream.

Studies in humans demonstrate that a subset of people with alcohol use disorder (AUD) in fact have increased intestinal permeability, measured using a method called Cr-EDTA, which examines excretion of orally administered chromium (Leclercq et al. 2014). In addition, those people with AUD and with increased permeability are more likely to have liver disease (Keshavarzian et al. 1999), indicating that intestinal permeability may be a

mediator of organ damage in some people with AUD. Another study showed that not only is gut permeability increased in people with AUD, it is increased enough to allow large macromolecules through the intestinal barrier (Parlesak et al. 2000). Endotoxins—also known as lipopolysaccharides (LPS)—are large macromolecules and, as expected, the same study found that plasma endotoxin levels increased in parallel with increases in gut permeability (Parlesak et al. 2000).

But exactly how does alcohol induce intestinal permeability? The short

answer is by disrupting the epithelial cells themselves (transepithelial permeability) and by disrupting the spaces between the epithelial cells (paracellular permeability), which consist of tight junctions, the cytoskeleton, and several associated proteins (see figure below). Trans-epithelial permeability is caused by direct cellular damage. For example:

- Alcohol causes cell death (Pijls et al. 2013), which leads to changes in the intestine that include mucosal ulcerations, erosions, and loss of

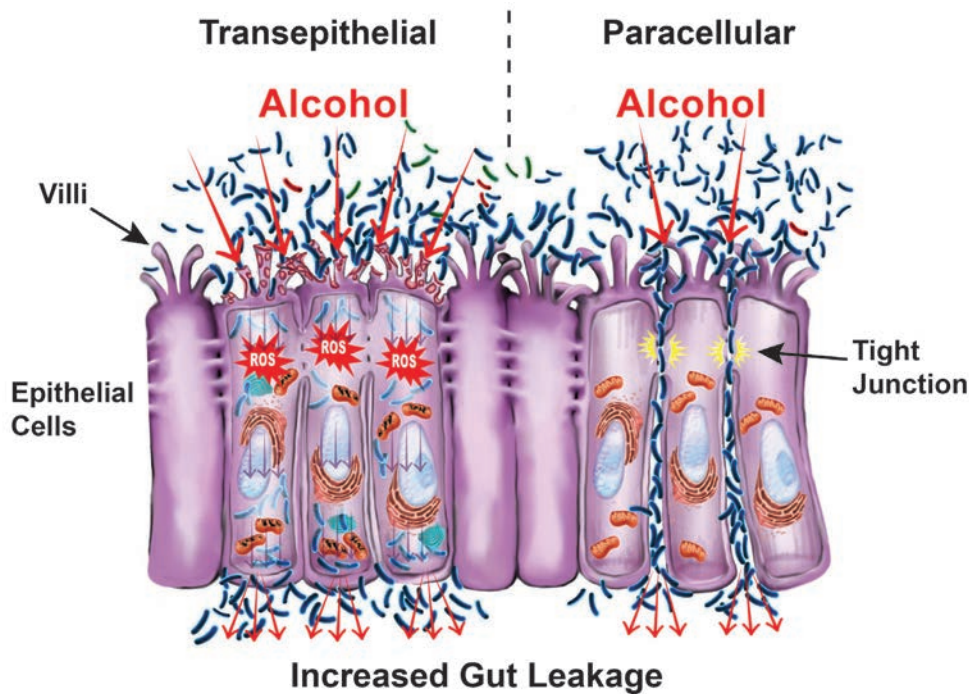


Figure The intestinal barrier regulates the passage of materials, including microbial products, between the inside of the intestine (where food and drink go) and the cells and blood vessels on the other side of the epithelial cell layer lining the inside of the intestine. Alcohol disrupts the intestinal barrier, increasing its permeability, in two ways: via transepithelial mechanisms (cells on the left), which allow material to pass directly through the epithelial cells, and paracellular mechanisms (cells on the right), which allow material to pass through the junctions between the epithelial cells. Alcohol and its metabolites trigger transepithelial mechanisms by damaging the cells directly and weakening cell membranes via several mechanisms including oxidative stress caused by reactive oxygen species (ROS). Alcohol’s metabolites trigger paracellular mechanisms by disrupting the proteins that create the tight junctions linking cells and proteins that stabilize cells’ cytoskeletons. Increased permeability of the intestinal barrier allows bacteria and the toxins they create to leave the gut and infiltrate other organs through the bloodstream.

epithelium mainly at the villi tips (Rocco et al. 2014);

- Acetaldehyde forms DNA adducts that cause direct cellular damage (Malaguarnera et al. 2014); and
- Reactive oxygen species (ROS) released during alcohol metabolism cause direct cellular damage via oxidative stress (Forsyth et al. 2014).

Alcohol and its metabolites cause paracellular permeability by acting on the tight-junction complex, which melds two adjoining cells together. For example, acetaldehyde destabilizes tight junctions by redistributing proteins (Dunagan et al. 2012); alcohol and its metabolites alter the expression of tight-junction proteins (Wang et al. 2014); and alcohol nonoxidative metabolites cause tight-junction redistribution, disrupting its barrier function (Elamin et al. 2013*b*). In addition, studies show that alcohol destabilizes cells' cytoskeletons, the cell borders that give them their structure (Elamin et al. 2014). There also is growing evidence that alcohol causes the overexpression of microRNAs (miRNAs), which are small stretches of noncoding RNA that silence gene expression (Tang et al. 2014). Specifically, alcohol can lead to overexpression of miRNAs that influence genes associated with gut-barrier integrity (Lippai et al. 2014).

Alcohol Modulation of Mucosal Immunity

Gut inflammation results from an inflammatory response mounted by the immune system against alcohol and its metabolites. Alcohol affects intestinal mucosal immunity via several mechanisms (see sidebar). In particular, it may first decrease the innate immune response in the mucosa, resulting in increased susceptibility to intestinal pathogens (Zhou et al. 2013). Subsequently, as found in studies in cell cultures, alcohol may trigger an immune system response and upregulation of

molecules that promote the inflammatory response, including a release of inflammatory immune cells, such as leukocytes and mast cells (Fleming et al. 2001).

As mentioned earlier, alcohol-related bacterial overgrowth and dysbiosis may lead to increased endotoxin production in the gut, which can bind to cells on the intestinal mucosa, causing local inflammation, and translocate to extraintestinal sites, causing systemic inflammation (Leclercq et al. 2014). Studies also show that alcohol can directly modulate both innate and adaptive immunity, further contributing to gut and gut-derived inflammation. For example, a study in mice found that alcohol inhibits the intestine's immune response for clearing hazardous bacteria (Sibley and Jerrells 2000), and other studies find that alcohol suppresses intestinal mucosal immune cell activity (Cook 1998). Additional studies find myriad ways that alcohol affects mucosal immunity, including the following:

- By reducing the amount of antimicrobial molecules intestinal cells secrete, which leads to bacterial overgrowth (Schnabl and Brenner 2014);
- By suppressing the signaling molecule, interleukin-22, which negatively affects antimicrobial peptides (e.g., Reg3 β and Reg3 γ) and intestinal mucosal integrity (Rendon et al. 2013); and
- By suppressing signal molecules and immune T cells and thereby suppressing the intestinal mucosal immune response and bacterial clearance (Trevejo-Nunez et al. 2015).

Modifying Factors for Alcohol-Induced Gut-Derived Inflammation

As described above, alcohol causes gut-derived inflammation, which is related to other alcohol-associated

pathologies. However, not all people with AUD develop disease, and those who do have varying degrees of disease severity. Although the extent of disease depends in large part on the extent of alcohol use and likely involves inherent individual characteristics, including genetics, race, and age, there are some adjustable factors that affect alcohol-induced intestinal inflammation and, therefore, may prevent or slow the progression of alcohol-related disease. Here, we discuss the roles of two adjustable environmental factors: circadian rhythm and diet.

Circadian Disruption

Circadian rhythm, also known as the biological clock, refers to an internal cycling of various biological processes. Chronic alcohol use can lead to a disrupted biological clock, which in turn can have a wide range of health-related consequences.

In terms of gut-related inflammation, studies in cell cultures, mice, and humans suggest that a disrupted circadian rhythm exacerbates alcohol-related gut leakiness. For example, one study (Summa et al. 2013) found that alcohol-fed CLOCK mutant mice—who have a disrupted circadian cycle—showed more evidence of gut leakiness than alcohol-fed wild-type mice. A study in humans (Swanson et al. 2015), including a group of shift workers who often have disrupted circadian rhythm, came to a similar conclusion. The researchers assessed circadian rhythm by measuring participants' blood melatonin levels, using low melatonin as a marker for disrupted circadian rhythm. They found that low melatonin correlated with gut leakiness in people with AUD.

Although it is unclear how circadian disruption amplifies alcohol-induced gut permeability, there are some hints from recent studies. For example, gut microbes have circadian oscillations, and circadian disruption can lead to dysbiosis in mice fed a high-fat diet (Voigt et al. 2014), which in turn can induce intestinal inflammation and hyperpermeability. In addition, timing

of lipid metabolism and bile-acid synthesis are regulated by the local hepatic circadian rhythm (Bailey et al. 2014). Together, the evidence on circadian rhythm suggests a looping cycle where circadian disruption promotes alcohol-induced intestinal inflammation and alcohol disturbs circadian rhythm, which may further propagate intestinal hyperpermeability and inflammation.

Diet

Various studies show that nutrition can modify alcohol-induced gut inflammation and, subsequently, extraintestinal organ damage. Because people with AUD typically have altered diet composition, a focus on changing dietary habits might attenuate alcohol-related diseases. The following section reviews

a sampling of studies on different diets and alcohol use.

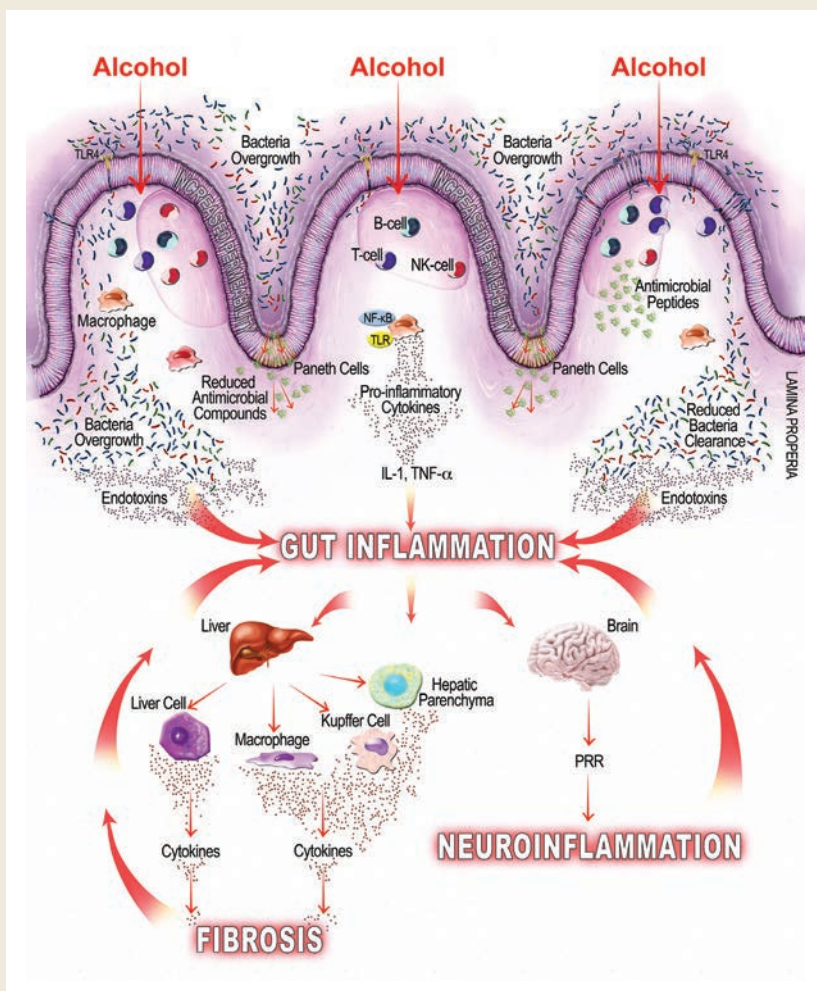
Fat. Studies examining high-fat diets find conflicting results. Some find fats propagate alcohol's effects on the intestine, and some find they attenuate alcohol's harmful effects. The contrast likely reflects the variety

Alcohol's Effect on Immunity and Inflammation

Alcohol can induce intestinal inflammation through a cascade of mechanisms that subsequently lead to inflammation and organ dysfunction throughout the body, in particular in the liver and brain. One mechanism is by increasing bacterial loads and the permeability of the intestinal wall (see figure) allowing bacteria to leak through, leading to local and systemic effects by affecting mucosal immunity and via endotoxin release, respectively. Alcohol also affects mucosal immunity by suppressing one of the intestine's main lines of defense against bacteria, Paneth cells that secrete antibacterial compounds. Suppressed Paneth cells secrete fewer antibacterial compounds, which can allow additional intestinal bacteria overgrowth and allow their byproducts (i.e., endotoxins) entrance through the intestinal barrier. The bacteria, via endotoxins, trigger an inflammatory response by the intestine's immune system, causing a release of proinflammatory cytokines. The endotoxins and cytokines can then enter the liver, directly interacting with hepatocytes and with liver immune cells, causing local cytokine release that leads to fibrosis and causes additional inflammation. The gut inflammation can also spread endotoxins

and cytokines into the bloodstream where they can enter the central

nervous system (CNS), causing neuroinflammation.



of fats found in high-fat foods. Generally, studies seem to support the idea that unsaturated fats increase gut permeability and some kinds of saturated fats are protective.

Studies have examined the effects of several types of saturated fats given as supplements to alcohol-exposed mice. One (Cresci et al. 2014) found that tributyrin, a triglyceride fat found in butter and margarine, prevented alcohol-induced tight-junction disruption, which in turn protects against intestinal hyperpermeability. Another (Chen et al. 2015a) examined saturated long-chain fatty acids (SLCFAs), which are found in coconut oil, peanut oil, and dairy products. The researchers observed that the intestinal bacteria in mice chronically fed ethanol produced far less SLCFAs than mice not fed ethanol, and they also had lower levels of tight-junction proteins. That changed after the researchers gave the ethanol-fed mice SLCFA supplementation. Indeed, the mice given supplementation had higher levels of tight-junction proteins than ethanol-fed mice without supplementation. SLCFA supplements also prevented dysbiosis (Chen et al. 2015a).

Unsaturated fats had less favorable effects. In one study (Kirpich et al. 2012), mice fed alcohol and unsaturated fats had increased fatty liver changes and suppressed mRNA expression of tight-junction proteins compared with mice fed alcohol and saturated fat. These findings suggest that an unsaturated-fat diet in conjunction with chronic alcohol use increases intestinal permeability.

Oats. Oats, which are rich in fat, fiber, protein, vitamins, and minerals, have long been associated with cardiovascular health and, more recently, examined for a possible role in gastrointestinal health. Several preclinical studies suggest that oats may attenuate alcohol's deleterious effects on the digestive system. In one study (Keshavarzian et al. 2001), two groups of rats received increasing doses of alcohol and either oats or regular rat

chow for a period of 10 weeks. The oats-fed rats had significantly lower endotoxin levels than the chow-fed animals. Another study (Tang et al. 2009) found that alcohol-fed rats given oat supplementation showed fewer signs of gut inflammation and alcohol-induced hyperpermeability than rats fed alcohol and regular rat chow. More recently, researchers examined supplementation with glutamine, an amino acid found in oats. The study in mice found that glutamine supplements ameliorated alcohol-induced intestinal leakiness and improved alcohol-induced liver injury (Chaudhry et al. 2016).

Vitamins and Minerals. People with AUD often are deficient in certain vitamins and minerals, including zinc and vitamin D, either from direct effects of alcohol consumption or poor diet. Those deficiencies, in turn, may have deleterious effects on the digestive system. A study in mice (Zhong et al. 2013) found a relationship between zinc deficiency and gut leakiness. The study compared mice fed alcohol and a zinc-deficient diet with mice fed alcohol and a zinc-adequate diet. The zinc-deficient mice showed increased intestinal permeability and higher plasma endotoxin levels (for more on zinc, see the article by McClain).

Another study, conducted in intestinal cell culture and mice, examined whether vitamin D might protect gut health from alcohol exposure. In the cells, treating with vitamin D protected the cells from ethanol damage. In the mice, higher vitamin D levels measured in blood correlated with increased resistance to changes that lead to intestinal injury (Chen et al. 2015b). These findings suggest that vitamin D deficiency may promote the deleterious effects of alcohol on the gut barrier and, perhaps, that vitamin D supplementation may attenuate those effects.

The Clinical Relevance of the Alcohol-Induced Gut-Derived Inflammation

Alcohol-induced gut inflammation is believed to promote several disease states both within the GI tract, in the form of gastrointestinal cancers and inflammatory bowel disease, and outside the GI tract, in the form of, for example, liver disease and neuroinflammation (Rao et al. 2004). The following section briefly reviews a sample of the conditions associated with alcohol-related gut inflammation.

Alcohol and GI Cancers

Chronic alcohol consumption is associated with increased risk of major gastrointestinal cancers including cancer of the esophagus, stomach, and colon (colorectal cancer). The risk generally increases as alcohol consumption increases and in combination with other lifestyle-related factors, such as smoking tobacco or metabolic syndrome. And although alcohol was initially thought to act as a direct carcinogen, research instead suggests that alcohol-induced gut inflammation may be at fault (Thrift et al. 2011).

Systemic inflammation seen in metabolic syndrome and obesity increases risk of several types of epithelial cancers, including those in the gastrointestinal tract (Feakins 2016), suggesting that the systemic inflammatory state created by alcohol-induced gut inflammation also may contribute to alcohol-induced carcinogenesis in the GI tract and other organs. This process can snowball because, as cells transition to a cancerous state, ADH activity increases while ALDH activity may decrease (Testino et al. 2011). This leads to an increased oxidation rate and a decreased ability to clear alcohol metabolites (Testino et al. 2011), which in turn can further promote carcinogenesis through direct effects on DNA, oxidative stress, and gut inflammation (Jelski and Szmítkowski 2008).

Alcohol and Inflammatory Bowel Disease (IBD)

Several lifestyle factors such as smoking and diet affect the incidence and severity of IBD, most likely by modulating gut inflammation (Swanson et al. 2010). Alcohol consumption also may influence the course of IBD through associated gut inflammation (McGuckin et al. 2009); however, its effect in patients with IBD only has been studied in a few small studies. One study (Swanson et al. 2011), for example, examined the impact of 1 week of moderate (24 g to 36 g ethanol daily) red wine consumption on clinical disease activity and other noninvasive markers associated with increased risk of future disease flare. The study found no significant changes in indices of clinical disease but did find subclinical increases in markers for disease activity, including intestinal permeability. Such findings suggest that chronic alcohol consumption could increase the long-term risk for disease flare in IBD and supports the need for additional study.

Gut–Liver Axis

Approximately 20 to 30 percent of heavy drinkers (people who drink more than 30 grams/day for at least 10 years) develop clinically significant alcoholic liver disease, including alcoholic steatohepatitis and cirrhosis (Gramenzi et al. 2006). Several factors, such as the amount and duration of alcohol consumption, obesity, and gender, seem to moderate a person's risk and progression of alcoholic liver disease. In addition, studies find that alcohol-induced gut inflammation can contribute to liver injury by increasing intestinal permeability and the likelihood that gut-derived endotoxins enter the liver. One study (Keshavarzian et al. 1999) found that people with AUD who also have liver disease are much more likely to have intestinal permeability: more than 40 times more likely than people without AUD and more than 20 times more likely than people

with AUD who do not have liver disease. In alcohol-fed rats, gut leakiness is evident 2 weeks after alcohol initiation; after another 2 weeks, endotoxemia develops and then liver injury, suggesting an intermediary role for endotoxemia on liver injury (Keshavarzian et al. 2009).

Once gut leakiness begins, endotoxins can enter the liver via the portal vein that drains from the gut. In the liver, gut-derived substances interact with the liver's hepatocytes, parenchymal cells, and immune cells. Alcohol exposure increases LPS levels in portal and systemic circulation (Wheeler et al. 2001), and that can have a host of deleterious effects:

- Initiating endotoxin-mediated hepatocellular damage by activating the innate immune system and leading to an increase in ROS and inflammatory cytokines, leukotrienes, and chemokines (Purohit et al. 2008);
- Activating signaling pathways that lead to proinflammatory cytokine release associated with liver fibrosis (Seki and Schnabl 2012); and
- Activating immune cells that can lead to liver inflammation and eventual fibrosis (Szabo et al. 2012).

Gut–Brain Axis

It is well established that the brain helps control the gut, and recently research suggests the opposite also is true: the gut can influence brain function (Hsiao et al. 2013). In fact, some evidence suggests that alcohol-induced intestinal permeability and LPS can influence psychological and cognitive function. For example, among a group of alcohol-dependent, noncirrhotic patients hospitalized for detoxification, the subset that showed signs of intestinal permeability and LPS also had higher scores on measures of depression, anxiety, and alcohol cravings and

scored worse on measures of selective attention (Leclercq et al. 2012). These findings suggest that some of the biological and behavioral changes seen in people with AUD may extend from the systemic inflammatory response triggered by changes in the gut.

Although the mechanisms by which the gut–brain axis conveys the effect(s) of alcohol on the central nervous system (CNS) are not well established, several studies suggest that systemic inflammation, like that caused by alcohol-provoked leaky gut, can influence the nervous system in several ways. For example, alcohol-induced gut inflammation can result in a systemic inflammation that subsequently affects neuronal function and may drive some symptoms of alcohol withdrawal, including autonomic disturbances and anxiety (Retson et al. 2015). In addition, elevated cytokines caused by the inflammatory response may be able to enter the brain and disrupt the blood–brain barrier, starting a vicious cycle that perpetuates alcohol's effects on the CNS (Banks et al. 2015). Alcohol-induced dysbiosis may have its own effect on the CNS via vagal afferent nerve fibers, which influence areas of the brain implicated in AUD, including the thalamus, hippocampus, amygdala, and prefrontal cortex. Specifically, accumulating evidence suggests that alcohol-induced dysbiosis and gut microbiome may contribute to modifications in the vagal response and neuroinflammation in the CNS linked with alcohol-associated behaviors (Gorky et al. 2016). Other studies link microbiota alterations and endotoxins with neuroinflammation (Szabo and Lippai 2014) and anxiety-like behavior (Bercik et al. 2011; Hsiao et al. 2013). Studies in mice and humans suggest that antimicrobials and probiotics can positively influence brain function in healthy people, holding out promise that targeting gut microbiota in people with AUD might help defray alcohol's influence on brain function (Bercik et al. 2011; Tillisch et al. 2013).

Conclusions

Through multiple pathways, alcohol induces gut inflammation, which in turn promotes broad-spectrum pathologies both inside and outside the GI tract. In fact, many alcohol-related disorders, including cancers, liver disease, and neurological pathologies, may be exacerbated or directly affected by this alcohol-induced gut inflammation. The inflammation itself results from oxidative and nonoxidative pathways of alcohol metabolism that lead to a leaky gut, bacterial overgrowth, dysbiosis, and alterations in the mucosal immune system. As research uncovers the mechanisms by which alcohol affects gut inflammation and how that inflammation influences disease, researchers may be able to develop better strategies to prevent, or treat, conditions associated with chronic alcoholism. Already, studies are suggesting ways to modify diet and intestinal flora that may help alleviate some of the risks associated with chronic heavy drinking. Controlled trials are needed to assess the use of dietary supplementation with micro-nutrients in preventing or reversing alcohol effects.

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Alcoholic Liver Disease: Pathogenesis and Current Management

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Excessive alcohol consumption is a global healthcare problem. The liver sustains the greatest degree of tissue injury by heavy drinking because it is the primary site of ethanol metabolism. Chronic and excessive alcohol consumption produces a wide spectrum of hepatic lesions, the most characteristic of which are steatosis, hepatitis, and fibrosis/cirrhosis. Steatosis is the earliest response to heavy drinking and is characterized by the deposition of fat in hepatocytes. Steatosis can progress to steatohepatitis, which is a more severe, inflammatory type of liver injury. This stage of liver disease can lead to the development of fibrosis, during which there is excessive deposition of extracellular matrix proteins. The fibrotic response begins with active pericellular fibrosis, which may progress to cirrhosis, characterized by excessive liver scarring, vascular alterations, and eventual liver failure. Among problem drinkers, about 35 percent develop advanced liver disease because a number of disease modifiers exacerbate, slow, or prevent alcoholic liver disease progression. There are still no FDA-approved pharmacological or nutritional therapies for treating patients with alcoholic liver disease. Cessation of drinking (i.e., abstinence) is an integral part of therapy. Liver transplantation remains the life-saving strategy for patients with end-stage alcoholic liver disease.

Key words: Alcohol consumption; heavy drinking; alcohol effects and consequences; abstinence; alcoholic liver disease; liver injury; hepatic lesions; steatosis; hepatitis; fibrosis; cirrhosis; treatment; pharmacological therapy; nutritional therapy; liver transplantation

Excessive alcohol consumption is a global healthcare problem with enormous social, economic, and clinical consequences, accounting for 3.3 million deaths in 2012 (World Health Organization 2014). Excessive drinking over decades damages nearly every organ in the body. However, the liver sustains the earliest and the greatest degree of tissue injury from excessive drinking because it is the primary site of ethanol metabolism (Lieber 2000). After a brief overview of alcohol metabolism in the liver, this article will summarize the mechanisms through which excessive alcohol consumption contributes to the development of various types of alcohol-induced liver

damage. It also will review modifiers of alcoholic liver disease (ALD) and discuss currently used treatment approaches for patients with ALD.

Hepatic Alcohol Metabolism

Beverage alcohol (i.e., ethanol) is chiefly metabolized in the main parenchymal cells of the liver (i.e., hepatocytes) that make up about 70 percent of the liver mass (Jones 1996). These cells express the highest levels of the major ethanol-oxidizing enzymes, alcohol dehydrogenase (ADH), which is located in the cytosol, and cytochrome P450 2E1 (CYP2E1), which resides in the

smooth endoplasmic reticulum (ER) (figure 1). Hepatocytes also express very high levels of catalase, an enzyme that inhabits peroxisomes. Catalase normally carries out the detoxification of hydrogen peroxide (H_2O_2) to water and oxygen. However, when ethanol is present, catalase has an accessory role in ethanol metabolism by using H_2O_2 to oxidize ethanol to acetaldehyde. Ethanol oxidation by catalase is a relatively minor pathway in the liver, but has a larger ethanol-oxidizing function in the brain (Aragon et al. 1992).

ADH is the most catalytically efficient ethanol-metabolizing enzyme. It reaches its half-maximal velocity when circulating ethanol levels are about 5 to 10 milligrams per deciliter, well below levels that cause intoxication.¹ ADH-catalyzed ethanol oxidation uses nicotinamide adenine dinucleotide (NAD^+) as a cofactor, generating reduced NAD^+ ($NADH$) and acetaldehyde. The latter compound is highly reactive and toxic. It can covalently bind to proteins (Donohue et al. 1983), lipids (Kenney 1982), and nucleic acids (Brooks and Zakhari 2014) to form acetaldehyde adducts, which, in turn, can disrupt the structure and function of these macromolecules (Mauch et al. 1986). One way that hepatocytes minimize acetaldehyde toxicity is by rapidly oxidizing it to acetate using the enzyme aldehyde dehydrogenase 2 (ALDH2) inside mitochondria. The ALDH2 reaction is another oxidation–reduction step that generates $NADH$ and acetate, the latter of which can diffuse into the circulation to be utilized in other metabolic pathways. The enhanced generation of $NADH$ by both ADH- and ALDH2-catalyzed reactions decreases the normal intrahepatocyte $NAD^+/NADH$ ratio, called the cellular redox potential. This change causes significant metabolic shifts from oxidative metabolism toward reductive synthesis, favoring the formation of fatty acids, which contribute to fatty liver development (Donohue 2007).

CYP2E1 is the other major hepatic enzyme that catalyzes ethanol oxidation to acetaldehyde. Although the catalytic efficiency of CYP2E1 is considerably slower than that of ADH, CYP2E1 has a 10-fold higher capacity for binding ethanol, becoming half-saturated at 46 to 92 milligrams per deciliter. Also important is that CYP2E1 is an

inducible enzyme; its hepatocellular content rises during chronic ethanol consumption (Dilger et al. 1997; Lieber and DeCarli 1968). Ethanol interacts directly with the CYP2E1 protein, causing it to assume a conformation that resists degradation by the ubiquitin-proteasome system and resulting in the accumulation of CYP2E1

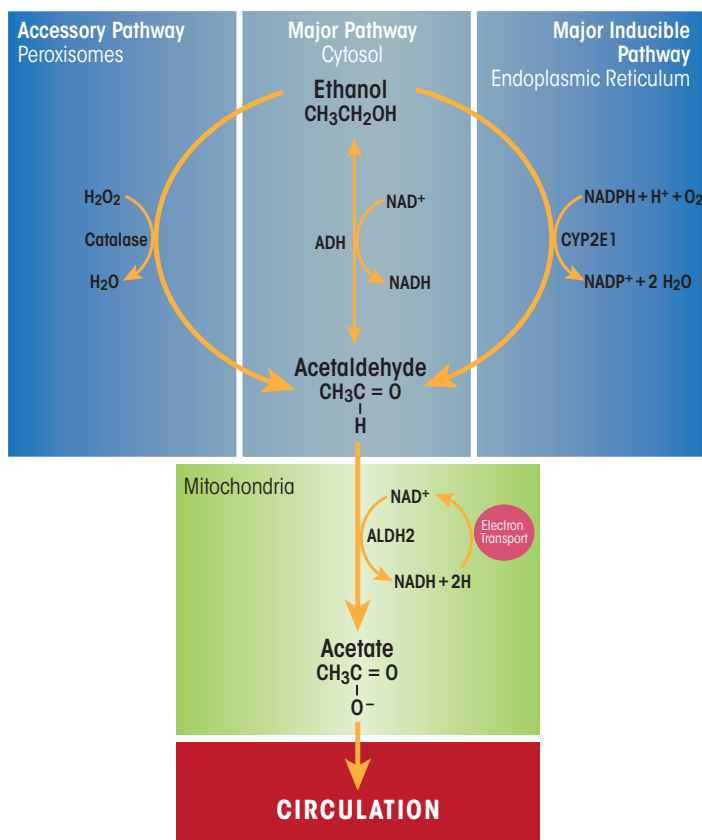


Figure 1 Major and minor ethanol-oxidizing pathways in the liver. Ethanol (i.e., ethyl alcohol) is oxidized principally in hepatocytes of the liver. **(Middle panel)** Alcohol dehydrogenase (ADH), a major enzyme in the cytosol, and aldehyde dehydrogenase 2 (ALDH2), which is located in the mitochondria, catalyze sequential oxidations that convert ethanol to acetate, producing two mole equivalents of reduced nicotinamide adenine dinucleotide ($NADH$). **(Right panel)** Cytochrome P450 2E1 (CYP2E1) is a major inducible oxidoreductase in the endoplasmic reticulum that oxidizes ethanol, in the presence of molecular oxygen (O_2), to acetaldehyde and converts reduced NAD phosphate ($NADPH$) to its oxidized form, generating water. **(Left panel)** Peroxisomal catalase is a minor hepatic pathway of ethanol oxidation that uses hydrogen peroxide (H_2O_2) to oxidize ethanol to acetaldehyde and water.

SOURCE: Figure adapted from Zakhari and Li 2007.

¹ People are legally inebriated when their blood alcohol levels reach 80 milligrams per deciliter.

molecules (Roberts et al. 1995). CYP2E1 induction has several major effects in heavy drinkers: First, because more CYP2E1 oxidizes ethanol, drinkers develop a “metabolic tolerance”—that is, they need to drink more alcohol to reach a level of intoxication that they formerly achieved after drinking less alcohol. Second, accelerated alcohol metabolism by higher levels of CYP2E1 puts liver cells in metabolic peril, because more CYP2E1 not only produces more acetaldehyde, but the induced enzyme also generates greater amounts of various other reactive oxygen species (ROS), including hydroxyethyl radicals (i.e., free-radical forms of ethanol), superoxide anions (O_2^-) and hydroxyl radicals ($\cdot OH$). Continuous generation of these reactive molecules in problem drinkers eventually creates the condition known as oxidant stress or oxidative stress. Under these conditions, the rate of ROS generation exceeds the liver’s capacity to neutralize them with natural antioxidants, such as glutathione and vitamins E, A, and C, or to remove them using antioxidant enzymes, including those listed in table 1 (Fang et al. 2002). Animal studies have revealed that chronic ethanol consumption decreases the activities and/or amounts of several antioxidant enzymes, which worsens the hepatocytes’ oxidant burden (Chen et al. 1995; Dong et al. 2014; Zhao et al. 1996). Oxidant stress further is exacerbated when the generated ROS

undergo secondary reactions with proteins and unsaturated lipids. The latter reactions result in the generation of lipid peroxides, which themselves interact with proteins and with acetaldehyde to form bulkier adducts (e.g., malondialdehyde-acetaldehyde [MAA] adducts) that are capable of generating an immune response (Tuma et al. 1996). Finally, because of CYP2E1’s broad substrate specificity, increased levels of the enzyme also accelerate the conversion of excess amounts of substrates other than ethanol, such as the analgesic and antipyretic medication acetaminophen. Following CYP2E1 induction by heavy drinking, acetaminophen is converted to a more toxic, reactive intermediate. This places the chronic drinker at substantial risk for liver disease or acute liver failure, especially after an acetaminophen overdose (Schiodt et al. 2002).

Alcohol’s Effects on Other Liver Cell Types

Although hepatocytes comprise most of the liver mass, nonparenchymal cells, including Kupffer cells (KCs), sinusoidal endothelial cells, hepatic stellate cells (HSCs), and liver-associated lymphocytes make up the remaining 15 to 30 percent of the liver mass. These nonparenchymal cells interact with hepatocytes and with each other via soluble mediators and by direct cell-to-cell contact. Each liver cell type

plays a specific role not only in normal hepatic physiology but also in initiating and perpetuating liver injury.

Spectrum of ALD

Heavy ethanol consumption produces a wide spectrum of hepatic lesions, the most characteristic being fatty liver (i.e., steatosis), hepatitis, and fibrosis/cirrhosis (see figure 2). Steatosis is the earliest, most common response that develops in more than 90 percent of problem drinkers who consume 4 to 5 standard drinks per day over decades (Ishak et al. 1991; Lieber 2004). (A standard drink is defined as the amount of alcoholic beverage that contains approximately 0.5 fluid ounces, or about 14 grams, of pure alcohol [figure 3]). However, steatosis also develops after binge drinking, defined as the consumption of 4 to 5 drinks in 2 hours or less. Steatosis was formerly considered a benign consequence of alcohol abuse. It is characterized by the deposition of fat, seen microscopically as lipid droplets, initially in the hepatocytes that surround the liver’s central vein (i.e., perivenular hepatocytes), then progressing to mid-lobular hepatocytes, and finally to the hepatocytes that surround the hepatic portal vein (i.e., periportal hepatocytes). If the affected individual ceases drinking, steatosis is a reversible condition with a good prognosis. However, patients with

Table 1 Hepatic Enzymatic Defenses Against Free-Radical Attack

Enzyme	Abbreviation	Cellular Location	Function	Effect of Chronic Ethanol Administration	References
Copper–Zinc-Superoxide Dismutase	Cu/Zn-SOD	Cytosol	Converts superoxide to H_2O_2	Decreases activity and content	Chen et al. 1995; Zhao et al. 1996
Manganese-Superoxide Dismutase	Mn-SOD	Mitochondria	Converts superoxide to H_2O_2	Decreases activity and content	Chen et al. 1995; Zhao et al. 1996
Catalase	Catalase	Peroxisomes	Converts H_2O_2 to H_2O	Increases activity	Chen et al. 1995
Glutathione Peroxidase	GSH peroxidase	Cytosol/ mitochondria	Scavenges peroxides and free radicals	Unaffected	Chen et al. 1995
Glutathione Reductase	GSSG reductase	Cytosol	Regenerates reduced GSH from GSSG	Decreases activity	Dong et al. 2014
Glutathione-S-Transferase	GST	Nuclei, cytosol, mitochondria	Transfers sulfur to acceptor molecules	Increases activity	Chen et al. 1995

chronic steatosis are more susceptible to fibrotic liver disease (Teli et al. 1995), because the presence of fat likely represents a greater risk for lipid peroxidation and oxidative damage.

Alcoholic hepatitis is a more severe, inflammatory type of liver injury characterized by swollen, dying hepatocytes (i.e., ballooning degeneration), neutrophilic infiltration, and the development of tangled aggregates of insoluble proteins called Mallory-Denk bodies within hepatocytes. Central to hepatitis development is the activation of KCs, the resident liver macrophages.

Fibrosis and its terminal or late stage, cirrhosis, refer to the deposition of abnormal amounts of extracellular matrix proteins, principally by activated HSCs. Patients initially exhibit active pericellular fibrosis, which may progress to cirrhosis, the late stage of hepatic scarring. However, some degree of hepatitis likely is always present in cirrhotic patients, whereas hepatic fat usually is not prominent in these individuals. The World Health Organization's (2014) *Global Status Report on Alcohol and Health* estimates that 50 percent of all deaths caused by cirrhosis were attributable to alcohol abuse.

The following sections provide a detailed description of the mechanisms involved in the development of these major lesions.

Mechanisms Involved in Alcoholic Steatosis

As the preceding section on ethanol metabolism stated, ethanol and acetaldehyde oxidations generate higher levels of NADH, which alters the cellular redox potential and enhances lipid synthesis (i.e., lipogenesis). However, ethanol-induced redox change alone does not fully explain why the liver rapidly accumulates fat. More recent studies now strongly support the notion that ethanol-induced steatosis is multifactorial as discussed below (see figure 4).

Alcohol Accelerates Hepatic Lipogenesis

Enhanced lipid synthesis results from a higher expression of lipogenic enzymes and cytokines (see table 2) that are encoded by genes regulated by two transcription factors, sterol regulatory element binding protein-1c (SREBP-1c) and early growth response-1 (Egr-1). SREBP-1c belongs to a family of transcription factors that control hepatic

cholesterol metabolism. However, in heavy drinkers, ethanol oxidation short-circuits hepatic lipid metabolism, converting the liver from a lipid-burning to a lipid-storing organ. Thus, hepatic SREBP-1c is relatively inactive in hepatocytes of abstinent people, residing mostly in the ER. However, in a person who binges or habitually drinks, hepatic ethanol oxidation triggers the translocation of SREBP-1c from the ER to the Golgi apparatus, where it

Table 2 Lipogenic Enzymes Regulated by SREBP-1c

Enzyme	Abbreviation	Function
Fatty Acid Synthase	FAS	Synthesizes fatty acids from acetyl coenzyme A (CoA) and palmitate
Acyl CoA Carboxylase	ACC	Synthesizes malonyl CoA from acetyl CoA
ATP Citrate Lyase	ACL	Converts citrate and CoA to acetyl CoA
Stearoyl CoA Desaturase	SCD	Synthesizes unsaturated fatty acids (oleate) from saturated fatty acids (stearate)
Malic Enzyme	ME	Generates reducing equivalents (NADPH) for triglyceride synthesis

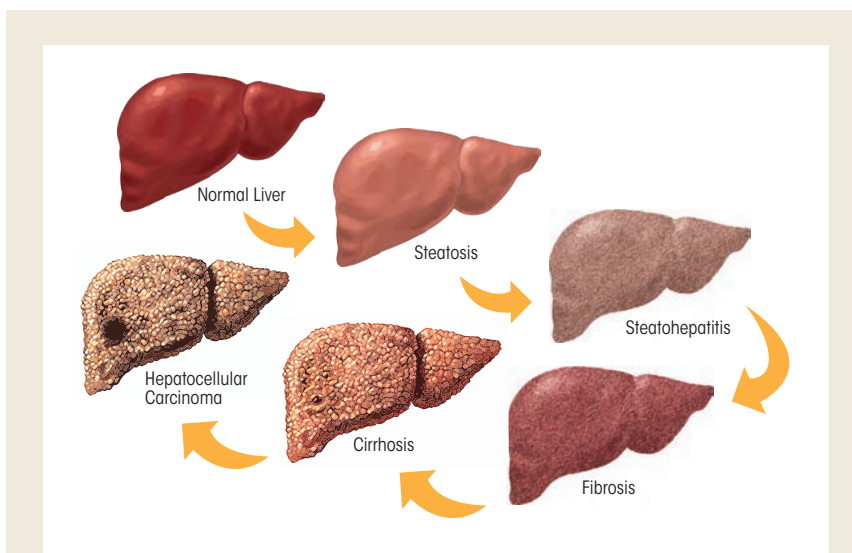


Figure 2 Spectrum of alcoholic liver disease. Heavy ethanol consumption produces a wide spectrum of hepatic lesions. Fatty liver (i.e., steatosis) is the earliest, most common response that develops in more than 90 percent of problem drinkers who consume 4 to 5 standard drinks per day. With continued drinking, alcoholic liver disease can proceed to liver inflammation (i.e., steatohepatitis), fibrosis, cirrhosis, and even liver cancer (i.e., hepatocellular carcinoma).

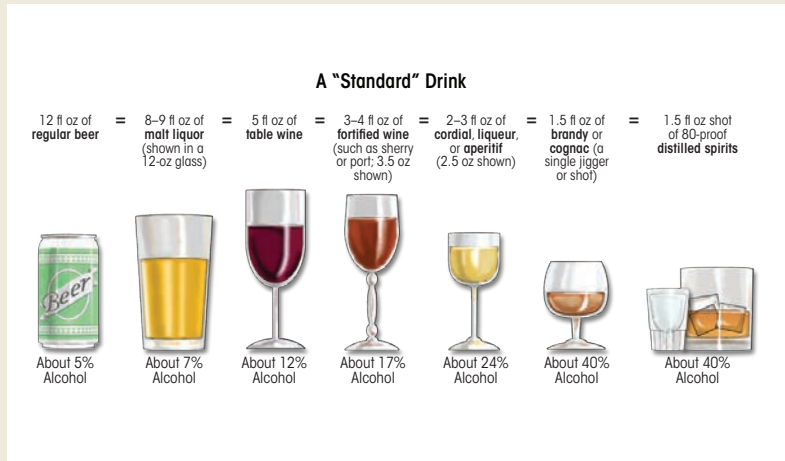


Figure 3 Illustration of "standard drinks" in order of increasing ethanol content among currently available alcoholic beverages. According to the National Institute on Alcohol Abuse and Alcoholism, the amount of beverage containing approximately 14 g of pure ethanol is defined as a standard drink. The percent of pure alcohol, expressed as alcohol by volume (alc/vol), varies by beverage. Thus, 12 ounces (360 mL) of beer at 6 percent alc/vol, 5 ounces (150 mL) of wine at 12 percent alc/vol, or 1.5 ounces (45 mL) of distilled spirits at 40 percent alc/vol each are equivalent to a standard drink. Although the standard-drink amounts are helpful for following health guidelines, they may not reflect customary serving sizes. In addition, although the alcohol concentrations listed are typical, there is considerable variability in actual alcohol content within each type of beverage.

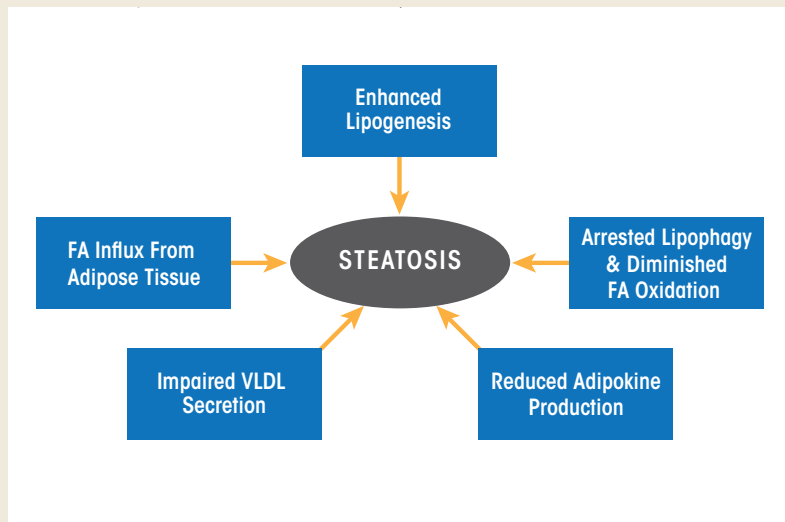


Figure 4 Hepatic and extrahepatic mechanisms that contribute to the development of alcoholic fatty liver (i.e., steatosis).

NOTE: FA = fatty acid; VLDL = very low density lipoprotein.

undergoes proteolytic maturation to its active form, generating a transcriptionally active SREBP protein fragment that enters the nucleus and enhances lipogenic gene expression (see table 2). Egr-1 controls the expression of genes that respond to cellular stress. It binds to gene promoter regions that are relevant to alcohol-induced liver injury and steatosis. The most notable of these is tumor necrosis factor alpha (TNF α), a lipogenic cytokine. Additionally, because Egr-1 is activated very early after ethanol administration (Donohue et al. 2012), it also regulates the expression of the SREBP-1c gene (Thomes et al. 2013). Figure 5 shows the postulated scheme of transcriptional control that contributes to enhanced lipogenesis in the liver.

In addition to enhanced hepatic lipogenesis, fat (i.e., adipose) tissue contributes to the development of steatosis. Adipose tissue normally is an important energy depot, storing excess calories derived from food consumption as fat. When necessary, high-energy fat then can be used to fulfill energy requirements during times of low nutrition (e.g., fasting) or high calorie utilization (e.g., exercise). Research with rodents subjected to chronic alcohol feeding has shown that ethanol consumption reduces adipose tissue mass by enhancing fat breakdown (i.e., lipolysis) in adipose tissue (Kang et al. 2007; Wang et al. 2016; Wei et al. 2013). The free fatty acids released from adipose tissue are taken up by the liver and esterified into triglycerides, thereby exacerbating fat accumulation in the liver (Wei et al. 2013). Clinical studies also have demonstrated that people with alcohol use disorder who have fatty liver have significantly lower body weight, body mass index, and body-fat mass content than control subjects (Addolorato et al. 1997, 1998).

Alcohol Decelerates Hepatic Lipid Breakdown

Because most lipids in hepatocytes are stored in lipid droplets, these organelles must first be degraded to extract

the lipids for their subsequent oxidation. Breakdown of lipid droplets is accomplished by lipophagy, a specialized form of the intracellular process that degrades cytoplasmic components (i.e., autophagy). During lipophagy, lipid droplets are engulfed within double-membrane-bound vacuoles called autophagosomes. These vacuoles transport the lipid-droplet cargo to lysosomes, where they are degraded by lipid-digesting enzymes (i.e., lipases), releasing free fatty acids that then undergo β -oxidation inside mitochondria. The rates of autophagy reportedly are retarded by chronic ethanol consumption, at least in part because ethanol is thought to cause faulty lysosome biogenesis. This results in fewer, more defective lysosomes (Kharbanda et al. 1995, 1996), thereby slowing the breakdown of lipid droplets in the steatotic liver.

It also is quite clear that once fatty acids are released from lipid droplets, heavy alcohol consumption reduces their rates of β -oxidation. There are several reasons for the slowdown: First, the enhanced generation of NADH by ethanol oxidation inhibits mitochondrial β -oxidation. Second, metabolically generated acetaldehyde inactivates the peroxisome proliferator activated receptor alpha (PPAR- α), a transcription factor that acts in concert with the retinoid X receptor (RXR) and governs expression of genes that regulate fatty-acid transport and oxidation. Acetaldehyde likely inactivates PPAR- α by covalently binding to the transcription factor (Galli et al. 2001), thereby blocking its ability to recognize and/or bind PPAR- α promoter sequences. Third, both acute and chronic ethanol oxidation cause mitochondrial depolarization, impairing their abilities to generate energy (i.e., adenosine triphosphate [ATP] molecules), and causing their outer membranes to leak, resulting in inefficient fatty-acid import and lower rates of β -oxidation (Zhong et al. 2014). Fourth, ethanol consumption reduces the production of the hormone adiponectin, which is secreted by fat cells

(i.e., adipocytes). One study demonstrated that the restoration of adiponectin to alcohol-fed animals re-establishes fatty-acid oxidation to normal (Xu et al. 2003). In addition, adiponectin appears to reduce the production of the cytokine TNF α , and there is evidence that TNF α also may regulate adiponectin production (You and Crabb 2004).

Alcohol Causes Defective Hepatic Lipid Export

It is well known that the liver exports triglycerides and cholesterol only as constituents of very low density lipoprotein (VLDL) particles; any impairment in either the synthesis or export of VLDL particles therefore contributes to fat accumulation within hepatocytes. VLDL assembly is regulated by the availability of triglycerides (which

make up more than 50 percent of the VLDL lipids) stored in cytoplasmic lipid droplets. Up to 70 percent of the triglycerides in VLDLs are derived from the pool of triglycerides stored in lipid droplets that first undergo lipolysis and then are re-esterified to constitute VLDL triglycerides. Although earlier reports implicated altered VLDL secretion in the development of alcoholic steatosis (Venkatesan et al. 1988), exactly how alcohol impairs lipolysis of triglyceride stores in lipid droplets for assembly of VLDL and its subsequent secretion is unknown. However, studies have shown that alcohol-impaired VLDL secretion is caused by a decreased synthesis of an essential constituent of VLDL (Kharbanda et al. 2007, 2009) as well as by reduced activity of an essential protein for its assembly (Shearn et al. 2016; Sugimoto et al. 2002).

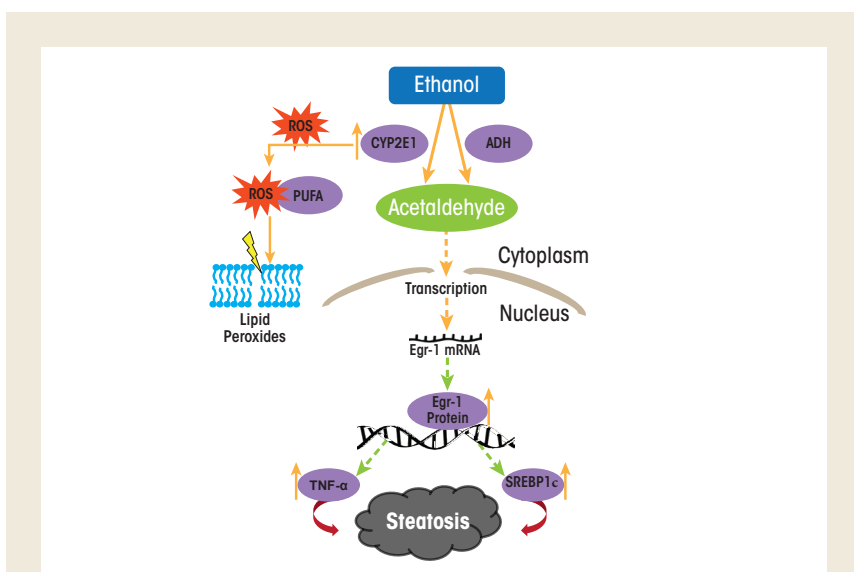


Figure 5 Proposed mechanism by which ethanol oxidation regulates early growth response-1 (Egr-1) and sterol regulatory element binding protein-1c (SREBP-1c) to enhance lipogenesis. Alcohol dehydrogenase (ADH) and cytochrome P450 2E1 (CYP2E1) each catalyze ethanol oxidation, producing acetaldehyde. This aldehyde enhances Egr-1 gene transcription by activating the Egr-1 promoter, thereby increasing the levels of Egr-1 mRNA and, subsequently, nuclear Egr-1 protein. It is believed that nuclear Egr-1 protein regulates transcription of SREBP-1c and tumor necrosis factor (TNF) genes to initiate ethanol-induced lipogenesis and fatty liver (i.e., steatosis).

NOTE: PUFA = polyunsaturated fatty acid; ROS = reactive oxygen species.
SOURCE: Figure adapted from Thomes et al. 2013.

Mechanisms Involved in Alcoholic Hepatitis

Alcoholic hepatitis occurs in about 30 to 40 percent of individuals reporting chronic alcohol abuse. It represents the most serious form of ALD and is associated with high short-term mortality. Ballooning degeneration of hepatocytes containing Mallory-Denk bodies, infiltrating neutrophils, and fibrosis are characteristic pathologic findings indicative of hepatitis (Lefkowitz 2005). Central to the progression of alcoholic hepatitis are resident and infiltrating immune cells called macrophages, which have important roles in inducing liver inflammation. KCs, the resident macrophages in the liver, represent up to 15 percent of liver cells and 50 percent of all macrophages in

the body. They reside in the liver sinusoids and provide the first line of defense, serving as potent innate immune cells. In contrast, infiltrating macrophages are recruited as immature cells from the bone marrow, and their differentiation into macrophages in the liver only occurs during inflammation.

The ability of macrophages to regulate inflammation depends on their polarization—that is, their ability to develop into one of two different functional states, namely M1 (i.e., proinflammatory) or M2 (i.e., anti-inflammatory) macrophages. The polarization to either phenotype depends on the microenvironment, including circulating growth factors, cytokines, and pathogen-associated molecular pattern (PAMP) as well as damage-associated molecular pattern

(DAMP) molecules. Because the liver is exposed to countless antigens, pathogens, and toxic substances that come from the intestine via the portal circulation, it must be protected from developing an immune response to such exposure. As a result, KCs usually have tolerogenic properties, meaning that they do not respond to all antigens with an immune response. However, excessive alcohol exposure can switch KCs to a proinflammatory M1 phenotype. Usually, ALD progression from liver steatosis to inflammation requires a second insult in addition to the alcohol exposure, such as another toxic insult, nutritional factor, or viral infection (Tsukamoto et al. 2009). More importantly, KCs can regulate the development of inflammation, depending on their ability to either induce or suppress proinflammatory changes. These effects are related to the stage and severity of the alcoholic hepatitis; in severe cases, KCs differentiate to the proinflammatory M1 phenotype, whereas in mild forms, KCs switch to the anti-inflammatory M2 phenotype. As inducers of inflammation, KCs release multiple proinflammatory cytokines, including $\text{TNF}\alpha$, interleukins, and chemokines that attract inflammatory cells from circulation. KCs also are an abundant source of ROS that exacerbate oxidative stress in the liver.

What factors trigger KC activity in patients with alcohol use disorder? One major factor is endotoxin, also called lipopolysaccharide (LPS), a cell-wall component of Gram-negative bacteria that translocates from the gut lumen into the portal circulation to reach the liver (figure 6). Accumulating data demonstrate that excess ethanol intake induces endotoxemia through two main mechanisms—by stimulating bacterial overgrowth and by increasing intestinal permeability (Bode and Bode 2003). Animal studies have revealed that increased circulating endotoxin levels correlate with the severity of liver disease (Mathurin et al. 2000). LPS is sensed by two types of receptors—CD14 and toll-like receptor 4 (TLR4)—

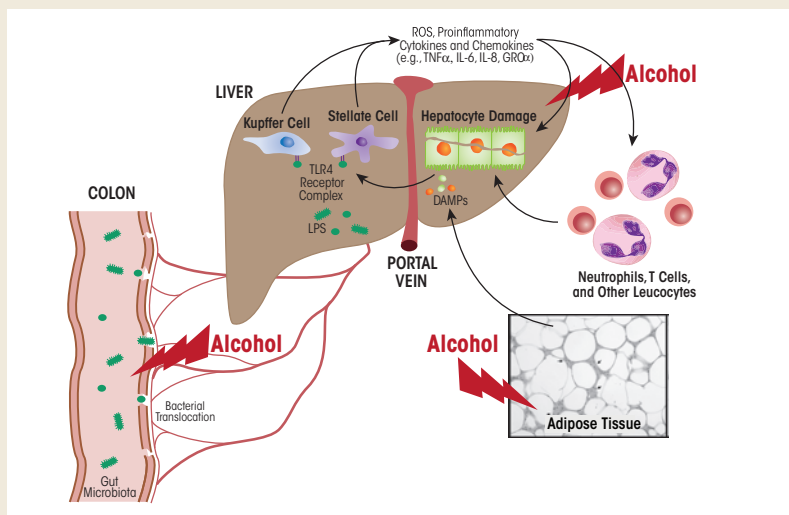


Figure 6 The gut–liver axis. A major factor in the initiation of the inflammatory response by resident macrophages of the liver (i.e., Kupffer cells) is endotoxin or lipopolysaccharide (LPS), a cell-wall component of Gram-negative bacteria that translocates from the gut lumen into the portal circulation to reach the liver. Enhanced circulating endotoxin levels in alcoholic hepatitis are caused by alcohol-induced qualitative and quantitative changes in the bacteria that inhabit the gut (i.e., gut microbiota) and increased gut leakiness. In the liver, LPS activates Kupffer cells and hepatic stellate cells by interacting with toll-like receptor 4 (TLR4). These cells produce reactive oxygen species (ROS) as well as proinflammatory cytokines and chemokines that together with alcohol contribute to hepatocyte damage. Other factors contributing to hepatocyte damage include alcohol-induced activation of various immune cells (i.e., neutrophils, T cells, and other leukocytes) as well as alcohol's effects on the fat (i.e., adipose) tissue, which results in the production of damage-associated molecular pattern (DAMP) molecules.

on the KC surface (Suraweera et al. 2015). These receptors activate KCs to produce proinflammatory cytokines and promote free-radical formation via induction of the reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and CYP2E1. The resulting reactive oxygen and nitrogen species promote the release of proinflammatory cytokines, which in turn increase inflammasome activation in KCs and the release of chemokines that attract circulating immune cells to the liver. Inflammasomes are innate immune-system sensors that regulate the activation of caspase-1 and induce inflammation in response to microbial/viral pathogens, molecules derived from host proteins, and toxic insults (e.g., alcohol exposure).

Other factors can exacerbate liver inflammation. Prominent among these are MAA adducts that are produced in alcohol-exposed hepatocytes. These adducts are taken up by scavenger receptors on KCs (Ambade and Mandrekar 2012), further promoting the proinflammatory response. Also, because macrophages metabolize ethanol via CYP2E1, the induction of oxidative stress by alcohol exposure activates macrophage-dependent release of proinflammatory cytokines, including TNF α . Although hepatocytes normally are resistant to TNF α , alcohol exposure sensitizes them to the cytokine, causing their death via apoptosis. The resulting release of small vesicles (i.e., exosomes) from dying hepatocytes provides activation signals to KCs (Nagy et al. 2016). Apoptotic hepatocytes are engulfed by KCs, thereby switching their phenotype to M1, which exacerbates inflammation. Inflammation-associated release of chemokines, in turn, attracts circulating macrophages, T-cells, and neutrophils (an additional source of oxidative stress) to the liver. These immune cells, by releasing proinflammatory cytokines and chemokines with direct cytotoxic effects, further promote hepatocyte cell death and the persistence of alcoholic hepatitis.

Recently, it was reported that HSCs also play a dual (i.e., stage-dependent) role in the regulation of liver inflammation (Fujita et al. 2016). An important function of HSCs is to transmit signals from sinusoid cells to the liver parenchyma. The proinflammatory cytokines and chemokines produced by activated KCs stimulate the production of proinflammatory cytokines by HSCs. In addition, LPS also can directly activate HSCs through TLR4 to promote the secretion of proinflammatory cytokines. The functions of HSCs are regulated by KCs. The dual role of KCs in the regulation of inflammation is not only related to production of proinflammatory substances. At the stage of the resolution

of inflammation, KCs produce anti-inflammatory substances, such as prostaglandin D2, which is sensed by HSC receptors. Prostaglandin D2 programs HSCs to switch their production to anti-inflammatory factors, including transforming growth factor- β 1 (TGF- β 1), which promotes fibrogenesis. The role of KCs and HSCs in promoting alcohol-induced inflammatory changes and progression to fibrosis/cirrhosis is schematically presented in figure 7.

Mechanisms Involved in Fibrosis/Cirrhosis

HSCs are the key players in the development of fibrosis. These cells normally

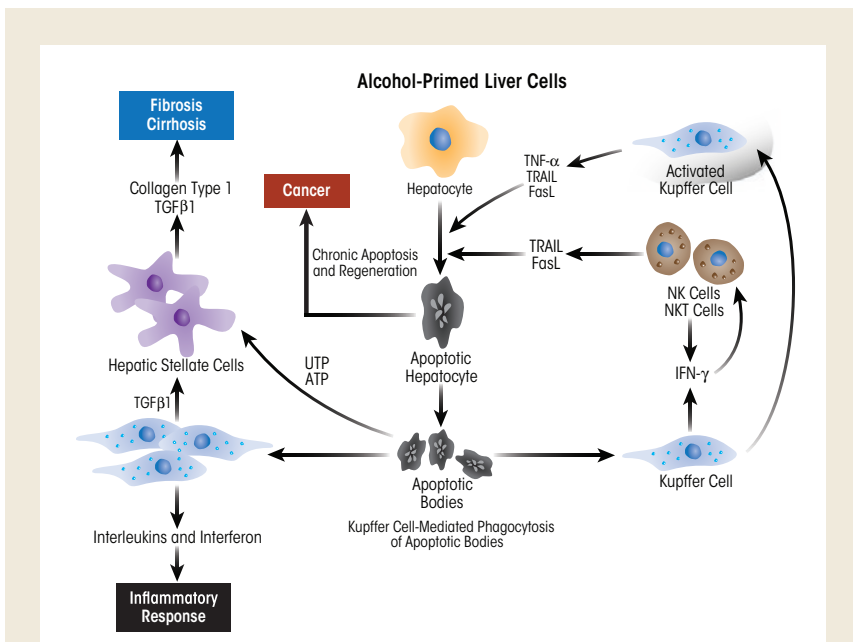


Figure 7 Schematic depiction of the role of Kupffer cells (KCs) and hepatic stellate cells (HSCs) in promoting alcohol-induced inflammatory changes and progression to fibrosis and cirrhosis. Injury begins with alcohol-induced hepatocyte damage and death (apoptosis), which generates apoptotic bodies that stimulate KCs to secrete inflammatory factors, such as tumor necrosis factor alpha (TNF α), interferon gamma (IFN- γ), tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), and Fas ligand (FasL). These factors attract immune cells (e.g., natural killer [NK] cells and natural killer T cells [NKT cells]) to the liver to exacerbate the inflammatory process. Activated HSCs secrete abundant extracellular matrix proteins (e.g., collagen type 1), forming scar tissue (fibrosis) that can progress to cirrhosis. In this condition, the scar tissue forms bands throughout the liver, destroying the liver's internal structure and impairing the liver's ability to regenerate itself and to function.

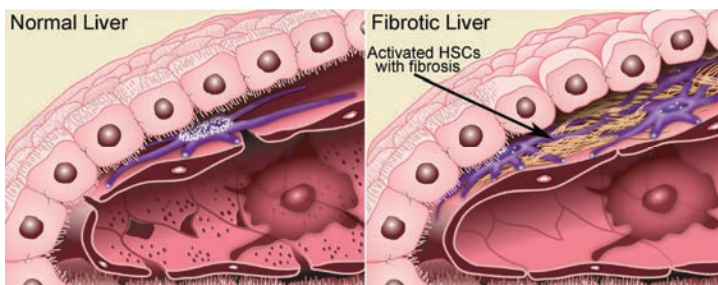


Figure 8 Hepatic stellate cells (HSCs) are key players in the development of fibrosis. HSCs normally reside in the space of Disse as quiescent, lipid (retinyl-ester)-storing cells. Chronic ethanol consumption initiates a complex activation process that transforms these quiescent HSCs into an activated state. Activated HSCs secrete copious amounts of the scar-forming extracellular matrix proteins. This, in turn, contributes to structural changes in the liver, such as the loss of hepatocyte microvilli and sinusoidal endothelial fenestrae, ultimately causing the deterioration of hepatic function.

SOURCE: Figure adapted from Friedman 2000.

reside in the space of Disse as quiescent, lipid (retinyl-ester)-storing cells (figure 8). Following hepatic injury, HSCs undergo a complex activation process (figure 9) and become the principal source for the increased and irregular deposition of extracellular-matrix components that characterize fibrosis. Activated HSCs also contribute to the inflammatory response, coordinating the recruitment and stimulation of leukocytes by releasing chemokines and proinflammatory cytokines as well as expressing adhesion molecules. The leukocytes, in turn, not only attack and destroy hepatocytes, but also activate quiescent and activated HSCs, thereby exacerbating the fibrogenic response (Friedman 2008).

Hepatic fibrosis is a transient and reversible wound-healing response, which may be restored to normal in some patients if alcohol intake ceases. However, if drinking continues, chronic inflammation and sustained fibrogenesis progress, resulting in the substitution of liver parenchyma by scar tissue that severely compromises the liver's vascular architecture. The main pathological feature of cirrhosis is the formation of regenerative nodules of hepatic parenchyma surrounded by fibrous septa. Cirrhosis development progresses from a compensated phase, in which part of the liver remains undamaged and functionally compensates for the damaged regions, to a decompensated phase, in which scar tissue fully envelops the organ. The latter is characterized by development of portal hypertension and/or liver failure.

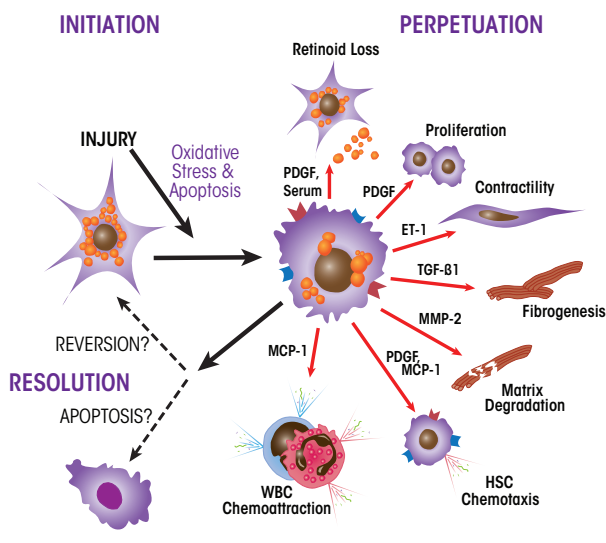


Figure 9 Pathways of hepatic stellate cell (HSC) activation. Following hepatic injury, HSCs undergo a complex activation process involving numerous signaling molecules that is characterized by loss of retinoids, increased proliferation, contractility, and chemotaxis. These activated cells are the principal cell source of increased and irregular deposition of extracellular matrix components, which characterize fibrosis. Activated HSCs also contribute to the inflammatory response by coordinating the recruitment and stimulation of white blood cells (WBCs) by releasing chemokines and proinflammatory cytokines, as well as expressing adhesion molecules.

NOTE: ET-1 = endothelin-1; MCP-1 = monocyte chemoattractant protein-1; MMP-2 = matrix metalloproteinase-2; PDGF = platelet-derived growth factor; TGF-β1 = transforming growth factor-beta1.
SOURCE: Figure adapted from Friedman 2000.

Modifiers of ALD Risk

Among problem drinkers, only about 35 percent develop advanced liver disease. This is because modifiers, as listed below, exist that exacerbate, slow, or prevent ALD disease progression.

- *Pattern of Consumption and Beverage Type.* The most important factors determining the progression

of liver disease are the beverage type consumed and the amount and pattern of drinking (e.g., outside mealtime or binges). Intake of 40 to 80 grams ethanol/day by males and of 20 to 40 grams/day by females for 10 to 12 years is a general predictor of more severe cases of ALD, including alcoholic steatohepatitis, fibrosis, and cirrhosis (Becker et al. 1996).

- **Gender.** Epidemiologic data show that women are more susceptible to alcohol-related liver damage than men. This appears to be related to higher blood alcohol concentrations in women than in men who ingest

the same amount of alcohol, resulting from a lower proportion of body water in females compared with males of equal weight (Mumenthaler et al. 1999). There also are reports that women possess a lower capacity than men to oxidize ethanol in the gut, a process called first-pass metabolism (Frezza et al. 1990). This deficit in women allows greater quantities of ethanol into the portal circulation, thereby exposing their livers to higher ethanol concentrations. Further, gender-based differences in the sensitivity of KCs to endotoxins and hepatic inflammatory responses have been related to higher susceptibility to ALD

progression in females than in males (Frezza et al. 1990).

- **Age.** It is not completely clear how age modifies ALD progression. It is, however, a predictor for ALD (Masson et al. 2014), because older adults (i.e., ages 65 and up) are more vulnerable to and show greater degrees of ethanol-induced impairments than younger people (Meier and Seitz 2008).
- **Race/Ethnicity.** Ethnicity is a major factor affecting the age at and severity of presentation of different subtypes of ALD (Levy et al. 2015). The

Glossary

Ascites: Accumulation of fluid in the abdominal cavity.

Autophagy: The breakdown of organelles (e.g., lipid droplets) and macromolecules (e.g., proteins and lipids) in lysosomes for maintenance of cell homeostasis.

β -Oxidation: The main metabolic process by which fatty acids are broken down in the cell.

Chemokine: Any of a group of small signaling proteins that are released by a variety of cells to stimulate the movement of *leukocytes* and attract them to the site of an immune response.

Cytokine: Any of a group of small, hormone-like proteins secreted by various cell types that regulate the intensity and duration of immune responses and mediate cell-to-cell communication.

Depolarization: Reduction in the difference in electrical charge across a membrane (e.g., between the inside and outside of a cell or a cell compartment, such as a mitochondrion), which can affect numerous cellular functions.

Encephalopathy: Any disorder of the brain; syndrome of overall brain dysfunction that can have many different organic and inorganic causes; for example, advanced liver cirrhosis can cause hepatic encephalopathy.

Endoplasmic Reticulum (ER): An organelle found in eukaryotic cells that forms an interconnected network of membrane-enclosed sacs or tube-like structures and is connected with the outer membrane of the cell

nucleus; the ER serves many functions, including the folding and transport of newly produced proteins that are then delivered to the *Golgi apparatus*.

Epigenetic: Pertaining to the regulation of gene expression without altering the DNA sequences; can include chemical modifications of the DNA or of the proteins (i.e., histones) around which the DNA is wound.

Golgi Apparatus: Membrane-enclosed organelle with tube-like structures that plays a role in the transport of newly produced proteins to their destination within the cell or out of the cell; the Golgi apparatus receives proteins packaged into small membrane-enclosed vesicles from the *endoplasmic reticulum* and transports them to their final destinations.

Hepatic Stellate Cell (HSC): Cell type found in the liver with several long protrusions that wrap around the *sinusoids*. HSCs play an important role in liver fibrosis; in normal liver, the HSCs are in a resting state but become activated upon liver damage, resulting in cell proliferation and secretion of collagen scar tissue.

Hepatorenal Syndrome: The occurrence of kidney failure in patients with liver disease.

Kupffer Cell (KC): Specialized immune cells (i.e., macrophages) that reside in the liver and are part of the immune system, particularly inflammatory responses; they play a central role in early stages of alcoholic liver disease.

reason(s) for these differences are not clear.

- **Genetics.** Both genetic and epigenetic influences govern the initiation and progression of ALD. Genome-wide association studies have identified specific genetic markers (i.e., single-nucleotide polymorphisms) in genes encoding alcohol-metabolizing enzymes, cytokines, and antioxidant enzymes that are related to the progression of ALD (Stickel and Hampe 2012). Most recently, an allele of patatin-like phospholipase domain-containing protein 3 (PNPLA3 I148M), a triglyceride-degrading enzyme, was identified as an independent risk factor for alcoholic cirrhosis (Anstee et al. 2016; Burza et al. 2014).
- **Nutritional Factors.** Dietary fat is a macronutrient and dietary modifier for ALD. In rodents, dietary saturated fat seems to protect against alcohol-induced liver damage, whereas dietary unsaturated fat that is enriched in linoleic acid reportedly promotes such damage (Kirpich et al. 2016).
- **Drugs.** Alcohol and other drugs (including prescription medications, over-the-counter agents, and illicit drugs) interact to enhance hepatotoxicity. For example, as described earlier, acetaminophen hepatotoxicity can be exacerbated by alcohol abuse.
- **Obesity.** Population-based studies have indicated a significant correlation between the risk of liver damage and alcohol consumption in people with a high body mass index (Ruhl and Everhart 2005).
- **Smoking.** Cigarette smoking can adversely affect certain hepatic functions and is associated with higher risk of alcoholic cirrhosis in humans (Klatsky and Armstrong 1992).

Glossary (continued)

Leukocytes: White blood cells that make up the immune system; they are found throughout the body and include five main types, one of which are the monocytes/*macrophages*.

Lipophagy: The selective *autophagy* of lipid droplets.

Macrophage: A type of *leukocyte* that act as phagocytes—that is, they ingest and destroy bacteria, foreign particles, and dead or diseased cells or other degenerating material in the body; they also release signaling molecules involved in the immune response.

Parenchymal Cells: The distinguishing or specific cells of an organ or gland that are contained in and supported by the connective tissue; parenchymal cells of the liver are the hepatocytes.

Peroxisome: A membrane-enclosed organelle found in many eukaryotic cells that contains various enzymes needed for the formation and degradation of hydrogen peroxide (H₂O₂); plays a role in breaking down fatty acids and detoxifying various molecules.

Portal Hypertension: Elevated blood pressure in the blood system supplying the liver (i.e., portal system); occurs in cirrhosis and other conditions that cause blockage of the portal vein.

Promoter: A region of DNA located in front of a gene that regulates and marks the starting point for gene transcription.

Proteolytic: Pertaining to or causing the breakdown of proteins (i.e., proteolysis).

Reactive Oxygen Species (ROS): Highly reactive chemical molecules containing oxygen, such as hydrogen peroxide or superoxide, that are formed as natural byproducts of various metabolic reactions but whose levels can increase during times of environmental stress; excess levels of ROS can damage macromolecules (e.g., proteins or DNA).

Sinusoid: A small, thin-walled blood vessel characterized by open pores between the cells lining the vessel, allowing small and medium-sized proteins to readily enter and leave the bloodstream; in the liver, *Kupffer cells* are located inside the sinusoids.

Space of Disse: In the liver, the small space that separates the walls of the *sinusoids* from the *parenchymal cells* (i.e., the hepatocytes).

Ubiquitin-Proteasome System: A system comprising multiple components that identifies and degrades unwanted proteins in all cells; is involved in cell growth and differentiation, cell death (i.e., apoptosis), and stress and immune responses.

Vacuole: A clear space within a cell that may surround an engulfed foreign particle and may degrade or digest that particle.

- *Viral Infections.* The course of hepatitis C (HCV) and hepatitis B (HBV) viral infections is worsened in alcohol-abusing patients, causing rapid progression to fibrosis, cirrhosis, and even hepatocellular carcinoma (Szabo et al. 2006). Several common mechanisms of viral infection and alcohol-induced damage have been suggested (Zakhari 2013); however, the exact mechanisms for this rapid disease progression are not completely understood. Because viral infections such as HCV or HBV affect more than 170 million people worldwide (Gitto et al. 2014), the following section will describe this topic in greater detail.

HCV and Alcohol

HCV and alcohol are the two most widespread causes of liver disease worldwide. Almost all patients with a history of both HCV infection and alcohol abuse develop chronic liver injury. Some studies report that 16.9 percent of HCV-infection cases progress to liver cirrhosis, which is twice the prevalence of cirrhosis from alcoholic liver disease. In HCV-positive alcohol abusers, cirrhosis prevalence is even higher at 27.2 percent (Khan and Yatsushashi 2000). A daily intake of 80 grams of alcohol increases liver-cancer risk 5-fold over that of non-drinkers, whereas heavy alcohol use by HCV-infected individuals increases cancer risk by 100-fold over uninfected heavy drinkers.

There are multiple mechanisms by which alcohol potentiates HCV-infection pathogenesis. For example, HCV proteins induce oxidative stress by binding to the outer membranes of mitochondria, stimulating electron transport and increasing the generation of cellular ROS (e.g., superoxide) (Otani et al. 2005). Coupled with the ethanol-induced depletion of the antioxidant glutathione and ROS-induced suppression of proteasome activity, this compromises cell viability (Osna et al. 2008), causing hepatocyte apoptosis

(Ganesan et al. 2015; Siu et al. 2009). Ethanol-induced oxidative stress also causes mutations in the HCV genome that increase resistance to interferon (IFN) treatment, the former standard of care for HCV (Serone et al. 2011). Only 9 percent of HCV-infected people with alcohol use disorder respond to IFN α therapy. There currently is little information on whether heavy drinking affects the outcomes of HCV treatment with the new generation of antiviral agents (Keating 2015).

Ethanol metabolites appear to stimulate HCV replication. CYP2E1-positive hepatoma cells exposed to ethanol show an increase in HCV RNA (McCartney et al. 2008). However, this rise is only temporarily sustained (Serone et al. 2007), because these heavily infected cells eventually die by apoptosis (Ganesan et al. 2015). The resulting cell fragments (i.e., apoptotic bodies) contain infectious HCV particles that spread the virus to uninfected cells, causing the production of proinflammatory cytokines by phagocytosing KCs (Ganesan et al. 2016). In addition to apoptotic bodies, another type of cell-derived vesicles (i.e., exosomes) that leak from dead cells enhances intracellular HCV replication in neighboring cells through an exosomal micro-RNA (miRNA 122). Because ethanol exposure also increases hepatic miRNA 122 levels (Bala et al. 2012), HCV replication in problem drinkers likely is augmented (Ganesan et al. 2016).

Innate immunity is the first line of antiviral protection in the liver. HCV commandeers this line of defense, and ethanol metabolism potentiates its takeover. For example, activation of antiviral IFN β production in liver cells occurs via the interferon regulatory factor 3 pathway, which requires participation of a protein called mitochondrial antiviral signaling protein (MAVS). HCV evades this innate-immunity protection by cleaving MAVS (Gale and Foy 2005), and ethanol metabolism further enhances this cleavage. There are other published examples of how ethanol consumption interferes with the immune response to HCV infection (Ganesan et al. 2015; Siu et

al. 2009). Thus, HCV and ethanol synergize in thwarting protective mechanisms that include both innate and adaptive immunity by increasing oxidative stress in liver cells, thereby accelerating the onset of cell death and facilitating the spread of the virus.

Current Management of ALD

There are no FDA-approved therapies for treating patients with ALD. The following therapies currently are used for optimal ALD management.

Abstinence

Drinking cessation is considered the most effective therapy in patients with ALD. Abstinence from alcohol not only resolves alcoholic steatosis but also improves survival in cirrhotic patients (Sofair et al. 2010). The effectiveness of abstinence is enhanced when it is combined with lifestyle modifications (e.g., behavioral interventions and dietary alterations) that are supervised by a nurse, primary care physician, or gastroenterologist/hepatologist (Addolorato et al. 2016; Pavlov et al. 2016).

Natural and Artificial Steroids

Corticosteroid treatment, including the use of prednisolone, has been the most extensively used form of therapy, especially for moderate to severe alcoholic hepatitis, based on their ability to suppress the immune response and proinflammatory cytokine response (Mathurin et al. 1996, 2013; Ramond et al. 1992). However, outcomes with steroids have been variable (Thursz et al. 2015). Current guidelines suggest discontinuation of therapy if there is no indication of a decrease in bilirubin levels by day 7 of treatment (European Association for the Study of the Liver 2012).

Nutritional Supplements

Nearly all patients with severe alcoholic hepatitis and cirrhosis are malnourished

and their degree of malnutrition correlates with disease severity and complications, such as variceal bleeding, ascites, infections, encephalopathy, and hepatorenal syndrome (Halsted 2004; Mendenhall et al. 1995; Stickel et al. 2003). Deficiencies in micronutrients (e.g., folate, vitamin B6, vitamin A, and thiamine) and minerals (e.g., selenium, zinc, copper, and magnesium) often occur in ALD and, in some instances, are thought to be involved in its pathogenesis (Halsted 2004). According to the current guidelines of the American Association for the Study of Liver Diseases, all patients with alcoholic hepatitis or advanced ALD should be assessed for nutritional deficiencies and treated aggressively with enteral nutritional therapy. A protein intake of 1.5 grams per kilogram bodyweight and 35 to 49 kcal per kilogram bodyweight per day is recommended for ALD patients (Frazier et al. 2011). Micronutrient supplementation should be considered if deficiencies are detected. Supplementation with one such micronutrient, zinc, has been shown to be therapeutic in animal models of alcoholic liver injury. Mechanistic studies have revealed that its protection is mediated by blocking or attenuating most mechanisms of liver injury, including increased gut permeability, oxidative stress, increased TNF production, and hepatocyte apoptosis (Mohammad et al. 2012). The few clinical studies conducted to date suggest that zinc supplementation could be an effective therapeutic approach for humans because liver function of ALD and HCV patients improved with 50 mg of elemental zinc (Mohammad et al. 2012).

Liver Transplantation

This procedure remains the standard of care for patients with end-stage liver disease. Some patients with ALD are not listed for the replacement of their own liver by a donor organ (i.e., orthotopic liver transplantation) for reasons such as continued alcohol consumption, improvement in liver func-

tion after abstinence, and a higher incidence of cancers of the upper airways and upper digestive tract. As a result, transplantation candidates with ALD often are screened for common malignancies and must undergo a formal medical and psychiatric evaluation. They also must abstain from alcohol for 6 months before being considered for liver transplantation. Data show that fewer than 20 percent of patients with histories of alcohol use as the primary cause of end-stage liver disease receive liver transplants (Lucey 2014). However, patient and organ survival is excellent in this patient population, with considerable improvement in their quality of life (Singal et al. 2012, 2013). Following transplantation, ALD patients return to consuming alcohol at rates similar to those transplanted for other reasons, although ALD patients may consume greater amounts (Bergheim et al. 2005). Because all transplant recipients exhibit increased levels of alcohol use over time, post-transplant interventions are deemed extremely valuable in supporting patients to maintain abstinence (Donnadiou-Rigole et al. 2017).

Unconventional and Herbal Remedies

Patients often turn to natural and herbal therapies based on their potential for hepatoprotection. A U.S. survey revealed that 41 percent of patients with liver disease used some form of complementary and alternative medicine. An extract of milk-thistle seeds (silymarin) and garlic were reported as the most commonly used herbs for liver disease, followed by ginseng, green tea, ginkgo, echinacea, and St. John's wort (Strader et al. 2002). As indicated in a recent review (Kim et al. 2016), these and other natural medicines, including betaine, curcumin, fenu-greek seed polyphenol, LIV-52, vitamin E, and vitamin C, have shown efficacy in experimental models of alcoholic liver injury but must pass the rigors of large randomized, controlled clinical trials.

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Alcohol–Organ Interactions: Injury and Repair

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Why Study Alcohol-Related Organ Damage?

The association between alcohol misuse and organ damage, specifically liver disease, has been recognized for more than 200 years (Maher 1997). Yet it was not until the early 1970s that researchers demonstrated a direct causal relationship between drinking and this serious—and sometimes fatal—condition. In their seminal paper, Rubin and Lieber (1973) established that baboons consuming up to 50 percent of their daily caloric intake in the form of ethanol developed the “entire constellation of histologic features characteristic of human alcoholic hepatitis,” p. 712. Because the animals’ diet otherwise provided adequate nutrition, the researchers concluded that ethanol was responsible for the development of liver disease. Subsequent studies determined that the risk of alcoholic liver disease (ALD), and particularly cirrhosis, increased with alcohol consumption levels.

Since those early studies, research has shown that excessive drinking can affect nearly all major organ systems, contributing significantly to alcohol-related morbidity and mortality. Indeed, the International Classification of Diseases, Tenth Edition (ICD–10) lists more than 20 disorders that are entirely attributable to alcohol, as well as a similar number of conditions for which alcohol consumption is a component cause (Shield et al. 2013).

According to the Centers for Disease Control and Prevention (CDC), a large proportion of alcohol-related deaths are the consequence of organ damage and disease. To quantify the impact of harmful drinking on morbidity and mortality, the CDC developed the Alcohol-Related Disease Impact (ARDI) application, which assesses the deaths and years of potential life lost (YPLL) attributable to alcohol misuse (CDC 2013). The most recent analyses of these data found that in the United States from 2006 through 2010, about 38,250 deaths annually could be attributed to chronic conditions related to alcohol misuse. Of these, more than 32,000 were related to alcohol-associated organ damage, including liver disease, various types of cancer, or cardiovascular disease. These deaths corresponded to 705,000 YPLL (Stahre et al. 2014).

Given these serious adverse effects of alcohol on organ function, it is crucial that we gain a deeper understanding of the relationship between alcohol consumption, organ function, and disease, as well as the physiological mechanisms underlying these effects. This issue of *Alcohol Research: Current Reviews* presents an overview of key findings in the field, with each article focusing on alcohol’s effects on either an organ or organ system.

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Alcohol's Impact on Organs and Organ Systems

Alcohol is a toxin that can damage tissues and organs both directly and indirectly through its metabolic byproducts. As the primary site of alcohol metabolism, the liver is particularly vulnerable to alcohol-induced damage. More than 90 percent of long-term heavy drinkers develop fatty liver, and about 35 percent develop advanced liver disease, underscoring the need for effective treatments for ALD (see article by Osna and colleagues in this issue).

The gastrointestinal tract is the primary site at which alcohol is absorbed into the blood, and it is also susceptible to alcohol-induced damage. Bishehsari and colleagues describe how alcohol and its metabolites contribute to intestinal inflammation, which may promote gastrointestinal cancers and inflammatory bowel disease. Damage to the pancreas—part of the digestive system, along with the liver and gastrointestinal tract—is also linked to alcohol consumption. Pandol reviews research showing that the relationship between heavy drinking and acute and chronic pancreatitis is well established. However, experimental data suggest that alcohol consumption alone does not initiate pancreatitis, but instead sensitizes the pancreas to disease from other insults.

The effects of alcohol on the brain also have been documented extensively. Zahr and Pfefferbaum review the structural brain changes found in people with alcohol-related neurological conditions, such as Wernicke's encephalopathy and Korsakoff syndrome, as a framework for understanding brain changes that occur in individuals with alcohol use disorder (AUD) in the absence of those conditions. They also discuss research demonstrating that although some alcohol-induced brain changes appear to be permanent, other patients recover over time with abstinence.

Skeletal muscle dysfunction (i.e., myopathy) is common in patients with AUD, and its incidence exceeds that of alcoholic liver cirrhosis. Simon and colleagues review the epidemiology of alcoholic myopathy, the pathophysiologic mechanisms underlying alcohol's effects on mechanisms involved in maintaining muscle mass, the clinical implications of these effects, and emerging literature on treatment options.

The association between alcohol and cardiovascular diseases (CVD), including hypertension, coronary heart disease, and stroke, has been studied extensively. Piano reports that the dose and pattern of alcohol consumption seem to be important moderators of the link between alcohol and CVD, with larger amounts of alcohol consumption being associated with increased CVD risk and low-to-moderate amounts with reduced CVD risk. The relationship between alcohol and CVD is complex, and ongoing research will help clarify the dose and health conditions for which alcohol may have potential benefits, as well as those for which alcohol may have adverse effects.

As described by Mehta and Guidot, people with AUD are particularly susceptible to lung diseases, such as pneumonia, tuberculosis, and acute respiratory distress syndrome. Researchers have identified several pathways through which alcohol misuse may interfere with lung function. This work has led to the identification of potential therapeutic targets for treating alcohol-related lung conditions.

Because hormones control virtually all important bodily functions and are essential for maintaining a constant internal environment, alcohol's effects on the endocrine system have potentially far-reaching impact. Rachdaoui and Sarkar review how the effects of alcohol on hormonal systems contribute to a broad range of debilitating disorders, including stress intolerance, reproductive dysfunction, thyroid problems, immune abnormalities, diabetes, cardiovascular disease, cancer, and psychological disorders. Alcohol's effects on hormone

function can be particularly harmful during puberty, resulting in delayed pubertal development (see article by Dees and colleagues in this issue).

Next Steps

The articles in this issue review the significant advances that have been made toward elucidating how alcohol affects organ systems and contributes to organ-related pathology. They also highlight the many gaps in our knowledge. For example, there is a relative dearth of research on the relationship between alcohol and kidney function. As Varga and colleagues note, although studies suggest several potential mechanisms by which alcohol may affect the kidneys, there is little experimental evidence demonstrating that alcohol consumption leads to kidney injury, and epidemiological data linking the two are inconclusive. This area is ripe for further investigation. Likewise, Piano notes that most data on the link between alcohol and CVD in humans are derived from epidemiological studies, pointing to the need for a prospective randomized controlled trial in this area.

Even with diseases such as ALD, in which the role of alcohol has been well established, the complex underlying pathophysiological mechanisms are not fully understood. Moreover, the body's organs do not operate in isolation—they interact with each other such that alcohol-induced damage in one organ can perturb others. For example, studies have shown that a single episode of binge drinking can cause bacterial toxins to leak from the gut into the bloodstream, inducing inflammation in other organ systems. Inflammation is implicated in much of the organ damage associated with alcohol misuse, and it is emerging as an important contributor to the development of AUD.

Developing a better understanding of the mechanisms by which alcohol affects organ function will lead to improved interventions for preventing and treating alcohol-induced organ damage. One promising area of study described by Barve and colleagues is the use of nutritional interventions. Although alcohol–nutrition interactions may contribute to various types of organ damage (e.g., liver, intestinal, and lung dysfunction), nutritional supplementation with micronutrients such as zinc may help prevent or ameliorate some of this damage. We hope that this issue will spark additional interest in this topic and lead to a greater menu of effective therapies for alcohol-related conditions.

Resources

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Effects of Alcohol on Tumor Growth, Metastasis, Immune Response, and Host Survival

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Most research involving alcohol and cancer concerns the relationship between alcohol consumption and cancer risk and the mechanisms of carcinogenesis. This review relates the amount and duration of alcohol intake in humans and in animal models of cancer to tumor growth, angiogenesis, invasion, metastasis, immune response, and host survival in specific types and subtypes of cancer. Research on the influence of alcohol drinking on human cancer patients is limited. Although there is more information in animal models of cancer, many aspects still are ill defined. More research is needed to define the mechanisms that underlie the role of alcohol on cancer progression in both animals and humans. Activation of the immune system can play a positive role in keeping cancer under control, but this also can facilitate cancer progression. Additionally, a functional immune system is required for cancer patients to achieve an optimal response to conventional chemotherapy. Insight into the underlying mechanisms of these interactions could lead to effective immunotherapeutic approaches to treat alcoholics with cancer. Defining the epigenetic mechanisms that modulate cancer progression also has great potential for the development of new treatment options not only for treating alcoholics with cancer but also for treating other alcohol-induced diseases.

Key words: Alcohol consumption; alcoholism; alcohol use duration; alcohol-induced disease; risk factors; cancer; cancer progression; tumor; metastasis; immune response; immune system; chemotherapy; host survival; angiogenesis; epigenetic mechanisms; treatment; animal models; human studies

Alcohol use and abuse have been implicated as etiological factors in the genesis of an increasing number of cancer types in both men and women. In 2012, the International Agency for Research on Cancer (IARC) listed both beverage alcohol (i.e., ethanol) and its major metabolite, acetaldehyde, as tumor-inducing substances (i.e., carcinogens) in humans. The most recent worldwide statistic from 2002 estimated that about 3.6 percent of all cancers, or 389,100 cases, are associated with alcohol consumption (Seitz and Stickel 2007). Cancers for which strong epidemiological evidence indicates that alcohol consumption is associated with an increased risk include, but are not

limited to, esophageal, laryngeal, pharyngeal, stomach, colorectal, liver, pancreas, lung, prostate, breast, central nervous system, and skin cancers (Berstad et al. 2008; Boffetta and Hashibe 2006; Brooks and Zakhari 2013; de Menezes et al. 2013; Haas et al. 2012; Kumagai et al. 2013; Longnecker et al. 1995; Nelson et al. 2013; Rota et al. 2014a; Watters et al. 2010). The risk of developing a second aerodigestive-tract cancer also is higher in alcohol drinkers (Day et al. 1994; Lin et al. 2005; Saito et al. 2014).

Increased risk of cancer often is associated with high alcohol consumption; however, the specific dose–response relationship varies according to the site

of cancer. A recent meta-analysis of 16 articles involving 19 cohorts of subjects with liver cancer (i.e., hepatocellular carcinoma) found a linear relationship between the amount of alcohol consumed and the risk of liver cancer compared with nondrinkers (Turati et al. 2014). Thus, consumption of three alcoholic drinks per day was associated with a moderate increase in risk, whereas consumption of about seven drinks per day was associated with an increase in risk of up to 66 percent. A similar linear relationship has been described for breast cancer risk (Scoccianti et al. 2014).

However, alcohol consumption does not increase the risk of all types

of cancer and may even be associated with a lower risk in some cases. For example, although alcohol consumption overall is associated with a higher risk of breast cancer in women, this association does not apply to all types of breast cancer. Thus, among women enrolled in the Women's Health Initiative the risk of estrogen-positive breast cancer was increased in those who drank alcohol, whereas the risk of triple-negative breast cancer¹ was reduced among drinkers compared with women who had never consumed alcohol (Kabat et al. 2011).

Interestingly, alcohol consumption also is associated with a lower incidence of several types of blood cancer, including non-Hodgkin's lymphoma (NHL) (Gapstur et al. 2012; Ji et al. 2014; Morton et al. 2005; Tramacere et al. 2012) and multiple myeloma (Andreotti et al. 2013). An analysis of 420,489 individuals diagnosed with alcohol use disorder (AUD) who were linked to the Swedish Cancer Registry also found a low risk of developing leukemia, multiple myeloma, and Hodgkin's disease (Ji et al. 2014). Another recent study also showed that alcohol drinking was not associated with increased risk of leukemia and that, in fact, light drinking (less than or equal to one drink per day) was associated with a modest 10 percent reduction in leukemia incidence (Rota et al. 2014b). In addition to blood cancers, alcohol consumption also is associated with a lower risk of thyroid cancer (de Menezes et al. 2013) and renal cell carcinoma (Song et al. 2012). In the case of renal cell carcinoma, a lower risk was noted even with consumption as low as one drink per day in both men and women, and higher alcohol intake conferred no further benefit. Finally, a retrospective, observational study of colon and rectum adenocarcinoma indicated that moderate alcohol consumption (less than 14 grams per day) was inversely

associated with the incidence of rectal cancer. The investigators also found that moderate intake of beer and especially wine was inversely associated with distal colorectal cancer (Crockett et al. 2011).

In summary, it is well established that alcohol use and abuse is associated with a wide variety of cancers, and the number of these associations continues to grow. At the same time, it now is becoming clear that alcohol can have a preventative effect for certain cancers. Whereas the role of alcohol as a carcinogen is well established, the mechanism(s) by which it prevents cancer are largely unknown and an area for further research. Also, despite the potential beneficial effects of alcohol in the prevention of some cancers, it is important to remember that the detrimental effect of chronic alcohol abuse cannot be disregarded.

Although extensive epidemiologic evidence links the etiology of cancer to alcohol, very little information addresses the critical question of whether and how alcohol modulates tumor metastasis, survival, and the response to cancer therapy. One of the components in these processes is the immune system. Much research regarding the role of the immune response in oncogenesis has centered on hepatocellular cancer (for excellent recent reviews, see Aravalli 2013; Stauffer et al. 2012; Wang 2011). However, less is known regarding the role and interaction among alcohol consumption, immune modulation of tumor growth, blood vessel formation (i.e., angiogenesis), metastasis, and survival. These issues form the major emphasis of this review. It is well established that immunosurveillance by the innate and adaptive immune systems plays important roles in the prevention of cancer and in controlling cancer survival (Fridmann et al. 2012; Rocken 2010). However, direct or indirect interactions of the tumors with their microenvironment can facilitate immune evasion so that the tumor is not detected by the immune system and thus can spread uncontrolled. Tumors also release factors

that can directly or indirectly suppress antitumor immune responses, thus facilitating angiogenesis, invasion of surrounding tissues, and metastasis to distant sites in the body (for a general review, see Jung 2011). (For more information on the processes involved in tumor metastasis, see the sidebar.) The following sections will review the role of alcohol in cancer growth and progression, both in humans and in animal models.

Alcohol, Tumor Growth, and Survival in Humans

Survival and Mortality

Statistics from 2002 indicate that approximately 3.5 percent of all cancer deaths are associated with alcohol (Seitz and Stickel 2007). A study of 167,343 adult subjects in rural southern India found that daily drinking for 30 or more years increased overall cancer-related mortality (Ramadas et al. 2010). Similarly, a study involving 380,395 men and women who were followed for 12.6 years as part of the European Prospective Investigation into Cancer and nutrition (EPIC) study indicated that compared with no or light-to-moderate consumption (i.e., 0.1 to 4.9 g alcohol/day), heavy (30 or more g/day) drinking in women and heavy to extreme (60 or more g/day) drinking in men was strongly associated with increased total mortality as well as deaths from alcohol-related cancers (Ferrari et al. 2014). However, the effect of alcohol on cancer-specific mortality is variable and depends on factors such as the amount of alcohol consumed, health status of the patient, and the type of cancer.

Survival of patients with oral cavity, pharyngeal, laryngeal, and esophageal cancer is generally reduced by drinking (Jerjes et al. 2012; Mayne et al. 2009; Thrift et al. 2012; Wang et al. 2012a; Wu et al. 2012; Zaridze et al. 2009). In Korean patients with head and neck and hepatocellular carcinoma the

¹ In estrogen-positive breast cancer, the cancer cells carry the estrogen receptor and depend on estrogen for growth. In contrast, in triple-negative breast cancer, the cancer cells carry neither estrogen nor progesterone or HER2 receptors.

death rate exhibited a dose-dependent relationship with consumption, with patients who drank between 124 and 289 g of alcohol per day showing the highest death rate (Park et al. 2006). Lower survival of patients with hepatocellular cancer also has been reported in Scotland (Dunbar et al. 2013), Russia (Zarizde et al. 2009), and Spain (Fenoglio et al. 2013). Shortened survival in drinkers as compared with nondrinkers with oral squamous cell carcinoma has been linked to the expression of hypoxia-inducible factor-1-alpha (HIF-1 α), a biomarker associated with tumor invasion, metastasis, and progression of a variety of human cancers that also plays a central role in angiogenesis. Drinkers showed higher HIF-1 α expression in the nucleus of their cancer cells than nondrinkers (Lin et al. 2008). Finally, although alcohol consumption lowers the incidence of NHL, it decreases patient survival of those with the disease (Battaglioli et al. 2006; Geyer et al. 2010; Talamini et al. 2008).

The effect of alcohol consumption on mortality of women with breast cancer is varied and difficult to interpret. In general, long-term low and moderate alcohol consumption does not seem to affect the survival of breast cancer patients (Flatt et al. 2010; Harris et al. 2012; Kwan et al. 2012; Newcomb et al. 2013). In fact, moderate drinking actually may benefit survival of young women with breast cancer (Barnett et al. 2008; Newcomb et al. 2013). On the other hand, several studies indicated that postmenopausal women with breast cancer who are high-intensity drinkers have lower survival than those with no or lower consumption (Holm et al. 2013; McDonald et al. 2002; Weaver et al. 2013).² In addition to patient age, the specific type of breast cancer may influence the effects of alcohol on survival. Thus, for women with estrogen receptor–positive breast cancer neither pre- nor postdiagnosis

alcohol consumption was associated with breast cancer mortality (Ali et al. 2014). In women with estrogen receptor–negative disease, however, mortality was slightly reduced. Another study investigated the effect of pre- and postoperative alcohol consumption over a 3-year period in 934 Swedish primary breast cancer patients who had breast cancer surgery (Simonsson et al. 2014). The study found that both pre- and postoperative consumption of any amount of alcohol was weakly associated with a lower risk of early distant metastases and death. The associations were found in patients with axillary lymph node involvement but not in patients without lymph node involvement.

The effect of alcohol consumption on the incidence as well as the mortality of patients with prostate cancer was evaluated in a prospective cohort study of 194,797 men from the United States aged 50–71 years in 1995–1996 (Watters et al. 2010). The incidence of nonadvanced prostate cancer increased with increasing number of drinks per day, with a 25 percent increase in risk observed after high alcohol consumption (six or more drinks per day). However, an inverse correlation existed between alcohol consumption and deaths from prostate cancer, suggesting that alcohol consumption likely does not affect advanced or fatal prostate cancer.

In summary, several reports indicate that alcohol consumption decreases survival of patients with cancer, whereas other studies did not observe this association. The effect of alcohol consumption on mortality of women with breast cancer is particularly complex and seems to differ according to age, estrogen receptor status, and extent of alcohol drinking. Clearly, more breast cancer–specific studies are needed that correlate mortality with the properties of the cancer and the level of alcohol consumption.

Tumor Growth and Metastasis

The actual influence of alcohol consumption on tumor growth and

metastasis is largely unknown in human cancer patients. Discriminant function analysis of 39 asymptomatic Italian patients with a total of 59 small hepatocellular carcinomas arising from cirrhosis revealed that, among other variables, alcohol intake was a good predictor of tumor doubling time and 2-year survival (Barbara et al. 1992). Another study of 35 Japanese patients with hepatocellular carcinoma and type C cirrhosis found that habitual drinkers consuming 80 g of ethanol per day for 5 years had a statistically significant ($P < 0.01$) shorter tumor-volume doubling time than did non-alcoholic patients (78 ± 47 days vs. 142 ± 60 days) (Matsushashi et al. 1996).

Basal cell carcinoma—a type of skin cancer—is the most common cancer in humans and continues to increase in incidence. Although the cure rate is high and mortality and morbidity rates are low, aggressive basal cell carcinomas are not rare. In a Spanish study, a significant positive association existed between moderate (5 to 10 drinks per week) and high (more than 10 drinks per week) alcohol consumption and the presence of aggressive basal cell carcinomas (Husein-Elahmed et al. 2012).

Alcohol, Tumor Growth, Invasion, and Metastasis in Animal Models

Several studies using animal cancer models indicate tumor specific differences in the effect of alcohol on tumor growth and metastasis. These models included various types of breast cancer, melanoma, lung cancer, colon cancer, and hepatocellular carcinoma (For more information, see the sidebar “Effects of Alcohol on Tumor Growth, Invasion, Metastasis, and Survival in Animal Models”). Taken together, these studies and animal models did not allow for general conclusions regarding the impact of alcohol on tumor growth, metastasis formation, and disease progression, as findings differed significantly depending on

² The exception to this is a study in Russia indicating an inverse association between alcohol consumption and mortality (Zarizde et al. 2009).

tumor type. The alcohol model used as well as the duration of alcohol administration also are important variables and can affect the overall outcome (D'Souza El-Guindy et al. 2010), as is the amount of alcohol administered. For example, in studies assessing alcohol's effects on metastasis formation, acute administration of high doses of alcohol, which mimics binge drinking, generally increased metastasis, whereas longer-term alcohol administration either had no effect or decreased metastasis formation, depending on the amount of alcohol consumed by the animal. Several mechanisms have been suggested as to how acute alcohol may enhance metastasis formation, including alcohol-induced formation of as

well as inhibition of various signaling molecules (i.e., cytokines and chemokines). However, although both of these mechanisms seem to contribute to the increase of metastases after acute administration, they do not account for the entirety of alcohol's effects. Another mechanism whereby alcohol could facilitate metastasis of certain cancers may involve disruption of the integrity of the cells lining the blood vessels (i.e., vascular endothelium). Thus, studies found that exposure to 0.2 percent (weight per volume [w/v]) ethanol in vitro, which promotes angiogenesis and invasion, interferes with the integrity of the vascular endothelium by inducing endocytosis of VE-cadherin (Xu et al. 2012). This

molecule is an important component of certain junctions between cells (i.e., cellular adherens junctions). These changes in the vascular endothelium have been shown to allow for increased migration of human A549 lung adenocarcinoma cells, MDA-MB-231 breast cancer cells, and HCT116 colon cancer cells through single-cell layers of endothelial cells (Xu et al. 2012).

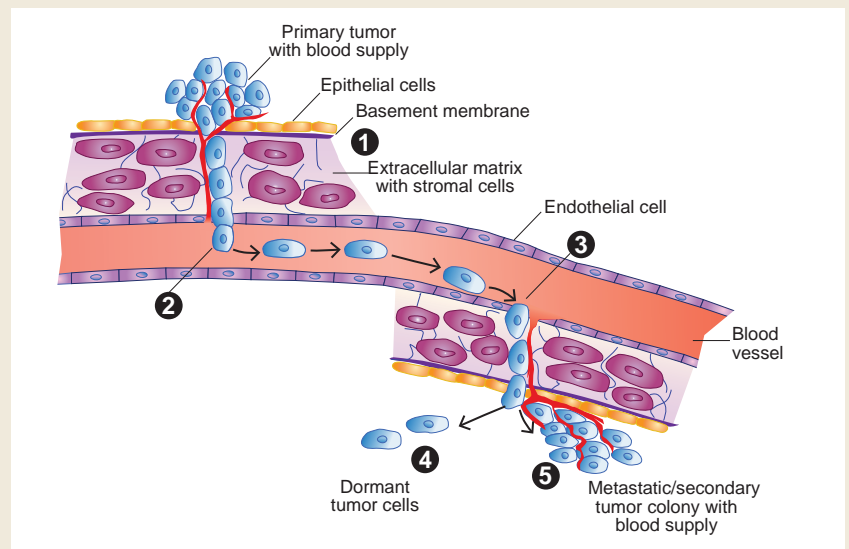
Researchers also examined the effects of alcohol administration on tumor growth. These studies found that high alcohol intake had no consistent effect on tumor growth across different tumors or within a specific tumor type. Low intake of alcohol generally has been associated with enhanced angiogenesis

Tumor Metastasis

Tumor metastasis is the ability of tumor cells to spread from their original site to other sites in the body and to re-establish growth, a new blood supply, and tumor colonies at the new location.

(1) Cells that escape from a primary solid tumor invade into the surrounding normal tissue by passing through the basement membrane and extracellular matrix (ECM). Several factors are involved in the invasion process, including the ability to activate enzymes called matrix metalloproteinases (MMP), which are important for the tumor cells to degrade basement membranes and underlying stroma.

(2) The escaped cells reach the blood either directly by actively passing through endothelial cells that line the blood vessels or passively through the lymphatic system, which ultimately carries the tumor cells to the blood. (3) Once in the blood, the tumor cells exit into tissues at the secondary site from small capillaries by passing through endo-



thelial cells and then invading the basement membrane of the ECM. (4) Once at the secondary site, the tumor cells can lay dormant for extended periods of time, or (5) they re-establish growth to form metastatic tumor colonies (by proliferation of cells from a single tumor cell), and finally form a new blood supply

(by stimulating the angiogenesis process) to nourish the metastatic tumor. Dormant cells also can proliferate at a future date and ultimately establish a new metastatic tumor. Factors that control the breaking of dormancy are largely unknown, and this is an active area of research.

(which promotes tumor growth), whereas high intake may have no effect.

As mentioned earlier, studies in humans found that alcohol's effects on breast cancer, its progression, and the associated mortality are influenced at least in part by the type of breast cancer involved, specifically its estrogen receptor status. However, animal models involving different breast cancer cell lines detected no consistent trend regarding the effect of alcohol consumption on tumor growth and progression associated with estrogen receptor expression. Estrogen generally suppresses breast cancer growth *in vivo* but increases *in vitro* migration of cells away from the original tumor. However, the relationship between estrogen supplementation, diet, caloric intake, and alcohol and their effects on subcutaneous breast cancer growth seem to be highly complex.

The effects of alcohol on *in vitro* invasion of surrounding tissue primarily have been studied in breast cancer and melanoma cells, with a variety of results. The evidence in melanoma suggests that ethanol can positively impact the extracellular membrane and augment expression of genes that suppress tumor metastasis, resulting in inhibition of metastasis. In addition, certain immune cells called natural killer (NK) cells seem to have some role in regulating the metastasis of breast cancers and melanomas. Clearly, more mechanistic research is needed in murine models to serve as a template for further examination of the complex interactions connecting alcohol to tumor growth, metastasis, and survival in humans.

Alcohol-Induced Immune Modulation and Tumor Progression

Although many factors influence tumor growth, metastasis, and survival in cancer patients, it is apparent that a functioning immune system plays an important role, not only because it helps control cancer progression but also because it is required for the

effectiveness of common cytotoxic chemotherapeutic drugs (Bracci et al. 2014). Evidence that directly implicates immune cells from both the innate and adaptive immune systems in control of cancer growth and progression continues to accumulate. This has stimulated research directed toward developing effective immunotherapeutic approaches to treat cancer (for a review of the tumor immune response as well as approaches being taken to develop immunotherapeutics for cancer, see Harris and Drake 2013).

The innate immune response reacts rapidly to recognize and destroy cancer cells. This response is characterized by inflammatory reactions involving various mediators, including chemokines and cytokines that are produced by a variety of immune cells, such as macrophages, neutrophils, NK cells, and dendritic cells. Macrophages and neutrophils can exhibit antitumor activity as well as suppress immune response against tumor cells (i.e., have immunosuppressive activity). NK cells can destroy tumors on contact, and their antitumor function can be further stimulated by cytokines. Dendritic cells are important in presenting molecules that identify a cell as harmful or foreign (i.e., antigens) to other immune cells and are a bridge between the innate immune response and the B-cell and T-cell responses that characterize the adaptive immune system.

B cells can recognize tumor-cell antigens to ultimately produce antitumor antibodies. They also can have immunosuppressive activity. T cells can be classified according to certain molecules they exhibit on their surfaces, such as CD4, CD8, or CD25. They also can be classified according to their specific functions (e.g., as helper, cytotoxic, regulatory, or memory T cells). CD4⁺ helper T cells can further be divided into Th1, Th2, and Th17 subpopulations based on the specific cytokines they produce and the reactions they induce in the body, which may either facilitate or suppress antitumor immune responses. Certain subsets of CD4⁺CD25⁺ T cells, known as

regulatory T cells, generally are immunosuppressive. Cytokines released by Th1 helper T cells, in turn, can activate CD8⁺ T cells, rendering them directly cytotoxic to tumor cells as well as enhance the activity of NK cells. Other populations of CD8⁺ cells (i.e., tumor-specific and memory CD8⁺ T cells) produce high levels of the cytokine interferon gamma (IFN- γ), which is important to the control of tumor metastasis and host survival. Finally, another population of T cells (i.e., NKT cells) that produce a wide variety of cytokines upon activation can function as immunoregulatory cells to either enhance or suppress antitumor immune responses, depending on the cytokine profile that they exhibit. Together, the cells of the immune response provide an intricate interactive control that governs tumor growth and progression. (For more information on the innate and adaptive immune systems and their responses, see the "Primer on the Immune Response," by Spiering.)

A Role for the Immune System in Control of Cancer Progression

Numerous findings with a variety of tumor types suggest that the numerous types of immune cells, particularly various T-cell subpopulations, are involved in controlling tumor progression, including the following:

- CD8⁺ T cells, in particular a subtype expressing the memory phenotype (CD8⁺CD44^{hi}) that produce high levels of IFN- γ , are key to controlling metastasis and host survival of different tumors (Erdag et al. 2012; Eyles et al. 2010; Fridman et al. 2012; Rosenberg and Dudley 2009).
- Increased tumor progression in patients with gastric cancer has been tied to increased peripheral blood levels of certain CD4⁺ T-cell subpopulations, including Th22 (CD4⁺IL-22⁺IL-17⁺IFN- γ) and

Th17 (CD4⁺IL-17⁺IFN- γ) cells (Liu et al. 2012).

- A multivariate analysis in metastatic breast cancer patients indicated that prolonged progression-free survival was correlated with increased CD3⁺CD4⁺ or CD8⁺CD28⁺ T cells. Conversely, elevated CD8⁺CD28⁻ T cells were associated with shortened progression-free survival (Song et al. 2013). These effects seem to be related to the cytokines produced by these cells, because patients with elevated CD8⁺CD28⁻ and CD4⁺CD25⁺ T cells had elevated levels of IL-6, and the patients that expressed elevated CD8⁺CD28⁻ T cells also exhibited decreased IFN- γ .

These data underscore the importance of immune cells in the progression of cancer.

Alcohol can modulate the body's immune responses, and it is possible that these alterations affect disease progression in cancer patients. For example, in a Chinese study of newly diagnosed NHL patients (Lin et al. 2009), alcohol addiction was associated with increased peripheral blood CD4⁺CD25^{hi}CD127(IL-7)^{lo} regulatory T cells, and these increases were higher in male than in female patients. However, the increased levels of these cells did not relate to the clinical features (e.g., age, tumor staging, cancer symptoms, pathological subtype, and short-term treatment efficacy). Therefore, the importance and significance of the elevated regulatory T cells is uncertain in NHL.

Another study of 25 patients with hepatocellular carcinoma in Japan (Yang et al. 2006) found an increase in CD4⁺CD25⁺ T cells in the tissue regions surrounding the tumor (i.e., the peritumoral region) compared with similar tissues in patients who had chronic hepatitis or liver cirrhosis but no hepatocellular carcinoma. The values were not correlated with the

stage of the tumor.³ These peritumoral CD4⁺CD25⁺ T cells had a regulatory phenotype, as indicated by an increased expression of several molecules (e.g., cytotoxic T lymphocyte antigen 4 [CTLA-4, CD152] and glucocorticoid-induced TNF receptor superfamily member 18 [GITR, CD357]), expression of a biomarker for regulatory T cells (i.e., FOXP3), and decreased expression of CD45RA. The numbers of these cells were inversely associated with the numbers of CD8⁺ T cells. Additional observations suggest that these regulatory T cells may contribute to the progression of hepatocellular carcinoma by interfering with normal immune responses. Thus, isolated peritumor CD4⁺CD25⁺ T cells that were incubated with peripheral blood T cells from the same person and stimulated with certain antibodies, suppressed T-cell proliferation and activation of CD8⁺ T cells (Yang et al. 2006).

The functionality of the innate immune system also can be correlated with tumor progression. A recent study compared innate immune-system functionality with the number of circulating tumor cells in patients with a variety of cancers. In patients with metastatic disease, these circulating tumor cells are promising as biomarkers for tumor progression and overall cancer survival, with relatively high circulating cell numbers correlated with a poor prognosis. The study, which included patients with metastatic breast, colorectal, and prostate cancer found decreased NK cell cytolytic activity and decreased expression of certain proteins (i.e., toll-like receptors 2 and 4) in patients with high circulating tumor cells compared with patients with relatively low numbers (Santos et al. 2014). Decreased NK cytolytic activity also has been linked with other types of cancer, including colorectal cancer (Kim et al. 2013), metastatic melanoma (Konjevic et al. 2007), and head and neck cancer (Baskic et al. 2013).

³ Interestingly, the same cell type was decreased in the peripheral blood in the cancer patients compared with control patients.

In addition to the effects of specific types of lymphocytes on cancer growth and metastasis, chemokines also have important roles in cancer progression, terminal growth arrest of tumor cells (i.e., tumor growth senescence), angiogenesis, epithelial mesenchymal transition,⁴ metastasis, and evasion of the immune system. Chemokines and their receptors often are altered in cancer patients, and their importance in cancer progression has been the subject of several recent reviews (Aldinucci and Colombatti 2014; de Oliveira et al. 2014; Sarvaiya et al. 2013).

Alcohol and Immune Effects in Patients with Cancer

A large body of literature indicates that alcohol consumption modulates many aspects of the innate and adaptive immune systems. Alcohol originally was described as immunosuppressive, and numerous studies support the immunosuppressive aspects of alcohol consumption on the innate and adaptive immune systems. However, it also is well documented that chronic alcohol administration can activate the immune system—especially dendritic cells, T cells, and NKT cells—in experimental animals as well as humans (Cook et al. 1991; Laso et al. 2007; Song et al. 2002; Zhang and Meadows 2005). This adds to the complexity of interpreting alcohol's effect on cancer progression and survival.

Few studies have specifically examined the interaction between alcohol and the immune response in cancer patients or in experimental animals implanted with cancer cells. Although human cancer patients often have immune deficits, few data are available that specifically address the effects of alcohol on immune parameters. The studies that are available examined the immune responses in patients with head and neck cancer. These patients often are immunodeficient because of

⁴ Epithelial mesenchymal transition is a process whereby epithelial cells lose their innate cellular polarity and cell-cell adhesive properties to become mesenchymal cells, which lack polarity and have the ability to migrate and to invade through tissues.

their alcohol abuse and heavy tobacco use; however, the contribution of continued alcohol abuse to altered immune parameters in these patients has largely not been assessed.

An early study of patients with head and neck squamous cell carcinoma and a history of smoking and significant alcohol use found a deficiency in the percentage of certain T cells (i.e., Th5.2⁺ IL-2–producing T cells) in peripheral blood compared with control patients who were hospitalized for elective surgical procedures (Dawson et al. 1985). The overall percentage of all T cells, as well as of CD4⁺ T-, CD8⁺ T-, B-, and NK cells, in contrast, did not differ between cancer and control patients. However, this effect cannot be clearly attributed to alcohol because the patients also were heavy tobacco users. Another study compared a different indicator of immune-system function (i.e., production of antigen-specific antibodies) using blood samples obtained from patients with squamous cell carcinoma of the oropharynx or larynx and healthy controls, some of whom had high alcohol consumption (i.e., 100 g/day) and/or excessive smoking (20 cigarettes per day for more than 5 years) (Wustrow 1991). The study found that among healthy participants, those with high alcohol consumption or smoking had a pronounced decrease of antigen-specific antibody production *in vitro*. The effect was more pronounced in heavy drinkers than in excessive cigarette smokers. Cancer patients who were heavy drinkers, in contrast, did not show any antigen-specific antibody production *in vitro*. However, after removal of a subset of white blood cells (i.e., mononuclear cells) from the peripheral blood, samples from two-thirds of the patients began to produce such antibodies, and antibody production reached the same level as that measured in the healthy subjects with high alcohol abuse and cigarette consumption. The author suggested that the decreased antigen-specific antibody production in the cancer patients could be related to

upregulation of suppressive cells in these patients (Wustrow 1991).

More recent studies have evaluated the role of a protein called macrophage migration inhibitory factor (MIF), which is an important regulator of the innate immune response. This factor has been studied in patients with lip or intra-oral squamous carcinoma as well as in patients who consumed alcohol regularly (Franca et al. 2013). The analyses found a significant relationship between the incidence of intra-oral cancer, alcohol use, and the number of MIF-positive cells in the stroma. Thus, MIF in the stroma of intra-oral tumors (i.e., tongue, floor of mouth, and alveolar ridge) was decreased in patients who consumed alcohol. The importance of these findings is unknown, although patients with tumors that did not express MIF had a worse prognosis than patients that did.

Alcohol and Immune Interactions in Animal Models of Cancer

If human tumor cells are introduced (i.e., inoculated) into animals with functioning immune systems, they do not form tumors because they are recognized as foreign by the animal's immune system. However, human tumors often grow in animals with compromised immune systems, and such animals can be used as models for a variety of research questions, including studies regarding the roles of various immune cells in controlling cancer and the impact of alcohol on this process. One such study specifically examined the role of CD4⁺ T cells in regulating tumor growth by implanting cells from a human lung cancer (i.e., the 201 T human lung adenocarcinoma cell line) into the lungs of a strain of mice called BALB/c (Hunt et al. 2000). In this study, the mice were administered alcohol chronically for 8 weeks and then were injected with an anti-CD4 monoclonal antibody to deplete CD4⁺ T cells. Initial experiments confirmed that normal, immunocompetent BALB/c

mice did not form lung tumors. To examine the effect of alcohol, the mice were administered ethanol in their food⁵ as well as 10 percent in their drinking water throughout the experimental period. After 8 weeks of ethanol administration or regular food, the mice were implanted with the tumor cells and also received one injection of the anti-CD4 antibody. Separate groups of mice were evaluated at 6 weeks and 13 weeks. Mice in the non-ethanol-fed control group injected with one dose of anti-CD4 antibody initially developed large tumors at 6 weeks, which significantly regressed thereafter. Compared with these control animals, the ethanol-fed mice exhibited significantly larger tumors at 6 weeks as well as a diminished ability to decrease their tumor size at 13 weeks. The findings suggest that this difference in the ability of the ethanol-fed mice to reduce their tumor burden results from an impaired immune system caused by chronic alcohol intake.

Another series of studies analyzed the interaction between chronic alcohol consumption and immune-system functioning in female C57BL/6 mice implanted with B16BL6 melanoma cells under the skin (i.e., subcutaneously). In these studies, the animals continuously received 20 percent w/v ethanol in the drinking water and generally were inoculated with B16BL6 melanoma after 12 weeks or longer of this treatment. The analyses found that in the alcohol-exposed, melanoma-bearing animals the overall numbers of peripheral blood lymphocytes (which include various types of immune cells) were lower than in water-drinking controls when determined 11, 14, and 17 days after tumor inoculation (Zhang et al. 2012). This was in contrast to normal mice not injected with melanoma cells, in which the number of lymphocytes was not altered by alcohol. The decrease in cells was not caused by cell death (i.e., apoptosis). Additional analyses demonstrated that the lowered lymphocyte numbers (i.e., lymphopenia)

⁵ The food included blocks of a jelly-like material (i.e., agar-agar) containing 40 percent alcohol and 0.5 g/kg peanut butter.

were associated with a two- to fourfold decrease in mature B cells as well as in CD4⁺ and CD8⁺ T cells. Further examination demonstrated that the decrease in mature B cells in the blood was associated with impaired B-cell circulation resulting from a down regulation in the formation of compound called sphingosine-1-phosphate and its receptors. Formation of sphingosine-1-phosphate is mediated (i.e., catalyzed) by an enzyme called sphingosine kinase 1, which is an important regulator of tumor progression in melanoma and several other cancers (Meng et al. 2014). This enzyme and other components of the sphingosine-1-phosphate pathway currently are being examined as potential targets for cancer drug development (Pyne and Pyne 2013; Tabasinezhad et al. 2013). Zhang and colleagues (2012) concluded that the severe decrease in mature B cells in

the blood of the alcohol-exposed and tumor-inoculated animals could result from inhibition of B-cell migration from the spleen to the blood resulting from impairment of the sphingosine-1-phosphate signaling pathway. The importance and role of mature B cells in antitumor immune responses is still unclear. They play a dual role by both inhibiting (Inoue et al. 2006) and facilitating antitumor immune response through production of cytokines and enhancement of T-cell activation (DiLillo et al. 2010). Thus, impaired circulation of B cells attributed to alcohol consumption (Zhang et al. 2012) could negatively affect T-cell function.

The investigators also analyzed the levels of the various types of blood cells in the spleen (Zhang et al. 2012). The spleen contains proportionally more B cells and fewer T cells than the peripheral blood; among the T cells, the spleen normally contains a higher

proportion of CD8⁺ T cells than the peripheral blood. The analyses found that alcohol consumption also led to a decrease in CD8⁺ T cells in the spleen; however, this reduction was less remarkable than in peripheral blood. No changes in these cells were observed in the bone marrow. Furthermore, alcohol consumption reduced the overall numbers of B cells in the spleen, although it did not affect all types of B cells equally. Thus, there was no effect on splenic follicular B cells, whereas the number of immature T1 B (CD19⁺CD93⁺CD23⁻) cells increased and the number of marginal zone B cells (CD19⁺CD1d^{hi}CD21^{hi}) decreased.

Other analyses (Zhang and Meadows 2010) investigated the effects of chronic alcohol consumption on various types of CD8⁺ T cells in mice with or without inoculation of B16BL6 melanoma

Glossary

Antibody: Immune molecule (protein) produced by *B cells* that recognizes foreign molecules that have entered the body (i.e., *antigens*), binds to these molecules, and marks them for destruction by the body's immune system.

Antigen: Any molecule that can bind specifically to an *antibody* and can induce an immune response.

B cells: One of the two main types of lymphocytes involved in the adaptive immune response; when activated by interacting with a specific *antigen*, they differentiate into specific subtypes and begin to produce *antibodies* that recognize the specific *antigen*.

Chemokines: Small proteins that serve as chemoattractants, stimulating the migration and activation of cells, particularly phagocytic cells and lymphocytes; they have a central role in inflammatory responses.

Cytokine: Any of a group of molecules, produced primarily by immune cells, that regulate cellular interactions and other functions; many cytokines play important roles in initiating and regulating inflammatory reactions.

Dendritic cell: A type of immune cell involved in the innate immune response that is characterized by a branched morphology; dendritic cells can bind to

antigens and present these antigens to *T cells*, thereby initiating an adaptive immune response.

Macrophage: A type of immune cell that ingests foreign particles and micro-organisms in a process called phagocytosis and which synthesizes *cytokines* and other molecules involved in inflammatory reactions.

Natural killer (NK) cell: A type of immune cell involved in the innate immune response that can kill certain harmful cells, particularly tumor cells, and contributes to the innate immune response to cells infected with viruses or other intracellular pathogens.

Neutrophil: A type of immune cell involved in the innate immune response that engulfs and kills extracellular pathogens in a process called phagocytosis.

T cells: One of the two main types of lymphocytes involved in the adaptive immune response after activation through the interaction with a specific *antigen*. T cells can be divided into several subgroups that support other immune cells (helper T cells), kill invading pathogens or infected cells (cytotoxic T cells), or help turn off the adaptive immune response (regulatory T cells).

cells. These analyses yielded the following results:

- CD8⁺CD44^{hi} T memory cells produced high levels of IFN- γ and were important in the antitumor response to B16BL6 melanoma. Mice not inoculated with melanoma that chronically consumed alcohol had higher levels of these memory cells than mice that drank water.
- After melanoma inoculation, these CD8⁺CD44^{hi} T memory cells increased over a 2-week period in water-drinking animals. However, in mice that chronically consumed alcohol, these memory cells failed to expand in response to melanoma inoculation.
- The lack of expansion of the memory T cells in response to melanoma inoculation in the alcohol-consuming mice resulted from a reduced ability of these cells to proliferate in response to melanoma. Additional experiments examined the ability of CD8⁺ T cells obtained from 2-week melanoma-bearing mice to proliferate *in vitro* in response to specific T cell stimulation (i.e., anti-CD3 and anti-CD28 antibodies). The analyses showed that proliferation of CD8⁺ T cells was reduced by more than one-half in alcohol-consuming mice compared with cells from water-drinking mice.
- The number of CD8⁺ T cells that specifically recognize a melanoma-specific antigen (i.e., gp100) was 2.5-fold lower in the spleen of the alcohol-consuming mice than in water-drinking control mice at three weeks after tumor inoculation, suggesting an impaired immune response.
- The percentage of IFN- γ -producing CD8⁺ T cells, which have tumor-suppressive effects, initially displayed a robust increase until day 11 after melanoma inoculation, but exhibited

an accelerated decay thereafter, suggesting enhanced inhibition of these cells related to an alcohol–melanoma interaction.

The investigators also analyzed the numbers of several types of cells whose production is induced by tumors and which produce factors that inhibit the antitumor functions of T cells, including myeloid-derived suppressor cells (MDSCs), tumor-associated macrophages, T regulatory cells (CD4⁺CD25⁺FOXP3⁺), regulatory B cells (CD1d^{hi}CD5⁺), and NKT cells (Zhang and Meadows 2010; Zhang et al. 2012). Of these, the percentage of CD11b⁺Gr-1^{int} MDSCs, as well as the percentage of the CD124⁺ subpopulation within the CD11b⁺Gr-1^{int} cells, was increased in the peripheral blood of alcohol-consuming mice as determined one week after tumor inoculation. These cells are known to suppress antitumor T-cell immune responses (Sinha et al. 2005; Terabe et al. 2005; Zhu et al. 2007). The percentages of T regulatory cells and tumor-associated macrophages did not differ between alcohol-consuming and water-drinking mice with melanoma tumors (Zhang and Meadows 2010).

The percentage and number of CD3⁺NK1.1⁺ invariant NKT cells was elevated in the blood of alcohol-consuming, B16BL6 melanoma-bearing mice especially at day 14 after tumor inoculation (Zhang et al. 2012). These cells have important regulatory functions and can either promote antitumor immune responses or inhibit them. Initially, these cells express a cytokine profile that favors antitumor immune responses (i.e., a high ratio of IFN- γ to IL-4). After repeated activation, however, these cells become anergic and switch to a cytokine profile that inhibits antitumor immune responses and favors tumor progression (i.e., a high ratio of IL-4 to IFN- γ) (Parekh et al. 2005). The invariant NKT cells from the alcohol-consuming, melanoma-bearing mice exhibit a high IL4/IFN- γ ratio, indicating that they express a cytokine

profile favoring immune inhibition and tumor progression (Zhang et al. 2015).

Overall, very few studies have addressed the role of and interaction among alcohol, cancer, and the immune system once the cancer is established. It is important to understand these interactions, however, because many alcoholics have immune deficiencies and because a competent immune system is important to the success of many conventional drug therapies for cancer. In addition, new immune-enhancing approaches to cancer therapy are being developed. Finally, evidence from animal models and human studies suggests that appropriately combined chemotherapy and immunotherapy may be more beneficial than either therapeutic approach alone (Ardiani et al. 2013; Shi et al. 2014; van Meir et al. 2014; Wang et al. 2014).

Additional Avenues for Future Research

The interactions between alcohol use/abuse, the antitumor immune response, tumor growth, and spread of cancer are complex. A negative impact of alcohol on the immune system can lead to increased cancer mortality; however, studies also indicate that alcohol, generally in low doses, can have beneficial effects on mortality, depending on the cancer. Clearly, more mechanistic research is needed to define the complex interactions between cancer and alcohol. Additional research is likely to uncover targets to mitigate the detrimental effects of alcohol on mortality and to identify specific biochemical and molecular mechanisms involved in the beneficial effects of alcohol related to enhancing survival of cancer patients. This research could translate into the development of more effective and specific targeted approaches to treat cancer patients in general and especially those who abuse alcohol.

Because cancer is a collection of different diseases with diverse underlying causes, it is important that research

take into account the diversity in gene mutations and alterations involved in uncontrolled growth. In addition, future analyses must address the genetic instability that fosters metastasis, the major cause of death from cancer. It is becoming increasingly clear that genes which suppress metastasis (Meadows 2012) as well as signaling pathways that inhibit metastasis (Singh et al. 2014) can be regulated through epigenetic mechanisms⁶ induced by the diet and dietary constituents, including alcohol. Alcohol-related epigenetic mechanisms include modulation of DNA methylation, histone acetylation/deacetylation, and expression of micro RNA (French 2013). These epigenetic mechanisms associated with alcohol also are known to affect the gastrointestinal-hepatic system (Shukla and Lim 2013) and may promote, for example, the progression of hepatic carcinoma. For the most part, alcohol-related epigenetic changes have not yet been associated with tumor growth, metastasis, and survival; however, alcohol-induced aberrant DNA methylation of certain genes plays a role in the control of breast cancer (Tao et al. 2011). Moreover, alcohol also can dysregulate the immune system through epigenetic mechanisms (Curtis et al. 2013), and this aspect of the association between alcohol, the immune system, and cancer progression needs to be explored further.

Another potential target for future research is a molecule called toll-like receptor 4, which is known to help regulate host innate immunity. This receptor recognizes the lipopolysaccharide (LPS) endotoxin, a molecule found on by certain bacteria that are part of the intestinal microflora. In the blood, LPS can induce strong immune reactions. Alcohol is known to facilitate the release of LPS from the gut into the systemic circulation, and this is a key factor in the pathogenesis of

alcoholic liver disease (Petrasek et al. 2010). In addition to its response to LPS, toll-like receptor 4 can facilitate antitumor immune responses; however, emerging evidence also suggests that overactivation of this receptor is associated with tumor progression as well as tumor development (Mai et al. 2013). Although these observations need to be explored further, they suggest that this receptor could be a target for future agonist or antagonist targeted treatment for cancer, particularly for patients that abuse alcohol.

Continued research into the detrimental and beneficial effects of alcohol in human cancer patients and animal models of cancer is a key factor to understanding the complex interactions that affect tumor progression and survival, particularly in the context of alcohol use. This research has a strong potential to discover new immunotherapy and epigenetic approaches to cancer treatment as well as treatment of other alcohol-induced diseases.

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⁶ Epigenetic mechanisms are processes that alter the expression of certain genes without permanently altering the DNA building blocks (i.e., nucleotides) making up the genes; examples of epigenetic mechanisms include the temporary chemical modification (e.g., methylation or acetylation) of nucleotides or of the proteins (i.e., histones) around which the DNA is wrapped in the cell nucleus.

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Effects of Alcohol on Tumor Growth, Invasion, Metastasis, and Survival in Animal Models

Several studies using animal cancer models indicate tumor-specific differences in the effect of alcohol on tumor growth and metastasis. These models included various types of breast cancer, melanoma, lung cancer, colon cancer, and liver cancer (i.e., hepatocellular carcinoma).

Lung Cancer

One early study (Capel et al. 1978) investigated the effect of alcohol exposure on the growth and metastasis of Lewis lung carcinoma. Male animals from a type of mouse strain called C57BL/6 were exposed to 10 percent ethanol in their drinking water for 2, 4, 5, or 8 weeks before tumor cells were implanted into their thighs. Ethanol administration then was continued for 2 more weeks. The study found that ethanol exposure before tumor injection did not affect tumor growth. Furthermore, metastases were significantly reduced in the 2-week and 8-week ethanol groups but not in the 4-week and 5-week groups. Administration of ethanol for 2 weeks after tumor inoculation affected neither tumor growth nor metastasis. These investigators also evaluated the effect of 2-week pre- and postimplantation ethanol exposure (10 percent in drinking water) on the growth of another type of tumor called Ehrlich ascites carcinoma, which is a spontaneous murine mammary adenocarcinoma adapted to grow in fluid in the abdominal cavity (i.e., ascites). Exposure to ethanol before but not after tumor injection significantly decreased the tumor cell number.

Colon Cancer

Gu and colleagues (2005) assessed the effects of alcohol on human HT1080 colon cancer cells in a chick embryo model, focusing on variables related to the blood supply of the tumor. One of the variables analyzed was the expression of vascular endothelial growth factor (VEGF)—a growth factor that promotes blood vessel formation (i.e., is proangiogenic) and enhances tumor vascularization. The study found that exposure to 0.25 g/kg ethanol per day for 9 days resulted in a 2.2-fold increase in tumor volume as well as a 3.9-fold increase in the expression of VEGF mRNA in the tumor cells and a 2.1-fold increase in the amount of the blood vessels in the tumor (i.e., intratumoral vascular volume) compared with control embryos. Exposure of isolated tumor cells to 10 mM and 20 mM ethanol for 19 hours also increased VEGF mRNA and protein expression. The increased intratumoral vascular volume strongly correlated with the increase in tumor volume as well as with intratumoral connective tissue volume density. Finally, invasion of HT1080 cells from the tumor into blood vessels (i.e., intravasation), which occurs during metastasis, increased more than eightfold in response to ethanol. Ethanol did not have a direct effect on cell proliferation.

Hepatocellular Carcinoma

Because excessive alcohol consumption often is associated with liver disease, investigators also have examined the effects of alcohol on hepatocellular carcinoma. One study (Thompson et al. 2013) evaluated

the effects of alcohol as well as a high-fat diet on hepatocellular carcinoma progression in male C57BL/6 mice with diet-induced obesity as well as in nonobese control mice. Both groups were injected with Hepa1-6 tumor cells. Ethanol was administered in drinking water at daily alternating concentrations of 10 percent and 20 percent for 6 weeks before the animals were injected with the tumor cells. The alcohol and dietary regimens then were continued for an additional 8 weeks. Gross numbers of tumors as well as tumor burden were lower in mice fed the high-fat diet with and without ethanol or the control diet with ethanol than in control animals receiving neither ethanol nor high-fat diet, with ethanol exerting a greater protective effect than the high-fat diet. However, the high-fat diet both alone and in conjunction with ethanol enhanced mortality in the tumor-bearing mice. Further analyses found that all tumor-bearing mice exhibited increased numbers of inflammatory white blood cells in their livers, regardless of whether they had received ethanol. The expression of some cytokines and their receptors also was altered in the tumor-bearing mice, and these changes in some cases depended on the animals' diet. Thus, tumor necrosis factor (TNF)- α and interleukin (IL)-6 receptor alpha mRNA expression were increased and transforming growth factor (TGF)- β mRNA expression was decreased in tumor-bearing mice receiving the high-fat diet compared with mice not injected with tumor. An analysis of seven cytokines (IL-1 β , IL-6, IL-10, IL-12, IL-13, interferon gamma [IFN- γ], and TNF- α) in the pooled plasmas from each experimental group indicated no changes across the groups except

Effects of Alcohol on Tumor Growth, Invasion, Metastasis, and Survival in Animal Models (*continued*)

in the water-drinking, high-fat diet group, which exhibited depressed cytokine levels. There was no correlation with ethanol consumption compared with mice not injected with tumor cells.

Breast Cancer

The effect of ethanol on mammary cancer growth has been studied in a number of animal models, using both rodent and human tumor cell lines.

Studies Using Rodent Tumor Cell Lines

Wang and colleagues (2012) examined the effect of ethanol on the growth of the aggressive estrogen receptor–positive E0771 mouse mammary cancer in female C57BL/6 mice. The mice were given 2 percent ethanol in drinking water for half a day on each of 3 consecutive days before the E0771 tumor cells were inoculated into breast tissue (i.e., secondary mammary fat pad), and the ethanol feeding regimen then was continued for 24 days. The study found that the ethanol group exhibited higher primary tumor growth rates, increased final tumor weights, and a twofold increase in lung metastases compared with the water-drinking control group. Immunohistochemical analyses of the mammary tumor tissues also showed a higher density of tiny blood vessels in the ethanol group, indicating that ethanol promoted tumor angiogenesis. Finally, the investigators found increased expression of a chemokine called monocyte chemoattractant protein-1, or MCP-1 (also known as CCL2), which has been implicated in tumor development and angiogenesis, in tumor tissues from the

ethanol group and in E0771 cells exposed to 0.2 percent ethanol. MCP-1 plays an important role in suppressing antitumor immune functions and facilitating tumor metastasis (Kudo-Saito et al. 2013), indicating another mechanism through which alcohol could promote breast cancer progression.

A related study using the same alcohol-feeding regimen confirmed alcohol's effects on growth and angiogenesis of E0771 inoculated into other female C57BL/6 mice (Lu et al. 2014). In that study, a molecule that can inhibit VEGF receptor 2 blocked alcohol's stimulatory effect on tumor growth, indicating that alcohol acts via a VEGF pathway.

Holcomb and colleagues (2012) examined the effects of various diets and supplements on the growth of estrogen receptor–positive mammary tumor cells (derived from mammary tumor virus-Wnt-1 transgenic mice) inoculated subcutaneously into female C57BL/6J mice that had their ovaries removed. Conditions tested included (1) 20 percent weight per volume (w/v) alcohol in drinking water; (2) a calorie-restricted diet with 30 percent fewer calories than normal; (3) a high-fat diet where 60 percent of calories were derived from fat; (4) a low-fat diet where 10 percent of calories were derived from fat; and (5) estrogen supplementation. The diets and alcohol were started when the animals were 8 weeks old and continued for 27 weeks. Estrogen pellets were implanted after 19 weeks of alcohol consumption, and tumors were implanted after 22 weeks. The results on tumor growth were similar to those obtained by Hong and colleagues (2011), with the high-fat diet and alcohol promoting tumor growth

and estrogen suppressing it. Tumor growth was greatly inhibited in the mice receiving a high-fat diet as well as estrogen supplements. The calorie-restricted diet also inhibited tumor growth independent of the effects of alcohol and estrogen supplementation. Neither the diets nor alcohol affected VEGF.

Researchers also studied the effects of alcohol on estrogen receptor–negative mouse mammary tumors. One study involving estrogen receptor–negative Met-1 cancer cells used female FVB/N mice that consumed 20 percent w/v ethanol in drinking water for 18 weeks before they were injected subcutaneously with the cancer cells (Hong et al. 2010). Compared with water-drinking control mice, the ethanol-drinking animals developed palpable tumors earlier and also developed larger tumors. Several other parameters (i.e., insulin sensitivity, leptin levels in the blood, and estrogen levels) were elevated in the alcohol-consuming mice. These researchers also examined the effect of ethanol *in vitro* on the migration of the estrogen receptor–positive T47D breast cancer cell line. The results showed that cells exposed to different concentrations of ethanol from 0.1 percent to 0.5 percent exhibited increased migration, as did cells exposed to estrogen (20 nM). The combination of estrogen and 0.5 percent resulted in higher migration than either treatment alone.

In another study (Hong et al. 2011), the researchers examined the effects of alcohol on Met-1 tumor growth as a function of a diet (i.e., low fat vs. high fat) and estrogen supplementation (low dose vs. high dose). Mice consuming the high-fat diet developed larger tumors than did mice fed the low-fat diet; moreover, alcohol ingestion increased the

Effects of Alcohol on Tumor Growth, Invasion, Metastasis, and Survival in Animal Models (*continued*)

final tumor volume in both dietary groups. Estrogen treatment suppressed tumor growth regardless of diet and alcohol consumption; however, mice treated with high-dose estrogen had slightly larger tumors than did mice treated with the low dose. Tumor tissues also were analyzed for the levels of various regulatory molecules hypothesized to potentially influence tumor progression, including phosphoinositide 3 kinase (PI3K/p85 α); a protein kinase called Akt; the phosphorylated form of Akt (p-AktSer⁴⁷³), a signaling molecule associated with cell proliferation, survival, and invasion; proliferating cell nuclear antigen (PCNA); and cleaved caspase-3, a molecule involved in programmed cell death (i.e., apoptosis), with the following results:

- PI3K (p85 α) was not affected by alcohol, high-fat diet, or estrogen treatment.
- Alcohol and the high-fat diet increased expression of p-AktSer⁴⁷³. In contrast, estrogen supplementation reduced phosphorylation of Akt in both the alcohol and high-fat diet groups.
- The high-fat diet and alcohol decreased the level of cleaved caspase-3, suggesting decreased apoptosis in the tumor tissue. Interestingly, estrogen reduced this signaling molecule in tumors from mice fed alcohol and high-fat diets.
- PCNA was not altered by any of the variables, indicating that cell proliferation was not affected.

Other researchers investigated the effects of alcohol on metastasis of

the estrogen receptor–negative and natural killer (NK) cell-sensitive rat MADB106 mammary adenocarcinoma (Yirmiya et al. 1992). In this study, male Fischer 344 rats were administered only one alcohol dose (1.5 to 3.5 g ethanol/kg body weight into the peritoneal cavity) 1 hour before intravenous tumor inoculation. The higher ethanol doses (i.e., 2.5 g/kg and 3.5 g/kg) significantly increased the number of lung metastases, whereas the lowest dose (1.5 g/kg) did not. Administration of naltrexone, an opioid receptor antagonist used to treat alcohol dependence, did not modify the alcohol-related increase in metastasis. Ethanol's effects on lung metastasis seem to depend on the exact administration schedule. Thus, in a related study these researchers found that administration of 2.5 g/kg ethanol 24 hours before or after tumor inoculation did not affect lung metastasis (Ben-Eliyahu et al. 1996). Yirmiya and colleagues (1992) also administered ethanol in a liquid diet for 2 weeks before and 3 weeks after tumor inoculation and found that lung metastases were increased.

Alcohol's effects also seem to depend on the tumor type studied. When rats were injected intravenously with rat CRNK-16 leukemia cells instead of MADB106 cells, acute administration of 1.5 to 3.5 g of ethanol/kg/body weight reduced survival in a dose-related fashion, whereas maintenance on a liquid diet containing 5 percent w/v ethanol did not (Ben-Eliyahu et al. 1996; Yirmiya et al. 1992).

One of the ways in which the body defends itself against tumor cells involves their destruction by NK cells. The investigators also analyzed alcohol's effects on NK-cell activity, finding that neither acute

injection nor dietary administration of ethanol in these experiments affected NK-cell activity against MADB106 cells when determined in an in vitro assay (Yirmiya et al. 1992). When MADB106 and CRNK-16 cells were incubated with ethanol in vitro, the numbers of these cells were reduced after 5 days. These effects were significant for MADB106 cells at ethanol concentrations of 0.2 percent, 0.5 percent, and 1.0 percent ethanol and for CRNK-16 cells at 0.5 percent and 1.0 percent ethanol. Ethanol had no effect in rats that were depleted of NK cells, and metastasis was not affected after injection of the NK-insensitive C4047 rat colon cancer cell line (Ben-Eliyahu et al. 1996).

Tumor growth and metastasis also were studied in the highly metastatic estrogen receptor–negative 4T1.2 cells implanted into the mammary fat pad of female Balb/c that had consumed alcohol in drinking water for 4 weeks (Vorderstrasse et al. 2012). The analyses indicated that continuous availability of 1 percent, 5 percent, and 18 percent ethanol w/v did not affect either the establishment of tumors or the final mammary tumor weight, indicating a lack of effect on tumor growth. However, the animals receiving 18 percent ethanol exhibited a marked reduction in metastasis to secondary mammary glands and to the lung. Tumor-associated increases in spleen size (i.e., splenomegaly) also were significantly reduced in the 18-percent ethanol group. Lung metastasis tended to be lower in mice consuming 5 percent ethanol, but there was no effect among those consuming 1 percent ethanol. Although the animals receiving 18 percent ethanol showed suppressed metastasis, they were less healthy compared with the

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other treatment and control groups as indicated by appearance, body condition, natural behavior, and provoked behavior. In vitro studies indicated that exposure of cells to 0.3 percent w/v ethanol did not affect 4T1.2 cell proliferation, colony formation, or invasion but did reduce cell migration twofold. Reduced cell migration may be related to changes in expression of CXCR4, a chemokine receptor that is important to migration and metastasis of breast and other cancers (Sarvaiya et al. 2013). Vorderstrasse and colleagues (2012) showed that expression of CXCR4 was suppressed 60 to 80 percent in tumors of mice consuming 18 percent ethanol; however, a correlation analysis indicated a lack of relationship between CXCR4 expression and metastasis. When the 4T1.2 cells were grown in 0.3 percent ethanol, however, CXCR4 expression was not altered, suggesting that expression was indirectly modulated by alcohol consumption in vivo.

Studies Using Human Tumor Cell Lines

Several studies examined the specific effects of ethanol on various aspects of disease progression in human breast cancer cell lines, including proliferation of cells. Singletary and colleagues (2001) found that incubation in 0.4 percent w/v ethanol increased cell proliferation in the estrogen receptor–positive MCF-7 and ZR75.1 breast cancer cells but not in the estrogen receptor–negative BT-20 and MDA-MB-231 cells. The effect of ethanol on MCF-7 cells also was correlated with increases in estrogen receptor alpha content. However, alcohol's effects are complex. When the MCF-7 cells were cultured

together with human skin fibroblasts in 0.4 percent ethanol for 72 hours, ethanol suppressed estrogen receptor alpha expression compared with untreated cells (Sanchez-Alvarez et al. 2013). Thus, the tumor micro-environment is important in determining estrogen-receptor status and the effects of alcohol on breast cancer. Whether this study is relevant to patients with breast cancer is not known. However, additional studies are warranted, because estrogen receptor–negative breast cancer generally is more aggressive, and patients have a worse prognosis than patients with estrogen receptor–positive breast cancer. Moreover, conversion from estrogen receptor alpha positive to estrogen receptor alpha negative can occur (Hoefnagel et al. 2010).

Other studies focused more on the invasion and migration in vitro of estrogen receptor–positive and estrogen receptor–negative human breast cancer cells. One study (Ma et al. 2003) compared the effects of incubation in 0.4 percent w/v ethanol for 48 hours on various breast cancer cell lines. This treatment increased invasion of the estrogen receptor–positive MCF-7 and T47D breast cancer cells as well as the estrogen receptor–negative HS578T, MDA-MB231, and MDA-MB435 cells. Similarly, incubation for 48 hours in 0.1 percent and 0.2 percent w/v ethanol stimulated invasion of estrogen receptor–negative SKBR3 and estrogen receptor–positive BT474 breast cancer cells. In contrast, ethanol exposure did not affect invasion of HB2, an immortalized normal human breast tissue cell line, or estrogen receptor–negative BT20 breast cancer cells. The effects of ethanol may depend not only on the specific cell line examined but also

on the ethanol concentration used. Thus, studies from another laboratory demonstrated that exposure to 0.1 percent, 0.2 percent, and 0.5 percent w/v ethanol enhanced invasion of T47D, MCF-7, and MDA-MB231 cells in a dose-dependent manner (Wong et al. 2011). Similarly, Aye and colleagues (2004) examined the effects of exposure for 48 hours to different ethanol concentrations on estrogen receptor–negative SKBR3 and estrogen receptor–positive BT474 breast cancer cells. For both SKBR3 and BT474 cells exposure to 0.1 percent and 0.2 percent w/v ethanol stimulated invasion. A higher dose of 0.4 percent w/v ethanol, however, inhibited invasion of SKBR3 cells and created mixed results for BT474, with one study (Aye et al. 2004) detecting no effect on invasion and another study (Xu et al. 2010) detecting increased invasion.

Invasive ability generally was related to the expression of ErbB2/neu, an epidermal growth factor (EGF) receptor that is amplified in 20 to 30 percent of breast cancer patients, with higher ErbB2/neu levels indicating higher risk of lymph node metastasis and poor prognosis. More detailed studies of the relationship between alcohol, ErbB2/neu, and invasion in the human breast cancer cell line T47D found that activation of the EGF receptor by addition of EGF did not significantly affect ethanol's ability to enhance invasiveness (Luo and Miller 2000). Conversely, prevention of ErbB2/neu production inhibited the ability of ethanol to increase migration (Luo and Miller 2000).

Numerous studies have sought to identify the mechanisms through which ethanol affects growth and migration of breast cancer cells or

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the molecules that mediate these effects. The results of such investigations include the following:

- Studies in MDA-MD231 and T47D cells determined that ethanol induced altered adhesion to fibronectin, increased development of lamellipodia,¹ increased phosphorylation of a protein called focal adhesion kinase, and increased production of several proteins (e.g., ribosomal protein L7a, smooth muscle myosin alkali light chain), all of which can promote cell migration (Xu et al. 2010; Zhu et al. 2001).
- Exposure of T47D cells to 0.5 percent w/v ethanol decreased mRNA expression of *NM23*, a known metastasis suppressor gene (Wong et al. 2011). However, gene expression levels of two other metastasis suppressors, *KTSS1* and *MKK4*, were increased by ethanol. Ethanol treatment also modulated the expression of more than 80 other genes associated with regulation of the extracellular matrix and cell adhesion. For example, ethanol induced expression of the gene encoding fibronectin receptor subunit integrin alpha 5 (*ITGA5*). This receptor activates signaling pathways supporting invasion, and reduced expression of *ITGA5* significantly inhibits invasion. The ethanol-induced expression of this gene was blocked by over-expression of the *NM23* gene, indicating an important relationship between these two genes in controlling invasion of T47D breast cancer cells.

- Studies also assessed alcohol's interactions with enzymes called matrix metalloproteinases (MMPs) such as MMP-2 and MMP-9 that are involved in the breakdown of the extracellular matrix in normal physiological processes as well as in disease processes, such as metastasis. Studies to date indicate no interactions between alcohol and MMPs (Aye et al. 2004; Ranuncolo et al. 2002).

Melanoma

In one of the first experiments conducted in melanoma, 6- to 8-week-old female CDBA/2F1 mice consumed water or 20 percent alcohol for 52 weeks before being inoculated in a leg with the Cloudman 8-91 melanoma tumor (Ketcham et al. 1963). When the tumors reached a size of 1.5 to 2.0 cm (about 28 days after tumor inoculation), the groups were divided in half, and half of each group had the primary tumor-bearing leg amputated. At 56 days after tumor implantation, the number and size of pulmonary metastases were recorded for all animals. The study detected no substantial or consistent effect of alcohol on the size or incidence of pulmonary metastases. However, surgical removal of the tumor-bearing leg decreased pulmonary metastasis in both ethanol-drinking and water-drinking groups.

Other studies in mice assessed the effects of acute and chronic alcohol consumption on tumor growth and metastasis using B16 melanoma and its more metastatic variants, B16F10 and B16BL6. An early study (Capel et al. 1978) found that mice given 10 percent ethanol in drinking water for 2 weeks before inoculation with B16 melanoma into the thigh showed

no altered tumor growth or metastasis compared with water-drinking controls. In another study (Tan et al. 2007), tumor growth and angiogenesis were examined in C57BL/6 mice implanted subcutaneously with B16F10 melanoma cells. The mice had access to regular drinking water and to 1 percent ethanol in their drinking water for 12 hours each per day for 4 weeks, with tumor cells being implanted during the second week of ethanol administration. Compared with animals who only drank water, those who had access to ethanol developed palpable tumors sooner and had 2.2 times greater tumor weights at the end of the study. Analysis of the tumors indicated an increase in VEGF mRNA and VEGF protein, as well as increased tumor angiogenesis. Moreover, another marker of angiogenesis, VEGF-R1 (Flt-1), also was found in a greater number of tumor cells and endothelial cells in the surrounding tissue from the ethanol group compared with the control group.

Wu and Pruett (1999) determined the effects of acute ethanol administration (5 or 6 g/kg body weight) given through a tube inserted into the mouth (i.e., by oral gavage) on melanoma metastasis. Ethanol administered to female B6C3F1 mice 1 hour before intravenous inoculation of B16F10 melanoma cells greatly increased lung metastasis. The number of metastases also was increased when administration of 6 g/kg ethanol occurred 4 or 10 hours after tumor inoculation but not when it occurred 6 hours before tumor inoculation. About half of the increase in lung tumor nodules seemed to be related to ethanol-induced corticosterone production in the adrenal glands. Experiments

¹ Lamellipodia are projections on the mobile edge of a cell that allow the cell to move across a substrate.

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analyzing whether ethanol-related inhibition of some aspect of NK-cell function also might contribute to the increased metastasis produced found no indication of significantly reduced NK-cell numbers or activity. However, further studies directed at determining the role of NK cells in the acute ethanol-induced increase in melanoma lung metastasis showed that ethanol inhibited experimental induction of NK-cell activity (in response to poly I:C), resulting in approximately a twofold increase in lung tumor nodules compared with control mice (Wu and Pruett 1999). Additional mechanistic studies indicated that a single dose of ethanol increased corticosterone levels (Collier et al. 2000) and also suppressed experimental induction of mRNA for a variety of cytokines (i.e., interferon alpha [IFN- α], interferon beta [IFN- β], IFN- γ , IL-6, IL-12, and IL-15) (Pruett et al. 2003). As a result, concentrations of IFN- α and IL-12 in the blood were decreased. Conversely, ethanol greatly enhanced the levels of IL-10 in the blood. However, these changes in serum cytokines were not mediated by corticosterone. Overall, these studies suggest that the increase in melanoma lung nodule formation after acute ethanol administration is complex and related to effects associated with some aspect of NK-cell inhibition, increased suppression of NK-cell activity by corticosterone, and corticosterone-independent effects on cytokines favoring tumor immune escape.

The effects of chronic alcohol consumption on tumor growth and metastasis of the highly invasive and spontaneously metastatic B16BL6 melanoma inoculated subcutaneously were studied in female C57BL/6 mice administered ethanol

in drinking water. In an initial study, consumption of 2.5 percent, 10 percent, or 20 percent w/v ethanol in drinking water for 6 to 8 weeks before tumor inoculation and continuing thereafter did not affect primary tumor growth (Blank and Meadows 1996). However, the animals receiving 20 percent ethanol in their drinking water exhibited consistently reduced survival, lower tumor weight, and lower final body weight compared with the other groups. Alcohol exposure also affected metastasis. All three ethanol-exposed groups had reduced metastasis to the axillary lymph nodes, with the 10-percent and 20-percent ethanol groups showing reduced lung metastasis, and the 20-percent ethanol group showing reduced superficial metastasis to the kidneys. Metastasis did occur, however, in the draining inguinal lymph nodes in mice consuming 20 percent weight per volume ethanol for 12 weeks (Zhang et al. 2011*b*).

More specific experiments sought to determine the effect in mice on metastasis of B16BL6 melanoma of short-term and long-term exposure to 10 percent and 20 percent w/v ethanol before and after intravenous inoculation into a tail vein or inoculation into the skin of the pinna of the ear² (Meadows et al. 1993*a,b*; Spitzer et al. 2000; Zhang et al. 2011*a*). These experiments showed that 10 percent w/v ethanol did not affect metastasis after intravenous tumor inoculation in female C57BL/6 mice consuming alcohol for 2 weeks or spontaneous metastasis in mice injected 1 week after initiating ethanol feeding. However, lung metastasis was inhibited if intravenous injection of tumor cells occurred at 4,

6.5, 7, and 12 weeks after initiation of 20 percent w/v ethanol. Similarly, spontaneous metastasis to the lung was significantly inhibited in mice injected with melanoma at 1, 4, 6.5, and 10 weeks of consuming 20 percent ethanol. Ethanol did not prevent spontaneous metastasis to the draining cervical lymph nodes.

The antimetastatic effect of ethanol on the B16BL6 melanoma tumor was confirmed after injection of tumor cells in male C57BL/6J mice that carry a gene for obesity and which had consumed 20 percent ethanol in drinking water for 10 weeks (Kushiro and Nunez 2012). Different groups were analyzed for lung tumor metastasis over a period of 16 to 21 days. All of the water-drinking animals had developed visible lung metastases at 16 days after tumor injection, whereas some of the ethanol-drinking mice did not develop lung metastases until 21 days. More-over, the numbers of lung metastases in the ethanol-drinking mice were significantly lower at 21 days.

The contribution of NK cells to the inhibition of metastasis was evaluated in mice consuming 10 percent or 20 percent w/v ethanol for 4 weeks (Meadows et al. 1993*a*). Whereas consumption of 10 percent ethanol did not alter the NK cells' ability to destroy other cells (i.e., decrease cytolytic activity), animals consuming 20 percent ethanol showed decreased NK cytolytic activity. And although experimental stimulation of NK cells could enhance their cytolytic activity 4.3-fold in the ethanol-drinking animals, compared with 2.6-fold in the control animals, overall cytolytic activity still was lower in the ethanol group than in the control group. Treatment of mice with an antibody against NK cells (i.e., anti-NK1.1 antibody)

² This method of tumor placement facilitates spontaneous metastasis.

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markedly decreased NK-cell cytolytic activity in both water- and ethanol-drinking animals. These same treatments were evaluated in mice injected with B16BL6 melanoma. Experimental stimulation of NK cells decreased the number of lung metastases in the water-drinking and 10-percent ethanol groups, but not in the 20-percent ethanol group. Inactivation of NK cells by administration of anti-NK1.1 antibody significantly increased lung metastases in the water-drinking and 10-percent ethanol groups, but not in the 20-percent ethanol group.

The data suggest that inhibition of NK-cell cytolytic activity in mice consuming 20 percent ethanol does not lead to enhanced metastasis following inoculation of B16BL6 melanoma. This lack of interaction between alcohol consumption and NK-cell cytolytic activity in B16BL6 melanoma lung metastasis was further confirmed in another strain of mice (i.e., beige mice) that naturally have low NK cytolytic activity (Spitzer et al. 2000). In other experiments to determine how ethanol decreases metastasis of B16BL6 melanoma, either isolated tumor cells grown in the presence of 0.3 percent ethanol or tumor cells from alcohol-consuming mice were inoculated into water-drinking mice (Blank and Meadows 1996). Mice that had received either tumor cells cultured in the presence of ethanol or derived from mice drinking 20 percent alcohol showed increased lung metastasis compared with control mice or those receiving tumor cells derived from mice drinking 10 percent ethanol. Thus, ethanol seems to increase the actual metastatic potential of melanoma cells. However, because alcohol drinking inhibits metastasis, there seem to be

other factors induced by ethanol that counter this metastatic potential.

Further research was directed at identifying factors involved in expression of the antimetastatic effect associated with chronic (12 weeks) consumption of 20 percent w/v alcohol consumption. Zhang and colleagues (2011*a*) found that chronic alcohol consumption increased the numbers of immune cells (e.g., NK, NKT, CD4⁺ T and CD8⁺ T cells) that produce IFN- γ . IFN- γ is an essential factor in the control of melanoma metastasis (Blankenstein and Qin 2003; Ikeda et al. 2002). IFN- γ also mediates the antimetastatic effects of chronic alcohol consumption, because mice that could not produce IFN- γ did not show this effect (Zhang et al. 2011*a*). The investigators attempted to determine whether one immune cell population was more important than another in producing the antimetastatic IFN- γ . However, through a series of specific cell depletion experiments, they found that it did not matter which cell type produced the IFN- γ .

Other investigators (Kushiro and Nunez 2012) found that B16BL6 melanoma cells grown in 0.1 percent, 0.2 percent, or 0.5 percent ethanol showed considerably reduced cell invasion and, at the highest ethanol concentration, reduced cell motility and anchorage-dependent growth. Ethanol did not affect cell proliferation, apoptosis, or necrosis of the melanoma cells. In addition, the highest ethanol dose altered the expression of several genes that play prominent roles in regulating melanoma metastasis (i.e., the IL6, Nfkb, snail1, E-cadherin, Kiss1, Nm23-m1, and Nm23-m2 genes). These changes also could contribute to the antimetastatic effect of alcohol on melanoma.

Summary

Animal models have yielded some insights into the effects of alcohol on tumor growth, survival, and metastasis of different cancers, including breast cancer, lung cancer, liver cancer, and melanoma. However, because cancer is a collection of many different diseases and subtypes, each cancer or cancer subtype might not respond similarly to alcohol, as is evident from the research discussed here.

The relationship between alcohol and tumor progression is complex. Tumor metastasis involves many steps and also is controlled by many different signaling pathways. Because each cancer is unique, the specific connections between alcohol and the steps involved in cancer progression also by nature are complex. Thus, it is important to relate experimental results to specific types/subtypes of cancer as well as the amount of alcohol consumed, the route of alcohol administration, and the duration of alcohol administration. Clearly, more studies are needed to define the effect of alcohol on tumor progression and to determine the mechanisms associated with individual cancers and subtypes.

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Alcohol and Viral Hepatitis

Role of Lipid Rafts

Angela Dolganiuc, M.D., Ph.D.

Both alcohol abuse and infection with hepatitis viruses can lead to liver disease, including chronic hepatitis. Alcohol and hepatitis viruses have synergistic effects in the development of liver disease. Some of these involve the cellular membranes and particularly their functionally active domains, termed lipid rafts, which contain many proteins with essential roles in signaling and other processes. These lipid rafts play a central role in the lifecycles of hepatitis viruses. Alcohol's actions at the lipid rafts may contribute to the synergistic harmful effects of alcohol and hepatitis viruses on the liver and the pathogenesis of liver disease.

Key words: Alcohol abuse; alcohol use and misuse; alcohol disorder; liver; liver disease; hepatitis; hepatitis B virus; hepatitis C virus; lipid rafts

Alcohol is the most used and abused psychoactive drug worldwide. Alcohol use and misuse, including alcohol use disorder, can have devastating effects and account for 5.9 percent of deaths and 5.1 percent of the global burden of disease and injury, thereby also imposing a significant social and economic burden on society (World Health Organization 2015). Moreover, treatments for alcohol abuse have shown limited effectiveness (Grant et al. 1988; National Institute on Alcohol Abuse and Alcoholism 1998). Alcohol use disorder is a systemic disease that affects all organs and systems. Evidence suggests that risk of alcohol-related organ damage occurs with excessive alcohol intake, which is defined as binge drinking or heavy drinking. According to the National Institute on Alcohol Abuse and Alcoholism, binge drinking is defined as a pattern of alcohol consumption that brings the blood alcohol concentration (BAC) level to 0.08 percent or more. This pattern of drinking usually corresponds to consumption of 5 or more drinks on a single occasion for men and 4 or more drinks on a single occasion for women, generally within about 2 hours. Heavy drinking typically is defined as consuming 15 drinks or more per week for men and 8 drinks or more per week for women (Centers for Disease Control and Prevention [CDC] 2014). The liver is particularly susceptible to alcohol-induced damage. However, although many chronic heavy drinkers develop alcoholic liver disease (ALD), no consump-

tion levels have been identified that predictably result in ALD. Factors that influence the susceptibility to ALD include gender, co-exposure to other drugs, genetic factors that either favor the development of addiction or affect alcohol-metabolizing enzymes, immunological factors, nutritional status, and infection with viruses targeting the liver (i.e., hepatotropic viruses).

Hepatitis viruses, and particularly hepatitis B virus (HBV) and hepatitis C virus (HCV), are responsible for most cases of chronic hepatitis in the United States. In 2013, almost 20,000 new cases of HBV infection and almost 30,000 new cases of HCV infection were estimated to occur in the United States (CDC 2015a). Worldwide, approximately 350 to 400 million people, or about 5 percent of the population, are chronically infected with HBV and about 180 million people, or 2 percent of the population, with HCV (El-Serag 2012). In chronic alcoholics, the prevalence of HCV infection as indicated by the presence of anti-HCV antibodies is higher than in the general population (Takase et al. 1993). Co-occurring viral hepatitis and alcohol use disorder adversely affect disease course and are associated with increased mortality and death at an earlier age (Kim et al. 2001; Sagnelli et al. 2012; Tsui et al. 2006; Wiley et al. 1998). The most serious complication of ALD is liver cirrhosis, which often progresses to hepatocellular carcinoma (HCC); indeed, about 20 percent of heavy drinkers develop cirrhosis during their lifetime, and this risk is much increased in the presence of co-occurring viral hepatitis (El-Serag 2012; Ishak et al. 1991). End-stage liver disease from viral hepatitis, together with ALD, is the main reason for liver transplantation in the United States (El-Serag 2012).

The mechanisms how alcohol and viral hepatitis together accelerate liver disease have been researched extensively over the last several decades. It is becoming clear that alcohol exposure, infection with hepatitis viruses, and the host's defense mechanisms against these offenders combine to contribute to the pathogenesis of liver disease and thus could be targets of therapeutic interventions. New antiviral drugs against HCV have been developed in recent years, and reasonably effective HBV treatments also are available (American Association for the Study of Liver Diseases 2015; Lok and McMahon 2009). Yet many patients either do not qualify for or cannot afford newer antiviral treatments. Further, exposure to alcohol, whether acute or chronic, light or heavy, may preclude eligibility for treatment of viral hepatitis. Additionally, many patients cannot achieve abstinence from alcohol or experience recurrent relapse (Becker 2008). Therefore, novel approaches are needed for the

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diagnosis and treatment of patients with coexisting alcohol use and viral hepatitis.

This article reviews some of the mechanisms underlying alcohol-induced liver injury and also explains the contributions of hepatitis viruses to liver disease, as well as the synergistic effects of alcohol and hepatitis virus infections on the liver. This discussion particularly focuses on the roles that the cellular membranes, and especially membrane domains called lipid rafts, play in these processes. Both alcohol and viral infections influence the functions of lipid rafts and the functional proteins they contain, which may exacerbate disease progression. The specific mechanisms underlying the effects of alcohol and hepatitis viruses on the cellular membranes and their contribution to liver disease pathogenesis, however, still remain to be fully elucidated.

Alcohol-Induced Liver Injury

Liver injury in ALD occurs as a result of multiple synergistic mechanisms, including impaired function of the main parenchymal liver cells (i.e., hepatocytes), imbalanced local (i.e., nonparenchymal) and systemic immune responses, and altered cross-talk between parenchymal and nonparenchymal cells in the liver.

Alcohol has diverse effects on the hepatocytes that result in significant disturbances of the cells' abilities to synthesize needed molecules and detoxify harmful products (Van Horn et al. 2001; Videla et al. 1973), pronounced deficits in antioxidant levels (Fernandez-Checa et al. 2002; Lauterburg and Velez 1988), and marked oxidative cellular stress (Tsukamoto 1993). These effects, together with additional changes in hepatocyte metabolism, lead to the accumulation of lipids in the alcohol-exposed hepatocytes (i.e., hepatic steatosis). The affected cells consume oxygen inefficiently, have reduced detoxifying ability, fail to synthesize needed compounds, and are more likely to undergo apoptosis (Nanji and Hiller-Sturmhoefel 1997). As a result of all of these changes, the cells also become more susceptible to other harmful influences, such as infections with hepatotropic viruses and dietary insufficiencies. Finally, alcohol exposure greatly enhances tumorigenesis in hepatocytes (Morgan et al. 2004).

Alcohol exposure also affects local immune responses by both hepatocytes and resident and nonresident immune cells. Hepatocytes are the first cells to encounter hepatotropic viruses, and activation of their cytokine signaling systems—including proinflammatory cytokines such as tumor necrosis factor alpha (TNF- α), interleukin (IL)-1 and IL-6, and interferons (IFNs)—is key to the initiation of immune responses. Alcohol exposure has diverse effects on these immune responses. On the one hand, alcohol suppresses intracellular expression of type I IFNs (IFN- α/β) in human hepatocytes by reducing the expression of key positive regulators of type I IFN signaling pathways and inducing the expression of key negative regulators of IFN- α/β signaling (Plumlee et al. 2005; Ye et al. 2010). On the other hand, alcohol-exposed hepatocytes increase the expression of

proinflammatory TNF- α (Mandrekar 2007; Plumlee et al. 2005). In addition, alcohol exposure results in differential activation of IL-1 pathways in hepatocytes versus other nonparenchymal cells (e.g., Kupffer cells). Thus, certain active molecules (i.e., the active fragment of caspase-1 and IL-1 β) are elevated only in liver immune cells but not in alcohol-exposed hepatocytes. Innate immune pathways in hepatocytes also may regulate hepatocyte steatosis and hepatocellular injury. A signaling molecule called IRF3, which is an essential component of innate immunity and is required for hepatocyte apoptosis, may play a unique role in the processes leading to hepatocyte apoptosis in ALD and tying together alcohol-induced liver inflammation, metabolic disturbances, and cell death (Petrasek et al. 2013).

Alcohol-induced cross-talk between parenchymal and nonparenchymal liver cells (e.g., Kupffer cells) is another key component of liver disease (Cohen and Nagy 2011). The activation of Kupffer cell-specific signaling pathways involving innate immune molecules called toll-like receptors (TLRs), and in particular TLR4, is emerging as a required step in the progression of liver disease from steatosis to steatohepatitis in ALD. In addition, TLR4-mediated activation of Kupffer cells seems to be important for the formation of scar tissue (i.e., fibrogenesis) in the liver after chronic alcohol treatment (Adachi et al. 1994; Inokuchi et al. 2011). Other TLRs also influence the development of ALD. Thus, alcohol exposure augments signaling via TLR8 and TLR7, thereby inducing both IL-10 and TNF- α and downgrading IFN expression in myeloid cells (Pang et al. 2011). These effects may contribute to the persistent inflammation and impaired antiviral responses in ALD. Kupffer cells seem to govern the course of ALD, especially in the early stages of the disease, because deletion of these cells protects against alcohol-induced liver injury. The mechanisms underlying these effects are not fully understood but likely are multifactorial and include cell cross-talking between innate immune cells and other liver cells, such as stellate cells (Adachi et al. 1994). Stellate cells, in turn, can develop into myofibroblasts that play a central role in alcohol-induced fibrogenesis. Alcohol exposure triggers stress signals from hepatocytes that can activate myofibroblasts, which favor excess type 1 collagen synthesis and lead to progression of fibrosis (Siegmund and Brenner 2005). Additionally, TLR4 is a key molecule involved in signaling to, from, and inside of stellate cells, suggesting that innate immune pathways also contribute to this stage of ALD (Paik et al. 2003; Seki et al. 2007).

Hepatitis Viruses

Hepatitis viruses are a heterogeneous group of five unrelated hepatotropic viruses that cause inflammation of the liver. They include hepatitis viruses A, B, C, D, and E. Of

these, HBV and HCV are clinically most relevant in Western countries.¹

HBV

HBV reproduces exclusively in hepatocytes. Each HBV particle contains a 3.2-kb open circular DNA encapsulated in a protein shell made of three envelope proteins and the enzymes HBV polymerase and cellular protein kinase C alpha (PKC α) (Wittkop et al. 2010). This complex is called the core particle or nucleocapsid. The nucleocapsid is further surrounded by a membrane derived from the previous host cell. When infecting cells, the viral envelope interacts with liver-specific receptors, leading to uptake into the cell (i.e., endocytosis) of the virus particle and release of the nucleocapsid (see figure 1). The nucleocapsid is transported to the nucleus, where the HBV genome is released and then transcribed into mRNAs that gives rise to three envelope proteins. In parallel, another viral mRNA is translated in the cytosol into the HBV core protein and viral polymerase. Then, the viral mRNA and the various viral proteins assemble to immature core particles in a membrane-enclosed cell structure called the Golgi apparatus. The HBV genomes mature within these core particles via reverse transcription of the pre-genomic mRNA to DNA. As soon as the mature virus is assembled, the viral particle release begins. Each virus particle is packaged into a cellular membrane coat from the Golgi apparatus and then released from the host cell, taking a bit of the cell membrane with it as an envelope.

Immune cells sense virus-infected cells, inducing a cytotoxic immune response. This response, combined with ongoing strong HBV DNA replication, often results in persistent, strong inflammatory disease (i.e., hepatitis), progressive fibrosis of the liver, and potentially in HCC (El-Serag 2012; Koziel 1998).

HCV

HCV is a positive-sense, single-stranded RNA virus that, like HBV, is thought to reproduce exclusively in hepatocytes (Paul and Bartenschlager 2014).² HCV replicates in humans and high-level primates; it causes acute infections and has very high propensity to progress to chronic infection. The HCV viral particle includes the HCV RNA genome, the core, and an envelope made up of two glycoproteins (i.e., E1 and E2), which are key to the initial viral attachment to its cellular receptor/co-receptors (Flint and McKeating 2000; Rosa et al. 1996). Numerous molecules can serve as HCV receptors, such as scavenger receptor class B

type I, low-density lipoprotein receptors, CD81, claudin-1, occludin, epidermal growth factor receptor, and Niemann-Pick C1-like 1 cholesterol absorption receptor (for a review, see Lindenbach and Rice 2013). Following attachment to the entry receptors, HCV is internalized into the host hepatocyte via endocytosis (Bartosch et al. 2003; Blanchard et al. 2006) and the RNA genome is released into the cytoplasm (see figure 2). The HCV RNA serves as template for the translation of a single large precursor protein that is processed further into 10 individual viral proteins. The translation, folding, processing, and function of these viral proteins depend on a specific intracellular structure in the hepatocytes called a membranous web, which also hosts viral RNA replication to generate new HCV genomes and assists in the assembly of new infectious viral particles (Chao et al. 2012). The assembly and release of these virus particles is closely linked to lipid metabolism (Paul et al. 2014). Thus, the lipid composition of the viral envelope is dependent on cholesterol biosynthetic pathways and resembles several types of cholesterol (i.e., low-density lipoprotein and very-low-density lipoprotein, with associated apolipoprotein E and/or B). In fact, the virus particles share the outer lipid coat with certain structures (i.e., lipid rafts, which will be discussed below) in the cell membrane surrounding the host hepatocytes (Chang et al. 2007; Gastaminza et al. 2008; Merz et al. 2011; Miyanari et al. 2007).

HCV replication is kept in check by the combined efforts of innate and adaptive (i.e., cellular and humoral) immune responses. In some people, the acute infections are mild and with limited clinical manifestations. In about 70 percent of infected individuals, however, the HCV infection is not cleared and a chronic infection is established. Possible mechanisms contributing to chronic HCV infection include failure of several types of immune cells, including natural killer cells, dendritic cells, and CD4 T cells (Dolganovic et al. 2012; Koziel 2005; Szabo and Dolganovic 2008). Persistently high viral replication that leads to steatotic transformation of hepatocytes and the subsequent death of some of the infected cells as well as an exaggerated inflammatory response to the infection can promote the development of fibrosis and induce disease progression from chronic hepatitis to end-stage liver disease and HCC.

Synergistic Effects of HBV/HCV Infection and Alcohol Abuse on Liver Disease

HBV and Alcohol Abuse

The prevalence of drinking in the general population is high, with more than 70 percent of people over age 18 in the United States reporting that they drank alcohol in the past year (National Institute on Alcohol Abuse and Alcoholism 2015). Accordingly, a significant portion of patients with chronic HBV infection are believed to have concomitant ALD. Alcohol use disorder is one of several conditions that

¹ The hepatitis A virus usually only causes self-limiting infections. Hepatitis D virus requires the helper function of HBV to replicate and thus hepatitis D virus infections only occur in people infected with HBV. Hepatitis E virus primarily is found in Asia and Africa and is less common in Europe and the Americas. Chronic hepatitis E virus infection only has been observed in people receiving immunosuppressant treatment after an organ transplant. The effects of alcohol on hepatitis A, D, and E viruses are largely unknown.

² Some reports suggest that viral replication outside the liver may also occur (Revie and Salahuddin 2011).

may co-occur with chronic HBV infection and contribute to rapid progression of liver disease, increased likelihood of tumorigenesis, and accelerated progression of HCC (Ribes et al. 2006; Sagnelli et al. 2012). Thus, heavy alcohol intake in chronic HBV-infected patients is associated with a higher risk for developing liver cirrhosis; the prevalence of cirrhosis is about 2.5 times higher in patients with co-occurring HBV infection and alcohol abuse than in patients with only one of these conditions (Sagnelli et al. 2012). The prevalence of HCC and liver-related mortality also is higher in people with chronic HBV infection and concurrent heavy alcohol

consumption (Hughes et al. 2011; Niro et al. 2010). Other co-occurring conditions that increase morbidity and mortality associated with chronic HBV infection and accelerate disease progression include infection with HCV, hepatitis D virus, and HIV (Ribes et al. 2006; Sagnelli et al. 2012).

Other studies found that alcohol promotes the presence of HBV particles in the blood (i.e., HBV viremia). For example, ethanol-fed mice showed up to sevenfold increases in the levels of HBV surface antigens (i.e., HBsAg) and viral DNA in the blood compared with mice fed a control diet. In addition, the ethanol-fed mice had elevated levels of

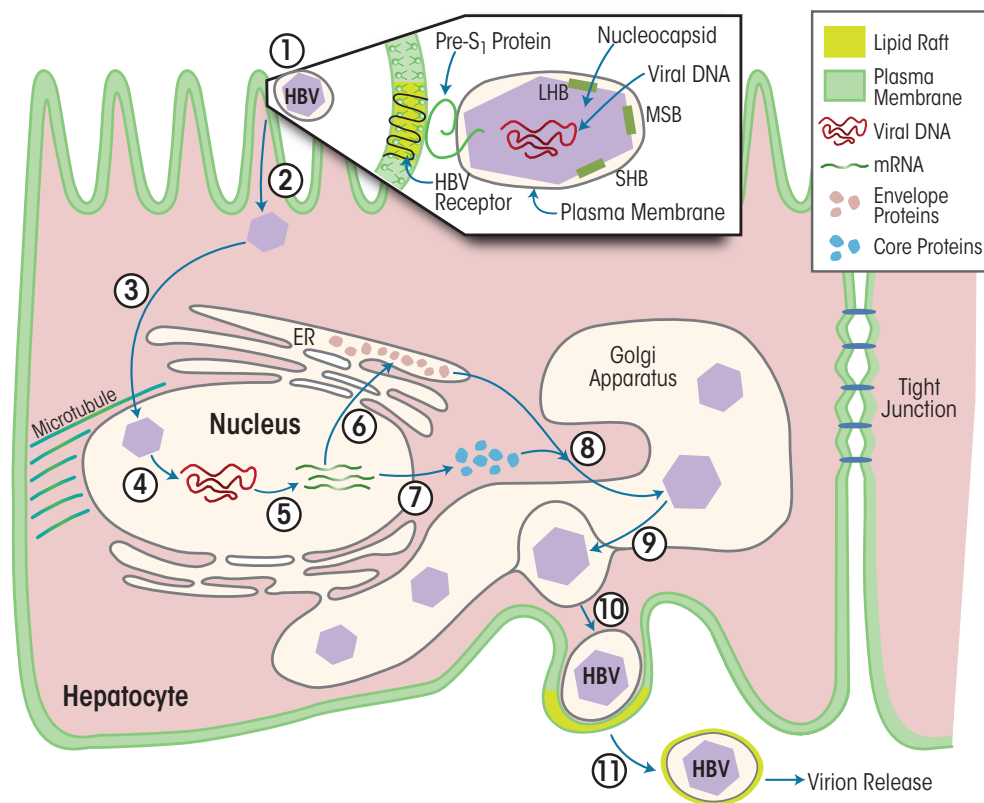


Figure 1 The life cycle of hepatitis B virus (HBV) and the role of lipid rafts. The HBV particles consist of an inner core particle (i.e., the nucleocapsid) that is made up of several envelope proteins, core proteins, and viral DNA. It is surrounded by a membrane derived from the previous host cell. (1) The virus particle attaches, presumably via the Pre-S1 protein, to unknown HBV receptors in the membrane of the cell. These receptors are located in membrane regions with characteristic lipid composition (i.e., lipid rafts). (2) The virus particle is taken up into the cell via a process called endocytosis and the nucleocapsid is released. (3) The nucleocapsid is transported into the nucleus, where (4) the DNA is released and (5) transcribed into mRNAs. (6) Some of the mRNAs are translated into the envelope proteins in the endoplasmic reticulum (ER). (7) Other mRNAs are translated into core proteins in the host cell's cytosol. (8) Envelope proteins, core proteins, and mRNA move to the Golgi apparatus and assemble into immature core particles. (9) The immature particles mature in the Golgi apparatus, including reverse transcription of viral mRNA into DNA. (10) The mature particles become surrounded by the Golgi apparatus membrane. (11) The mature particles are released from the host cell, taking a piece of cellular membrane with them as an envelope, including lipid raft pieces.

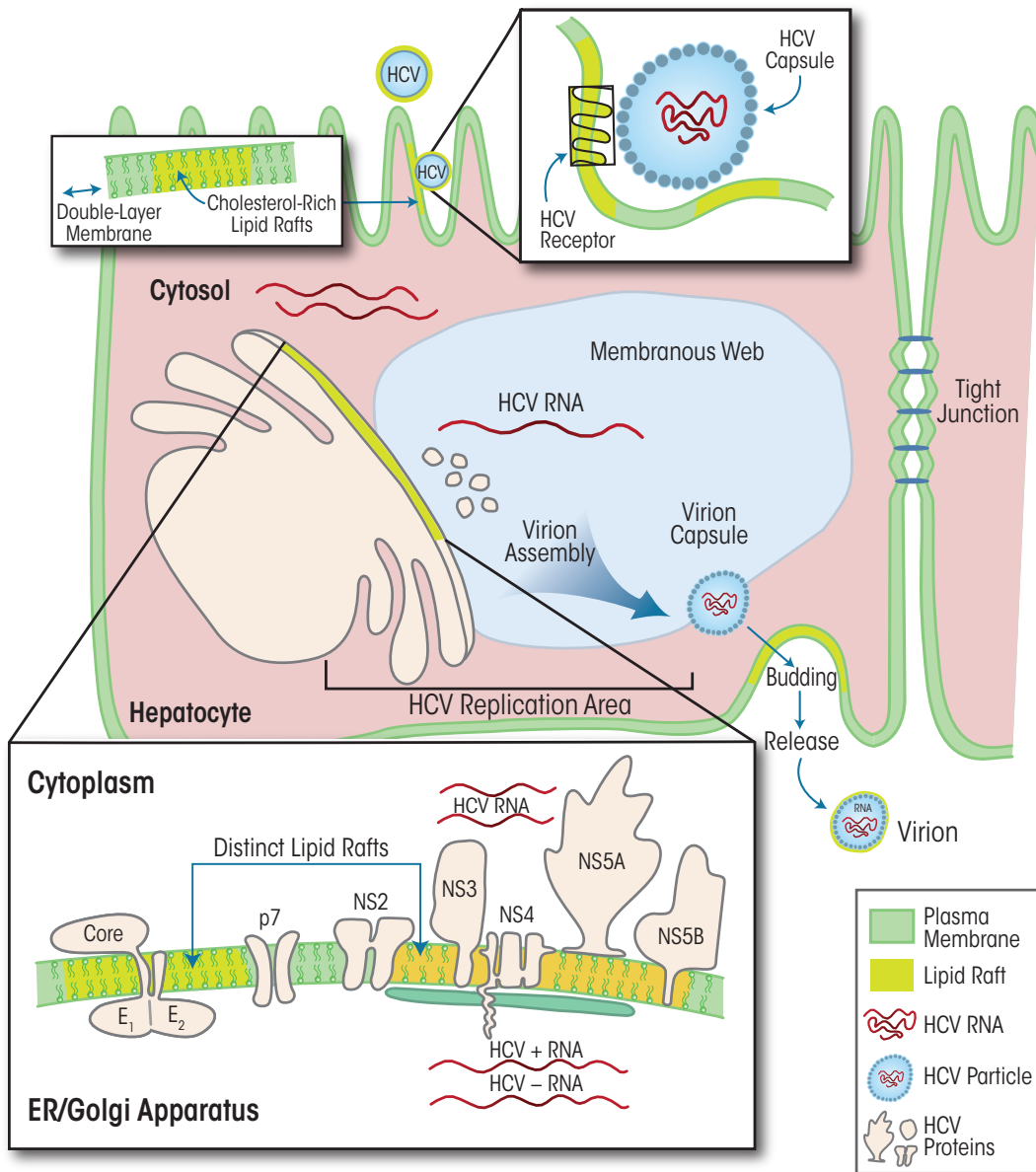


Figure 2 The life cycle of hepatitis C virus (HCV) and the role of lipid rafts. HCV particles attach to receptors in lipid-raft regions of the hepatocyte membrane, and the virus particles are taken up into the cell via endocytosis. The viral RNA is released and serves as template for the production of viral proteins at a structure called the membranous web, which also includes the membranes surrounding the endoplasmic reticulum (ER) and Golgi apparatus. The membranous web also is the site of assembly of new virus particles. During assembly and subsequent release of the viral particles, the particles obtain pieces of the cellular membrane as an outer envelope that shares the lipid composition of the membrane, particularly of the lipid rafts.

HBV RNA as well as increased expression of various viral proteins (i.e., surface and core proteins) and X antigens in the liver (Larkin et al. 2001).

HCV and Alcohol Abuse

The prevalence of chronic HCV infection is significantly elevated among people with alcohol use disorder (Fong et al 1994; Novo-Veleiro et al 2013) compared with the general population (prevalence of 1 to 2 percent) (CDC 2015*b*). Variables independently associated with HCV infection include female gender, injection drug use, and the presence of ALD (Novo-Voleiro et al. 2013). At the same time, patients with HCV infection have a higher prevalence of alcohol abuse and a longer duration of alcohol consumption compared with the general population (Degos 1999; Nevins et al. 1999; Pessione et al. 1998).

Chronic HCV infection results in the development of HCC in about 1 to 3 percent of patients after 30 years (Grebely and Dore 2011), contributing to the morbidity associated with HCV. The rate of HCC is substantially higher in people with HCV-related cirrhosis, reaching 2 to 4 percent per year in the United States, and even higher rates of up to 7 percent have been reported in Japan. Risk factors for the development of HCV-related HCC include male gender, age older than 55 years, and high levels of alcohol consumption (Grebely and Dore 2011; Hajarizadeh et al. 2013; Kim and Han 2012). Alcohol intake of 40 grams ethanol per day or more is associated with more rapid progression of liver disease associated with chronic HCV infection, including a more rapid increase in fibrosis and a doubled incidence of cirrhosis compared with patients with lower consumption levels (Wiley et al. 1998). Similarly, the risk of developing HCC is twice as high in patients with chronic HCV infection who drink heavily. Even small amounts of alcohol lead to an increased level of serum HCV RNA in patients with HCV infection (Cromie et al. 1996).

Alcohol, Cellular Membranes, and Lipid Rafts

Biological membranes surround the cells and create compartments within the cells, such as the endoplasmic reticulum and Golgi apparatus. Current models view cellular membranes as tri-dimensional lipid–protein complexes that are easily disturbed. Thus, even small stimuli, such as changes in pH, ion environment, or binding of a molecule to a protein receptor, can lead to profound changes in the composition, function, and integrity of the cellular membrane. Not surprisingly, therefore, alcohol also can alter the state of the cellular membranes and may thereby affect cellular function. At the same time, proteins embedded in the cellular membranes may serve as receptors and points of entry for viruses, such as HBV and HCV.

The specific structure and function of hepatocyte membranes contributes to the ability of hepatitis viruses to infect the cells. In contrast to nonparenchymal liver cells,

hepatocytes are polarized cells—that is, they have two clearly defined ends (i.e., an apical and a basolateral side), which is reflected in the membrane structure. Thus, the apical and basolateral membranes each have characteristic components that cannot mix, partially because the two cellular domains are separated by structures called tight junctions that also ensure the connection between a hepatocyte and its neighboring cells. The composition of polarized membranes differs between both ends of the cell with respect to both protein composition and lipid repertoire. Additionally, the membranes of both polarized and nonpolarized cells can be divided into lipid rafts and non-lipid-raft domains. Lipid rafts are membrane sections ranging in size from 10 to 200 nm that are enriched in specific lipids (i.e., sterols, sphingolipids, or ceramide). The specific structure of these lipid rafts promotes protein–protein and protein–lipid interactions; in addition, many cellular processes occur in these membrane regions. In both hepatocytes and other cell types, the overall protein concentration in the lipid rafts is relatively low, although certain proteins are highly concentrated in these membrane sections (Prinetti et al. 2000). The association with a lipid raft can influence the function of a protein (Paik et al. 2003; Pike 2006; Sonnino and Prinetti 2013). For example, proteins within lipid rafts are less able to move to other membrane areas, which favors more stable interactions with other proteins in the same domain. Thus, the activation of a cellular protein that serves as a receptor in a lipid raft facilitates clustering of the receptor with its co-receptors. Because the outer envelope of animal viruses such as HBV and HCV is derived from the host membranes, the lipid composition of the viral envelope resembles that of the membrane from which the virus buds (Laine et al. 1972). The cellular lipids and lipid rafts obtained from the host often modulate the membrane fusion between virus and host cell that is mediated by viral proteins (Teissier and Pécheur 2007) and therefore could become important targets for efforts to disrupt the viral life cycle. In general, the viruses seem to attach primarily to membrane areas containing lipid rafts; it remains to be determined whether viruses gain infectivity advantage if they attach to lipid rafts located in the apical or basolateral domain of the cell (Lindenbach and Rice 2013).

Influence of Alcohol on Cellular Membranes and Lipid Rafts

The effect of alcohol exposure on cellular membranes, and lipid rafts in particular, depends on the cell type and its activity state as well as on the alcohol concentration and duration of exposure. It is important to remember, however, that alcohol's effects on the cellular membrane do not occur in isolation; rather, they are part of alcohol's global effects on the cell and on the tissue as a whole. In addition, liver-cell membranes may adapt to alcohol consumption (Rottenberg 1991), although it is difficult to determine which of those changes represent an adaptation and which represent pathological changes. Whether the adaptive changes of membrane

composition, structure, and function delay or accelerate the onset of the pathological changes in the liver of human alcoholics also still is unclear.

Alcohol's effects on cellular membranes can be indirect or direct (see figure 3). Indirect effects include, for example, the binding of acetaldehyde—which is a major metabolic product of ethanol and is found in high concentrations in the serum during alcohol abuse—to hepatocyte membranes. The acetaldehyde affects the structure of the cellular membrane, which leads to disruption of tight junctions, increased immune recognition of certain antigens, cell damage, DNA damage, and mutagenesis (Setshedi et al. 2010; Thiele et al. 2008). Alcohol's direct effects on the cellular membrane can be subdivided further into effects on the lipids and effects on the protein components. Of these, alcohol's effects on protein function probably have the greatest impact on both

parenchymal and nonparenchymal liver cells. They occur during both the acute and the chronic phase of alcohol exposure and lead to significant functional impairment of the cells, which can cause cell death, tumorigenesis, altered intercellular communication, and increased susceptibility to additional insults, including viral infections. All of these can contribute to liver dysfunction. Studies have demonstrated that alcohol can impair the functions of proteins in cellular membranes and lipid rafts in liver cells in multiple ways, including actions on lipid-raft-associated signaling pathways (Dai and Pruett 2006; Dolganiuc et al. 2006). However, these studies have focused primarily on the outer cellular membrane and its lipid rafts; the effects of alcohol on intracellular lipid rafts (Chao 2012) remain to be characterized. Nevertheless, it is clear that as a result of the complex actions of alcohol on lipid-raft-associated signaling, the

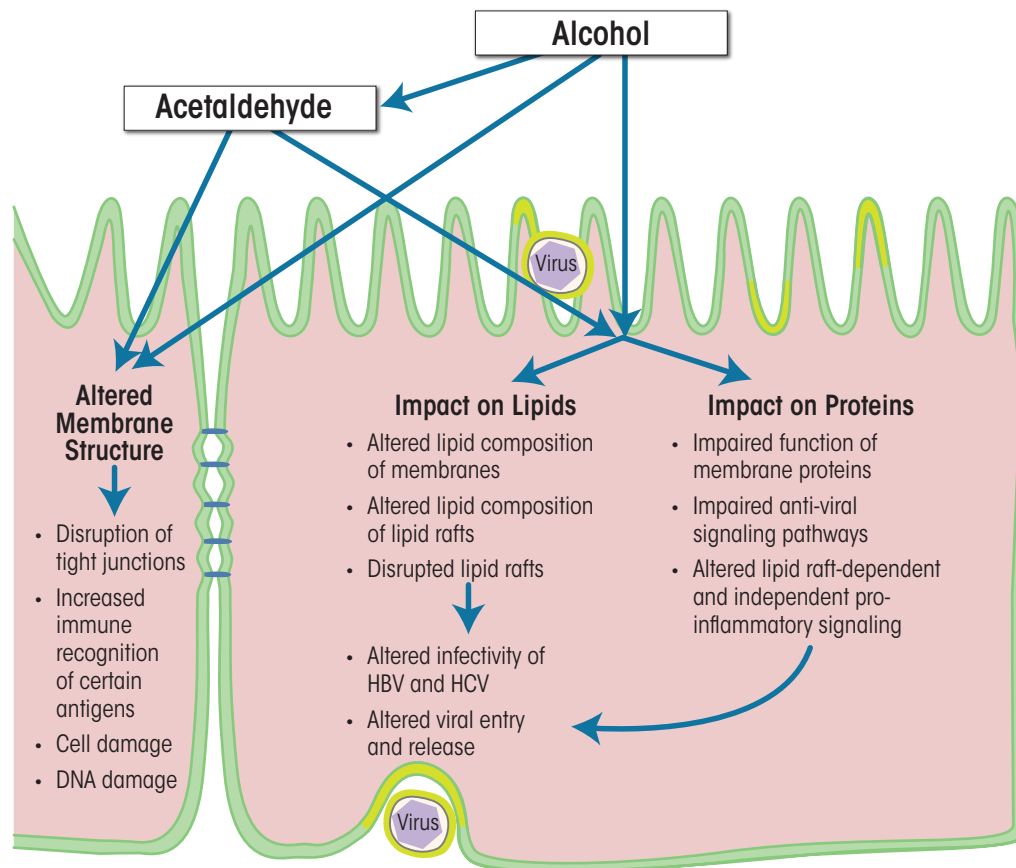


Figure 3 Alcohol's effects on virus-infected hepatocytes. Alcohol may exert its effects both directly and indirectly. Indirect effects are, for example, related to the actions of the alcohol metabolite, acetaldehyde. Alcohol can directly affect both lipids and proteins in the cell. Through a variety of mechanisms, these effects may alter the infectivity of and the cell's response to HBV and HCV, affecting both viral entry into the cells and release of viral particles from the cells.

liver cells are more likely to create a proinflammatory milieu and downregulate their antiviral defense mechanisms. For example, studies have detected interference with signal transduction systems (Aliche-Djoudi et al. 2011; Dolganiuc et al. 2006; Nourissat et al. 2008) as well as enhancement of oxidative stress (Nourissat et al. 2008). Additionally, the cells spend excessive resources on efforts aimed at maintaining cellular homeostasis (e.g., remodeling the cellular membrane or re-ordering metabolic priorities) and on mechanisms to counteract cell death (Dolganiuc et al. 2012; Donohue and Thomes 2014). More importantly, exposure to alcohol, especially prolonged exposure, increases the liver cells' vulnerability to second hits, including hepatitis viruses.

Effects of Alcohol Abuse and Hepatitis Virus Infection on Cellular Membranes

As described above, the cellular membranes and lipid rafts are important targets of alcohol's actions in the liver (Lieber 1980; Tsukamoto 1993) and are key in many aspects of alcohol-induced liver-cell dysfunction. Concurrent infection with HBV, HCV, and/or other viruses exacerbates alcohol's detrimental effects on liver function and leads to an accelerated course of liver disease (Ribes et al. 2006; Tsui et al. 2006). The mechanism underlying the synergistic effects of hepatitis viruses and alcohol, and particularly the role of cellular membranes and lipid rafts, are not yet fully understood.

For HBV, alcohol's effects on the membranes are relevant because the virus acquires its envelope from the membrane of the endoplasmic reticulum (Gerlich 2013). This envelope has a relatively high cholesterol content (Satoh et al. 2000), which is a key determinant of virus infectivity (Funk et al. 2007, 2008; Stoeckl et al. 2006). Thus, interference with the cellular membrane and lipid rafts during the viral life cycle, whether it is at the level of the host hepatocyte or cholesterol depletion from the virus membrane, has detrimental effects on the virus. Specifically, cholesterol-poor HBV virions take longer time to attach to, enter, and migrate inside the hepatocytes and are more likely to be cleared from the cells once they do enter (Funk et al. 2008). Alcohol exposure can distinctly alter the lipid composition of cellular membranes in general and lipid rafts in particular (Dolganiuc et al. 2006) and may thereby influence HBV infectivity. However, the precise effect of alcohol on the various steps of the HBV lifecycle remains largely unknown.

In addition to directly affecting both the virus and host parenchymal liver cells, alcohol influences anti-HBV immunity—an effect that also involves the cellular membrane as well as lipid rafts. HBV is known to interfere with normal T-cell function, and specifically with the T-cell receptor (TCR) that is responsible for recognizing and interacting with foreign antigens, thereby initiating an immune response. Thus, during HBV infection, the virus can impair the translocation of various components of the TCR (e.g., CD3f, ZAP-70, and Grb2) to lipid rafts; this is

a hallmark of defective adaptive immune responses during chronic HBV infection (Barboza 2013; Larkin et al. 2001). Similarly, lipid-raft-dependent TCR localization and function are altered when adaptive immune cells are exposed to alcohol (Ishikawa et al. 2011). In particular, ethanol inhibits lipid-raft-mediated TCR signaling in CD4 T cells, resulting in suppression of IL-2 production (Ghare et al. 2011). Thus, alcohol acts synergistically with HBV to limit antiviral immunity. The consequences of alcohol's effects on the TCR of HBV- and HCV-infected individuals are largely unknown but remain of high interest because adaptive immunity plays an important role in viral clearance (Barve et al. 2002; Heim and Thimme 2014; Loggi et al. 2014).

Compared with HBV, the life cycle of HCV depends on cellular membranes and lipid rafts to an even greater extent. HCV attaches to the cellular membrane and binds to a variety of cellular receptors that also serve as signaling molecules or receptors for other host proteins; most of these receptors reside in lipid rafts or are recruited there upon viral sensing and signaling. For example, one study found that compared with control cells, lipid rafts of cells expressing an HCV-1b genome showed altered levels of 39 proteins, including new or increased expression of 30 proteins and decreased expression of 9 proteins (Mannova and Beretta 2005). These alterations also affect a signaling pathway called the N-ras-PI3K-Akt-mTOR pathway (Peres et al. 2003; Zhuang et al. 2002); modulation of this pathway is one of the strategies by which HCV inhibits apoptosis and prevents elimination of infected cells. Alcohol can target these signaling platforms and may exert detrimental effects on lipid rafts that contain several putative HCV receptors, which may affect HCV replication and survival of HCV-infected cells. Thus, alcohol has been shown to affect the PI3K-mTOR pathway in non-liver cells (Li and Ren 2007; Umoh et al. 2014). However, the effect of alcohol on the PI3K-mTOR pathway in parenchymal and nonparenchymal liver cells remains to be determined.

Alcohol also adversely affects many of the immune cells and pathways that are considered key to antiviral immunity to HCV. Thus, alcohol exposure enhances signaling via TLRs that mediate inflammation and impairs signaling via TLRs that mediate production of antiviral molecules, including interferons. Of note, some of the same pathways are targeted in similar ways by HCV, thus producing synergistic effects that promote inflammatory reactions and support the viral lifecycle in both parenchymal and nonparenchymal liver cells (John and Gaudieri 2014; Koziel 2005; Pang et al. 2011; Szabo et al. 2010).

Conclusions

Alcohol exposure and hepatitis viruses exploit common mechanisms to promote liver disease. Some of these mechanisms focus on the cellular membrane and its most active domains, the lipid rafts, which play critical roles in sustaining the lifecycle of both HBV and HCV. For HBV, the cellular

membranes and lipid rafts are particularly involved in viral entry; for HCV, lipid rafts additionally are required for formation and/or maintenance of HCV viral replication, virion assembly, and virion release from the host cell. Lipid rafts additionally influence viral survival indirectly because they serve as signaling platforms for a proinflammatory signaling cascade as well as for antiviral pathways, and they help regulate intracellular lipid storage within the parenchymal liver cells. Moreover, cellular membranes and lipid rafts play a crucial role in the immune-mediated cell defense in nonparenchymal liver cells. Alcohol affects membrane and lipid-raft function both directly and indirectly by modulating the proinflammatory cascade as well as antiviral pathways and intracellular lipid storage within the parenchymal liver cells and hampering the function of nonparenchymal liver cells through both lipid-raft-dependent and -independent mechanisms. The synergistic effects of hepatitis viruses and alcohol on the cellular membranes lead to impaired antiviral immunity and a proinflammatory milieu in the liver, thereby helping to sustain the viral lifecycle and promoting rapid progression and a more severe course of liver disease.

A better understanding of lipid-raft function may contribute to new approaches to treatment of viral and alcohol-related hepatitis, but knowledge of the structure and function of these cell structures is only beginning to emerge. For example, lipid-raft formation still is an enigma, and researchers are only now starting to investigate and understand the processes underlying lipid-raft activation, protein-lipid interactions, lipid-raft-dependent signaling, and other mechanisms through which lipid rafts direct the bioactivity of the various membrane constituents. Eventually, however, better understanding of cellular membranes and lipid rafts and their involvement in health and disease may lead to novel treatment approaches, including cellular- and subcellular-based personalized medicine approaches that also may lead to improved outcomes for patients with viral and/or alcohol-related hepatitis.

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Alcohol's Burden on Immunity Following Burn, Hemorrhagic Shock, or Traumatic Brain Injury

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Alcohol consumption contributes to increased incidence and severity of traumatic injury. Compared with patients who do not consume alcohol, alcohol-consuming patients have higher rates of long-term morbidity and mortality during recovery from injury. This can be attributed in part to an impaired immune response in individuals who consume alcohol. Acute and chronic alcohol use can affect both the innate and adaptive immune defense responses within multiple organ systems; the combination of alcohol use and injury results in increased susceptibility to bacterial and viral pathogens. This review examines the major deleterious effects of alcohol on immunity following tissue damage or traumatic injury, with a focus on alcohol's influence on the ability of the immune and major organ systems to fight disease and to repair damaged tissues following injury.

Key words: Alcohol consumption; alcohol use, abuse, and dependence; chronic alcohol use; acute alcohol use; injury; traumatic injury; morbidity; mortality; immune response; impaired immune response; bacterial pathogens; viral pathogens; tissue; organs; disease

The incidence of traumatic injury in alcohol-intoxicated individuals continues to escalate. According to the Centers for Disease Control and Prevention (2012a), more than 38 million American alcohol users consume 5 or more drinks on the same occasion (i.e., binge drink) and do so about 4 times per month. This behavior is highly conducive to unintentional or accidental traumatic injury, which according to the National Vital Statistics Reports is the leading cause of years of potential life lost (YPLL) before age 45. Unintentional injury causes more YPLL than that attributed to cancer, intentional injuries, heart disease, and HIV individually (Centers for Disease Control and Prevention 2009). Data from the National Center for Injury Prevention

and Control, as well as data derived from prospective and retrospective studies, show that up to 40 percent of victims of traumatic injury have positive blood alcohol concentrations (BAC), with 35 percent presenting with blood alcohol levels above the legal limit of intoxication (Beech and Mercadel 1998).

The severity of trauma, reduced blood flow and oxygen delivery (i.e., hemorrhagic shock, referred to as shock in this article), and tissue injury is greater in intoxicated victims than in sober victims, resulting in higher mortality rates in the alcohol-consuming patient population (Pories et al. 1992). Although immediate mortality from traumatic injury has improved significantly as a result of aggressive

resuscitation, long-term morbidity and mortality continue to be unacceptably high during the recovery period. The prevalence of morbidity and mortality is particularly attributable to the altered immune response among impaired patients to subsequent challenges, such as surgery or infection, leading to multiple organ failure (Roumen et al. 1993; Sauaia et al. 1994). Acute alcohol intoxication complicates the initial management of trauma victims and is associated with greater incidences of pneumonia and respiratory distress, requiring ventilator assistance during hospitalization (Gurney et al. 1992; Jurkovich et al. 1992). In addition, major complications including tracheobronchitis, pneumonia, pancreatitis, and sepsis are significantly

increased in patients with high levels of carbohydrate-deficient transferrin (CDT), a marker for alcoholism (Spies et al. 1998). European studies show that, compared with nonalcoholics, alcoholics more frequently develop major complications and require a significantly prolonged stay in the intensive care unit (ICU) following trauma (Spies et al. 1996a).

Excessive acute and chronic alcohol consumption has significant effects at multiple cellular levels, affecting both innate and adaptive immune mechanisms (Molina et al. 2010). Both chronic and acute patterns of alcohol abuse lead to impaired immune responses, resulting in increased susceptibility to infectious diseases caused by bacterial and viral pathogens (Brown et al. 2006). Clinical and preclinical studies show that the combined effects of alcohol and injury result in greater immune disruption than either insult alone (Messingham et al. 2002). This article reviews the current understanding of the burden of alcohol on the immune response to three specific traumatic events: burn, shock, and traumatic brain injury (TBI). The major pathophysiological consequences of these injuries on other major organ systems—including the cardiovascular system, pulmonary system, and gastrointestinal tract—are highlighted with emphasis on the contribution of alcohol-induced immunomodulation to postinjury morbidity.

Reestablishment of homeostasis after a traumatic insult involves activation of host defense mechanisms for self-protection against toxic inflammatory processes and tissue repair. Trauma victims frequently are subjected to necessary invasive procedures, such as surgery and anesthesia. In addition, trauma victims frequently are exposed to subsequent challenges, particularly infection. These additional stresses to an already compromised inflammatory and neuroendocrine milieu further contribute to morbidity and mortality in this patient population. Traumatic injury and hemorrhagic shock produce a temporal pattern with early upregula-

tion of pro-inflammatory cytokine1 gene product expression and with later suppression of stimulated pro-inflammatory cytokine release (Hierholzer et al. 1998; Molina et al. 2001). Together, these alterations lead to generalized immunosuppression, ultimately resulting in an increased susceptibility to infection (Abraham 1993; Ertel et al. 1993).

Alcohol has been shown to affect multiple aspects of the host immune response, contributing to pathological processes (Szabo 1998). For example, alcohol alters the expression and processing of cytokines and a type of cytokine known as chemokines (D'Souza et al. 1989; Standiford and Danforth 1997), the expression of adhesion molecules (Zhang et al. 1999), inflammatory cell recruitment (Patel et al. 1996; Shellito and Olariu 1998) and accumulation, and oxidative capacity of macrophages (Nilsson and Palmblad 1988). The monocyte/macrophage production of cytokines and chemokines, in particular interleukin (IL)-8 and tumor necrosis factor- α (TNF- α), is critical in the regulation of the acute inflammatory host response to infectious challenge. The combined inhibition of pro-inflammatory cytokine production and neutrophil activation and migration to a site of infection has been suggested to contribute to the enhanced susceptibility to infection in alcoholic individuals (Nelson et al. 1991) and to the increased risk of trauma- and burn-related infections associated with alcohol intoxication (Arbabi et al. 1999). Several lines of evidence show that these alcohol-mediated alterations in host defense following injury lead to increased morbidity and mortality from infections during the recovery period (Faunce et al. 2003; Messingham et al. 2002; Zambell et al. 2004). In addition, considerable evidence suggests that the severity of disease processes is greater in intoxicated trauma victims than in nonintoxicated counterparts (Spies et al. 1996a,b, 1998). In

particular, immunoparalysis characterized by inhibition of stimulated pro-inflammatory cytokine release (Angele et al. 1999) and alterations of both cellular and humoral immunity (Napolitano et al. 1995; Wichmann et al. 1998) have been identified as risk factors for infection and progression to organ injury during the post-traumatic injury period (Abraham 1993; Ertel et al. 1993).

The systemic response to injury is associated with marked activation of neuroendocrine pathways that contribute to cardiovascular adaptation to blood loss, injury, and pain but also exert immunomodulatory effects (Molina 2005). Catecholamines (e.g., dopamine, norepinephrine, and epinephrine), and drugs that mimic their effects (i.e., adrenergic agonists), are especially known to exert important regulatory functions on macrophages as well as on B- and T-lymphocyte cytokine production, proliferation, and antibody secretion; dendritic cell function; cytokine and chemokine release; and nitric oxide (NO) production (Madden et al. 1995). The relevance of these control mechanisms and the implications of their dysregulation have been demonstrated by the high incidence of infection in patients who experience elevated temperature, increased heart rate, and perspiration (i.e., “sympathetic storm”) following acute brain trauma and myocardial infarction (Woiciechowsky et al. 1998). Alcohol intoxication produces marked disruption of several neuroendocrine pathways. Disruption of the homeostatic neuroendocrine counterregulatory response to shock impairs hemodynamic stability and recovery, contributing to compromised blood flow and increased end-organ injury (Molina et al. 2013). Specifically, binge alcohol use blunts central neuroendocrine and autonomic activation, and this seems to result from alcohol-accentuated NO production in the periventricular nucleus (PVN) of the hypothalamus (Whitaker et al. 2010). Alcohol-mediated impairment of neuroendocrine counterregulatory responses to traumatic injury not only

¹ Cytokines are proteins involved in cell signaling. They are produced by a variety of cells including immune cells and regulate the immune response.

exacerbates low blood pressure (i.e., hypotension) during hemorrhage but also attenuates blood pressure recovery during fluid resuscitation, leading to significant alterations in blood flow redistribution and notably affecting circulation in the gastrointestinal tract (Wang et al. 1993). Studies have shown that alcohol-intoxicated animals have greater reduction of blood flow to the liver, kidney, and small and large intestines than nonintoxicated animals, following shock and fluid resuscitation (Sulzer et al. 2013). These macro- and microcirculatory changes during trauma and hemorrhage have been implicated in the subsequent development of sepsis and multiple organ failure (Peitzman et al. 1995) and contribute to an increased host susceptibility to infection and tissue injury during recovery (Mathis et al. 2006; Xu et al. 2002). People who abuse alcohol, including both binge and chronic drinkers, have a higher incidence of traumatic injury such as burn, shock, and TBI. The host response to these diverse insults is markedly affected by both patterns of alcohol abuse and some systems—including gastrointestinal, cardiovascular, and pulmonary—are more affected than others according to the specific injury.

Alcohol and Burn Injury

Burn injury is a common type of traumatic injury that affects thousands of people in the United States every year (Bessey et al. 2014). Approximately 50 percent of burn-injured patients have detectable blood alcohol levels at the time of hospital admission (Haum et al. 1995; McGwin et al. 2000), and these patients have more complications, require longer hospital stays, and have greater mortality rates than those with a similar degree of injury who are not intoxicated at the time of injury (McGill et al. 1995). Most morbidity and mortality among patients who survive initial injury is attributed to complications stemming from infection (Baker et al. 1980). Therefore, the pre-burn

immunological condition of injured patients affects susceptibility to infection and survival. Several mechanisms contribute to infection in burn patients, including loss of barrier function, changes in normal flora, wound ischemia, and cellular immunosuppression resulting from pro-inflammatory processes. Neutrophil, helper T-cell, and macrophage dysfunction; increased pro-inflammatory cytokine production; and enhanced production of immunosuppressive factors have all been shown to contribute to the pathophysiological response to burn injury (Faunce et al. 1998; Messingham et al. 2000). The mechanisms that contribute to infection in burn patients are influenced by acute and chronic alcohol intoxication and will be discussed below (see figure 1).

Research by Kovacs and colleagues (2008) has offered insight into the combined effects of burn injury and alcohol intoxication on immunity (Bird and Kovacs 2008). Chronic alcohol abuse alone increases the risk for lung infection (Baker and Jerrells 1993), impairs the phagocytic activity of alveolar macrophages and clearance of infectious particles from the airways, and impairs oxidant radicals, chemokine, and cytokine release that are required for microbial killing (Brown et al. 2007; Mehta and Guidot 2012; Molina et al. 2010). Acute alcohol intoxication prior to burn injury significantly suppresses the immune response relative to the insult alone (Faunce et al. 1997) and causes greater suppression of T-cell proliferation and response, reduced IL-2 production, and increased IL-6 production and circulating levels (Choudhry et al. 2000; Faunce et al. 1998). The T-cell and cytokine impairment caused by the combined effect of alcohol and burn injury may further suppress cell-mediated immunity, resulting in even greater susceptibility to infection than burn alone. Alcohol-mediated immunomodulation contributes to tissue injury in target organs as described below.

Gastrointestinal Tract

A multitude of studies have demonstrated that the gut is a reservoir for pathogenic bacteria, which may contribute to increased susceptibility to infections following traumatic injury (Deitch 1990). The intestinal mucosal barrier serves a major role in the local defense against bacterial entry and the translocation of endotoxin to the systemic circulation (Xu et al. 1997). Increased permeability and immune dysfunction indicate the compromised state of the intestinal mucosal barrier to bacterial translocation following trauma (Deitch et al. 1990; Willoughby et al. 1996). Increased intestinal permeability enhances bacterial and endotoxin translocation from the intestinal tract to the systemic circulation, triggering a systemic inflammatory response (Xu et al. 1997). Activated macrophages and lymphocytes release pro-inflammatory cytokines including TNF- α , IL-1 β , and IL-6, thereby contributing to tissue injury (Fink 1991). Studies have determined that chronic alcohol consumption disrupts intestinal barrier function and induces gut leak (Li et al. 2008; Tang et al. 2009). In addition, reports have shown a loss of intestinal barrier function followed by an increase in endotoxin and bacterial translocation to the systemic circulation following burn injury alone (Carter et al. 1990; Deitch and Berg 1987; Horton 1994), alcohol intoxication alone (Keshavarzian et al. 1994; Tabata et al. 2002), and burn injury with alcohol intoxication (Choudhry et al. 2002; Kavanaugh et al. 2005; Napolitano et al. 1995). Acute alcohol intoxication at the time of burn injury enhances bacterial growth in the intestine and is reflected in a proportional increase in mesenteric lymph node bacterial count (Kavanaugh et al. 2005). Acute alcohol intoxication also modulates intestinal immune defense by suppressing T-cell proliferation and increasing bacterial accumulation in mesenteric lymph nodes, spleen, and blood, which suggests that T-cell suppression may play a role

in bacterial translocation from the lumen of the gut (Choudhry et al. 2002). Moreover, studies have shown that following shock, trauma, or burn injury, the gut leaks bacteria and pro-inflammatory factors that are carried by the mesenteric lymphatic system, which contributes to acute lung injury (ALI) (Magnotti et al. 1999). The possibility that alcohol exacerbates toxin delivery to the systemic circulation through the lymphatics is supported by studies demonstrating that alcohol regulates the contractile cycle of mesenteric lymphatic vessels modulating the driving force of lymph flow (Keshavarzian et al. 1994; Souza-Smith et al. 2010). Thus, the contribution of gut-lymph to end-organ

damage following burn injury and alcohol intoxication may be significant.

Collectively, studies indicate that alcohol consumption preceding burn injury (1) increases gut permeability; (2) enhances intestinal bacterial growth, translocation, and systemic accumulation; and (3) suppresses T-cell proliferation. Further, research supports the concept that the intestine is not only a source of infection but also the site of the initial immune perturbation leading to the development of multiple organ dysfunction or organ failure.

Cardiovascular System

Immediately following a burn injury, the cardiovascular system responds with a decrease in cardiac output

(Cuthbertson et al. 2001) as a result of low blood volume and reduced venous return (Kramer et al. 2007). This phase is associated with decreased cardiac contractility, mediated by the release of vasoactive and pro-inflammatory mediators (Williams et al. 2011). Subsequently, there is a surge in counterregulatory neuroendocrine mediators (catecholamines, glucagon, and cortisol) that contribute to the development of a hyperdynamic cardiovascular state—characterized by increased heart rate and cardiac output—and is associated with increased myocardial oxygen consumption and myocardial hypoxia (Williams et al. 2011). These pathophysiological processes enhance oxidative metabolism and increase the risk for free-radical generation, further

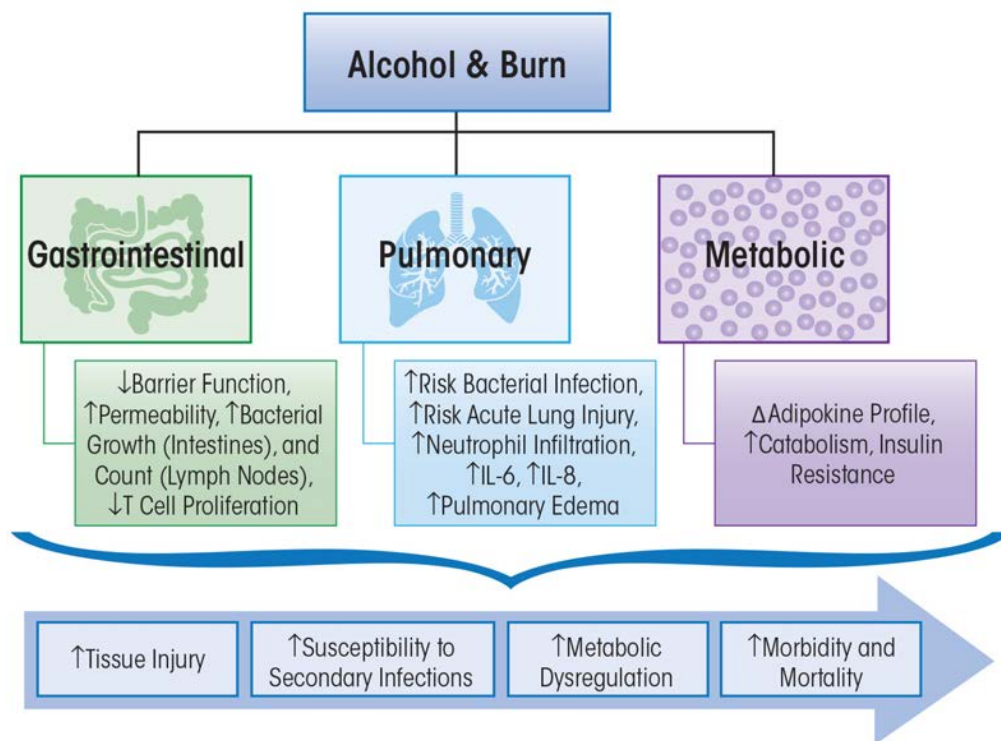


Figure 1 Salient gastrointestinal, pulmonary, and metabolic pathophysiological consequences of alcohol abuse prior to, or at the time of, burn injury. The decrease in gut barrier function leads to increased permeability and bacterial translocation that enhances the risk for bacterial infections and lung injury. Marked alterations in metabolic responses, characterized by altered adipokine profile consistent with increased insulin resistance, collectively contribute to greater morbidity and mortality post-burn injury.

exacerbating the pro-oxidative environment that has been proposed to contribute to impaired wound healing in burn patients (Herndon and Tompkins 2004). Chronic binge alcohol consumption also has been shown to promote a pro-oxidative and pro-inflammatory milieu (Rashbaste et al. 1993), and these factors may further impede wound healing in patients consuming alcohol prior to experiencing burn injury. Additional research is needed to better understand immunomodulation effects following the combined insults of alcohol and burn injury and the mechanisms underlying the more severe outcome of burn injury with alcohol abuse.

Pulmonary System

Adult respiratory distress syndrome (ARDS) is a frequent cause of death in burn patients. The lungs are one of the first organs to fail following traumatic injury (Turnage et al. 2002). Chronic and acute alcohol abuse impair pulmonary host defense to infection, thus increasing the risk of bacterial infection and acute lung injury (Boe et al. 2009; Happel and Nelson 2005). Lung injury as a result of the combination of alcohol intoxication and burn injury may be attributed to the delicate architecture of the lungs combined with other alcohol-related factors, such as bacterial and endotoxin leakage from the gut and a higher risk of contact with pathogens from the circulation and airways (Bird and Kovacs 2008; Li et al. 2007). Previous studies show that the combined insult of acute alcohol consumption and burn injury in mice leads to increased infiltration of the lungs by white blood cells, called neutrophils, and pro-inflammatory cytokine expression of IL-6 (Chen et al. 2013). Systemic and pulmonary IL-6 reflect the inflammatory state of the host and have been shown to be decreased in the absence of Toll-like receptor-4 (TLR-4) and intercellular adhesion molecule-1 (ICAM-1) (Bird et al. 2010). The role of IL-6 in lung injury has been demonstrated in

studies in IL-6 knockout mice or following neutralization of IL-6, both of which result in significantly reduced lung inflammation (Chen et al. 2013). Studies also have shown that acute alcohol intoxication at the time of burn injury induces an upregulation of IL-18 production and neutrophil infiltration within the lung compartment, all leading to pulmonary edema (Li et al. 2007).

Metabolism

The post-burn period is characterized by a hypermetabolic state (Pereira and Herndon 2005) consisting of increased oxygen consumption; increased breakdown of glycogen, fats, and proteins; elevated resting energy expenditure and glucose synthesis; and reduced insulin-stimulated glucose uptake into skeletal muscle and adipose tissue (Gauglitz et al. 2009). Previous studies suggest that development of this hypermetabolic state during the post-burn period occurs as a consequence of (1) increased plasma catecholamine and corticosteroid concentrations (Jeschke et al. 2008; Williams et al. 2009; Wilmore and Aulick 1978), (2) increased systemic pro-inflammatory mediator expression, favoring processes that release energy (i.e., catabolic) over those that store energy (i.e., anabolic) (Jeschke et al. 2004), and (3) increased adipose tissue mRNA (Zhang et al. 2008) and protein (Yo et al. 2013) expression of uncoupling protein-1 (UCP-1), enhancing heat production and metabolism. Further, circulating levels of TNF- α , a known anti-insulin cytokine, are increased (Keogh et al. 1990), and the post-burn period can be described as a state of marked insulin resistance (IR) (Gauglitz et al. 2009). Insulin sensitivity has been reported to be decreased by more than 50 percent at 1-week post-burn injury in pediatric patients (Cree et al. 2007) as well as in rodent models of burn injury (Carter et al. 2004). The relevance of insulin levels to overall outcome from burn injury is supported by results from clinical

studies showing that exogenous insulin therapy in pediatric burn patients decreased pro-inflammatory cytokines, increased anti-inflammatory cytokines, and increased serum concentrations of insulin-like growth factor-1 (IGF-1) and insulin-like growth factor binding protein-3 (IGFBP-3). Together, these changes could help to preserve organ function and better promote anabolic processes during the post-burn hypermetabolic state (Jeschke et al. 2004). Chronic alcohol consumption decreases insulin responsiveness and can alter insulin signaling through various mechanisms, including increased hepatic protein expression of the gene phosphatase and tensin homologue (PTEN), which directly inhibits insulin signaling through the phosphatidylinositol-5,5-bisphosphate 3-kinase (PI3K)/protein kinase B (Akt) pathway (de la Monte et al. 2012). In addition to the negative regulation of the pathway by PTEN proteins, the enzyme protein tyrosine phosphatase dephosphorylates and decreases activity of important molecules involved in the insulin signaling cascade, potentially contributing to impaired insulin action (Gao et al. 2010; Koren and Fantus 2007). In addition, Lang and colleagues (2014) demonstrated that chronic alcohol consumption reduces Akt and AS160 phosphorylation, reduces membrane localization of glucose transporter type 4 (GLUT-4) protein, and increases serine phosphorylation at serine-307 of insulin receptor substrate-1 (IRS-1), all of which will attenuate insulin-stimulated skeletal muscle glucose uptake and other insulin-mediated anabolic effects (Lang et al. 2014). These negative effects on insulin signaling occurred in conjunction with sustained increases in pro-inflammatory cytokines TNF- α and IL-6 following chronic alcohol exposure (Lang et al. 2014). Thus, both burn injury and chronic alcohol exposure alter metabolic pathways—favoring catabolic and opposing anabolic pathways—possibly resulting in long-lasting alterations in metabolic processes. The metabolic dysregulation

following burn injury is likely to produce more severe consequences in chronic alcohol burn victims. Previous studies assessing nutritional status of alcoholic patients have been discordant, with some studies suggesting that increased alcohol consumption increases the prevalence of malnutrition in alcoholic patients (Hillers and Massey 1985), whereas other studies do not show a role for excessive, or chronic, alcohol consumption in malnutrition (Nicolas et al. 1993; Urbano-Marquez et al. 1989). A study assessing the influences of aging and chronic alcohol feeding in mice on protein synthesis demonstrated that chronic alcohol feeding decreases gastrocnemius muscle protein synthesis, which provides a mechanism for loss of lean body mass (Korzick et al. 2013; Lang et al. 2014). Decreased anabolism during the post-burn period, which itself is a state of heightened catabolic processes, could significantly impair recovery for these alcoholic patients experiencing burn injury. Further, the hypermetabolic state of the post-burn period is thought to contribute to delayed or impaired wound healing, increased susceptibility to infections, and erosion of lean body mass (Pereira and Herndon 2005). Moreover, both binge alcohol consumption (Pravdova and Fickova 2006; You and Rogers 2009) and burn injury (Venkatesh et al. 2009; Wade et al. 2013) can contribute to dysregulation of cytokines secreted by adipose tissue (i.e., adipokines). Recent studies show that mice exposed to a single alcohol binge prior to burn injury have a dramatic increase in pro-inflammatory response and a decrease in anti-inflammatory response in adipose tissue (Qin et al. 2014). The heightened pro-inflammatory response during the post-burn period would be predicted to modulate leptin levels. Thus, recovery from burn injury is likely to be severely impaired in alcoholic individuals as a result of a greater disruption in metabolic processes as well as impairment of host defense mechanisms, leading to greater morbidity and health care costs

associated with the management of these patients. Therefore, further investigation is warranted to understand the modulation of the immune system by the combined effect of alcohol and burn that might result in dysregulation of adipose tissue and altered metabolism.

Alcohol and Hemorrhagic Shock

Studies from several investigators have provided evidence that traumatic injury and hemorrhagic shock produce an immediate upregulation of pro-inflammatory cytokine gene product expression (Ayala et al. 1991; Hierholzer et al. 1998). The early pro-inflammatory response is later followed by suppression of stimulated pro-inflammatory cytokine release (Angele et al. 1999; Xu et al. 1998) and alterations of both cellular and humoral immunity (Napolitano et al. 1995; Wichmann et al. 1998), leading to generalized immunosuppression, which ultimately results in an increased susceptibility to infection (Abraham 1993; Ertel et al. 1993). Along with marked alterations in hemodynamic homeostasis and neuroendocrine regulation, immunological derangements and subsequent infections are also a major cause of increased morbidity and mortality following hemorrhagic shock (Livingston and Malangoni 1988; Phelan et al. 2002).

Studies focused on the immune modulatory effects of alcohol exposure following hemorrhagic shock have demonstrated that even 24 hours after the post-hemorrhagic shock, alcohol-intoxicated animals had a marked suppression in cytokine release to an inflammatory challenge (Greiffenstein et al. 2007), affecting the ability to fight secondary infectious challenges. Conversely, findings observed at the tissue level determined that alcohol intoxication enhanced the pro-inflammatory milieu following hemorrhagic shock, priming tissues for injury. The burden of alcohol and hemorrhagic shock on specific target

organ systems is discussed below and summarized in figure 2.

Gastrointestinal Tract

Hemorrhagic shock produces similar alterations in gut barrier function to those resulting from burn injury. Alcohol intoxication at the time of hemorrhagic shock further exacerbates hemorrhagic injury-induced gut permeability and leakage (Sulzer et al. 2013). Chronic alcohol consumption has been shown to disrupt intestinal barrier function and induce gut leak (Li et al. 2008; Tang et al. 2009). The combination of greater hypotension and inadequate tissue blood flow (i.e., hypoperfusion) observed in alcohol-intoxicated animals and the increased gut leak observed in alcohol-intoxicated hemorrhaged animals are speculated to contribute to increased host susceptibility to infection and tissue injury during recovery (Molina et al. 2013). Alcohol-intoxicated, hemorrhaged animals have been shown to have greater reduction in hepatic, renal, and intestinal blood flow than that observed in nonintoxicated animals (Sulzer et al. 2013). This reduction in critical organ blood flow was associated with enhanced tissue damage. An additional mechanism that could contribute to tissue injury in the alcohol-intoxicated, hemorrhaged host is the disruption of gut-associated lymphoid tissue function, which has been shown to play a role in other disease states.

Cardiovascular System

Studies using a rodent model of binge-like alcohol consumption prior to hemorrhagic shock have shown that acute alcohol intoxication decreases basal mean arterial blood pressure (MABP), exacerbates hypotension, and attenuates blood pressure recovery during fluid resuscitation (Mathis et al. 2006; Phelan et al. 2002). Following fixed-volume hemorrhage, alcohol-intoxicated animals were significantly more hypotensive throughout the

hemorrhage and resuscitation periods (Mathis et al. 2006). In response to a fixed-pressure (40 mmHg) hemorrhage, a significantly lesser amount of blood was removed from the alcohol-intoxicated animals than controls (Phelan et al. 2002). Similarly, McDonough and colleagues, using a guinea pig model of ethanol exposure prior to hemorrhagic shock (loss of 60% blood volume) and resuscitation, demonstrated that a low dose of ethanol (1 g/kg) decreases MABP and heart rate and exacerbates the metabolic effects of hemorrhagic shock, as shown by increased glucose and lactate concentrations (McDonough et al. 2002). Despite the plethora of previous studies that have examined functional cardiovascular consequence of hemor-

rhagic shock and hemorrhage with alcohol intoxication, few studies have examined the combined effects of alcohol, hemorrhagic shock, and immune dysfunction on the cardiovascular system. However, exacerbation of pre-existing cardiovascular disease and prolonged recovery are anticipated outcomes of the combined effects of alcohol and hemorrhagic shock, all leading to an impaired immune response.

Pulmonary System

As mentioned previously, alcohol intoxication produces significant dysregulation of the host defense mechanism during the post-injury period. Lung IL-6 and TNF- α are suppressed, while granulocyte-colony

stimulating factor (GCSF) mRNA is increased in alcohol-intoxicated, hemorrhaged animals (Mathis et al. 2006; Ono et al. 2004). Moreover, isolated pleural cells and peripheral blood mononuclear cells (PBMCs) from alcohol-intoxicated, hemorrhaged animals display suppressed TNF- α , IL-1 β , and IL-6 release following lipopolysaccharide stimulation (Greiffenstein et al. 2007), suggesting greater impairment of humoral immune response than that resulting from hemorrhagic shock alone. The importance of these alterations in host defense mechanisms was demonstrated in animals inoculated with *Klebsiella pneumonia* following hemorrhagic shock. These studies showed suppressed neutrophil response,

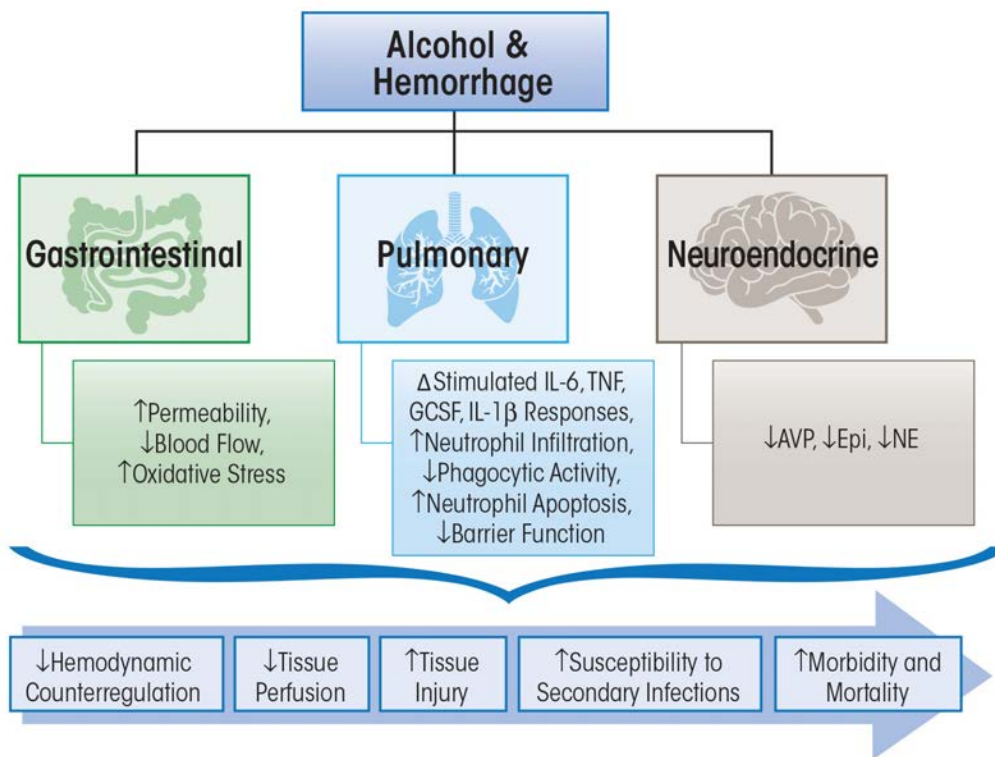


Figure 2 Salient gastrointestinal, pulmonary, and neuroendocrine pathophysiological consequences of alcohol abuse prior to, or at the time of, hemorrhagic shock. The decreased hemodynamic counterregulatory response leads to decreased tissue perfusion, accentuated oxidative stress, and enhanced tissue injury. In addition, the alcohol/hemorrhaged host shows greater susceptibility to secondary infections leading to increased morbidity and mortality during the post-injury period.

decreased phagocytic activity, and increased neutrophil apoptosis in hemorrhaged animals that were alcohol intoxicated at the time of injury (Zambell et al. 2004). This was associated with greater lung bacterial counts and prolonged elevation in TNF- α and IL-6 levels (18 h) post-infection. Furthermore, only 30 percent of alcohol-intoxicated, hemorrhaged animals survived compared with 70 percent survival of dextrose/hemorrhage animals (Zambell et al. 2004). In addition to cytokine dysregulation, alcohol impairs innate barrier functions of the lung by increasing epithelial cell permeability and altering the function of the ciliated epithelium (Elliott et al. 2007; Molina et al. 2010).

Neuroendocrine System

The pathophysiology of traumatic-hemorrhagic injury involves decreased blood volume (i.e., hypovolemia) and hypoperfusion, which results in signaling to central cardiovascular centers aimed at restoring hemodynamic stability through activation of descending autonomic neuroendocrine pathways (Molina 2005). Several mechanisms have been proposed to account for the increased hypotension and impaired hemodynamic stability observed with alcohol intoxication, with one proposed mechanism being blunted neuroendocrine activation. Studies demonstrated that acute alcohol intoxication at the time of injury results in significant attenuated release of counterregulatory hormones and potent vasoconstrictors such as arginine vasopressin (AVP), epinephrine, and norepinephrine in response to fixed-pressure hemorrhage (Phelan et al. 2002). A disruption in the neuroendocrine response with alcohol intoxication at the time of injury is associated with enhanced expression of lung and spleen TNF- α as well as suppression of circulating neutrophil function, which would be expected to enhance the risk for tissue injury (Whitaker et al. 2010). Conversely, Sato and colleagues

(2013) demonstrated that alcohol aggravates hemorrhagic shock in a dose-dependent manner not by triggering an immune response but by suppressing hormonal and neuro-humoral responses, thereby inhibiting hemodynamic auto-regulation and shortening the survival interval. Thus, both alcohol and hemorrhagic shock have detrimental effects on neuro-endocrine responses that are likely to modulate the host immune system in addition to impacting on hemodynamic stability and recovery and accentuating tissue hypoperfusion and end-organ injury.

Alcohol and Traumatic Brain Injury

Traumatic brain injury (TBI) accounts for approximately 50 percent of all trauma-related mortality (Centers for Disease Control and Prevention 2012*b*). TBI affects multiple sectors of the population, and young males have the highest rates of hospital visits and death (Faul et al. 2010). Falls are the first leading cause of TBI, followed by motor vehicle accidents and unintentional trauma sustained during sports activities such as football or boxing. TBI can be categorized as mild, moderate, or severe, and the majority of TBIs sustained in the United States are in the mild category (Centers for Disease Control and Prevention 2012*b*). In addition to the physical dysfunction caused by injury, TBI patients frequently experience lingering psychological symptoms, such as heightened anxiety, depression, sleep disturbances, and pain hypersensitivity (Whyte et al. 1996). These symptoms have been implicated in increased alcohol intake following TBI in humans (Adams et al. 2012). Furthermore, it is well accepted that alcohol consumption increases the risks of sustaining a TBI (Corrigan 1995; Hurst et al. 1994). Nevertheless, a comprehensive understanding of the influences of alcohol on TBI-induced inflammation, recovery from injury, and long-term damage

currently is limited and is summarized in the following section (see figure 3).

Neuroinflammation

The pathophysiology of TBI involves a primary mechanical injury followed by a secondary tissue injury resulting from neuroinflammation (Werner and Engelhard 2007). A large percentage of TBI victims show signs of further deterioration following the event (Suaia et al. 1995). This suggests the induction of a secondary brain injury and immune activation as the key cascades contributing to the pathophysiological processes of the secondary damage (Cederberg and Siesjo 2010). After TBI, a series of events occurs, including the activation of resident immune cells such as astrocytes and microglia, release of pro-inflammatory cytokines and chemokines, upregulation of endothelial adhesion molecules, and recruitment and activation of blood-derived leukocytes across the disrupted blood brain barrier (Feuerstein et al. 1998; Morganti-Kossmann et al. 2001; Ransohoff 2002). An increase in the levels of TNF- α in the serum or cerebrospinal fluid in victims of TBI also has been detected in rodents following closed head injury (Goodman et al. 1990; Ross et al. 1994; Shohami et al. 1994). IL-1 β is released after TBI (Fan et al. 1995) and induces nuclear factor-kappa B (NF- κ B), a key transcription factor that regulates the expression of genes encoding cytokines, as well as inducible NO synthase (iNOS), and cyclooxygenase-2 (COX-2) (Blanco and Guerri 2007; Woodrooffe et al. 1991; Ziebell and Morganti-Kossmann 2010). Following the rise of early cytokines, the release of IL-6 is associated with increased acute-phase proteins, as well as blood-brain barrier disruption (Kossmann et al. 1995; Shohami et al. 1994; Woodcock and Morganti-Kossmann 2013) and sustained elevation of chemokines such as chemokine (C-C motif) ligand-2 (CCL-2) in the cerebrospinal fluid for as long as 10 days post-injury (Semple et al. 2010). Although early

cytokine release is essential in mediating the reparative processes after injury (Ziebell and Morganti-Kossmann 2010), sustained elevation of pro-inflammatory mediators has been increasingly recognized to play a role in neuropathological changes associated with long-term degenerative diseases (Fan et al. 1995; Lyman et al. 2014). Accordingly, the additional risks of alcohol as a factor contributing to the alterations of TBI-induced neuro-inflammatory processes may affect the overall recovery.

Alcohol exerts a profound impact on neuroinflammation. Although there are some conflicting reports in the literature about the role of alcohol on recovery, the major findings are

summarized here. Some animal studies suggest that acute alcohol administration prior to TBI leads to an early reduction in the levels of pro-inflammatory cytokines and chemokines in the injured cortex, hippocampus, and hypothalamus, as well as in the serum shortly after TBI (Goodman et al. 2013; Gottesfeld et al. 2002). Recent studies also have confirmed that acute alcohol intoxication at the time of TBI does not exacerbate the expression of pro-inflammatory cytokines and chemokines at 6 hours post-injury. However, results obtained at a later time point (24 hours) show a sustained mRNA expression of IL-1 β , TNF- α , IL-6, and CCL-2 following a lateral fluid percussion injury in rodents that

were alcohol-intoxicated at the time of TBI (Teng and Molina 2014). Overall, some preclinical studies suggest that acute alcohol treatment prior to TBI may lead to a suppressed release of pro-inflammatory mediators during the early phase post-injury. Thus, the temporal pattern of neuroinflammatory responses and the impact of alcohol intoxication on neuroinflammatory responses are factors to consider when drawing conclusions on the role of alcohol in modulating the outcome from TBIs.

Because the literature surrounding the relationship between acute alcohol intoxication and response to trauma is conflicting, it is important to consider the pattern of alcohol abuse and the

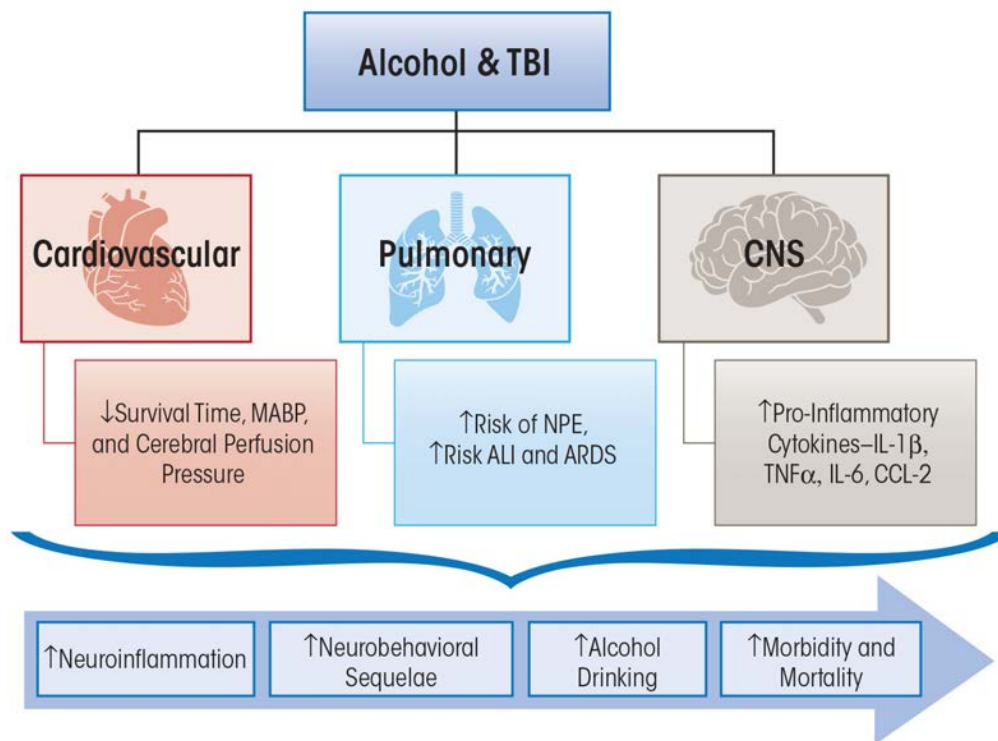


Figure 3 Salient cardiovascular, pulmonary, and central nervous system pathophysiological consequences of alcohol abuse prior to, or at the time of, traumatic brain injury (TBI). The disruption in hemodynamic homeostasis resulting from TBI contributes to decreased cerebral perfusion pressure. The lung is affected through neurogenic mechanisms leading to neuropulmonary edema (NPE) and associated risk for acute lung injury (ALI) and adult respiratory distress syndrome (ARDS). In the brain (CNS), alcohol accentuates neuroinflammation, which is associated with neurobehavioral dysfunction that can potentially promote alcohol drinking. Together, these pathophysiological consequences increase morbidity and mortality from TBI.

model used in different studies. In general, reports in the literature indicate that chronic alcohol exposure produces immune activation in the brain, inducing an enhanced pro-inflammatory state, as evidenced by the presence of CCL-2 and microglial activation in postmortem brains of human alcoholics (He and Crews 2008). Animal studies show that chronic, intermittent binge alcohol administration to rodents results in increased microglial activation and inflammatory cytokine expression in the cortex and hippocampus (Zhao et al. 2013). In addition, Crews and colleagues (2004) have found that chronic alcohol treatment induces expression of inflammatory cytokines such as TNF- α , which further activates resident glial cells to secrete additional pro-inflammatory cytokines and chemokines, resulting in an increased immune activation in the brain. The overall pro-inflammatory effects of alcohol also have been shown by Guerri and colleagues (2007) who reported alcohol-mediated stimulation of TLR-4 and IL-1 receptor signaling pathways, including extracellular regulated-kinase 1/2 (ERK1/2), stress-activated protein kinase/c-Jun N-terminal kinases (JNK), and p38 mitogen-activated protein kinase (MAPK), as well as the expression of NF- κ B, activator protein-1 (AP-1), iNOS, and COX-2 in cultured glial cells (Alfonso-Loeches et al. 2010; Fernandez-Lizarbe et al. 2009). The role of TLR4 has been identified in studies where 5 months of chronic alcohol administration increased glial activation and levels of caspase-3, iNOS, COX-2, and cytokines (IL-1 β , TNF- α , and IL-6) in the cerebral cortex of wild-type mice but not in the TLR4-deficient mice (Alfonso-Loeches et al. 2010). Another mediator of alcohol-mediated neuroinflammation is high-mobility group protein B1 (HMGB1), which has been reported to be increased along with TLR-2, TLR-3, and TLR-4 in postmortem brains of human alcoholics (Alfonso-Loeches et al. 2010). Despite a substantial amount of evidence showing

increased neuroinflammatory responses to chronic alcohol exposure, there have not been sufficient preclinical studies performed to determine the combined effect of chronic alcohol consumption and TBI on neuro-immune activation. Because both TBI and alcohol can induce inflammation in the brain, we speculate that the

Alcohol combined with traumatic injury can significantly affect morbidity and mortality through disruption in host immune responses.

combination of the two events would further accentuate neuroinflammation.

Retrospective studies have revealed that outside of the central nervous system, peripheral organ damage can contribute to the increased mortality rate among TBI patients as a result of cardiovascular, pulmonary, and endocrine dysfunction (Gennarelli et al. 1989; Shavelle et al. 2001). More specifically, TBI patients have an increased incidence of ALI, pulmonary infection, neuroendocrine alterations, and cardiovascular dysfunction during the post-injury period (Vermeij et al. 2013). Although the combined effects of alcohol and TBI and the role of local or systemic immune responses in peripheral organs are understudied, the current knowledge is summarized below (figure 3).

Pulmonary System

ALI, one of the most common nonneurologic complications following TBI, results from acute pulmonary edema and inflammation and can lead to ARDS (Holland et al. 2003; Johnson and Matthay 2010). ALI is characterized by hypoxemia, loss of lung compli-

ance, and bilateral chest infiltrates (Dushianthan et al. 2011). Development of ALI post-TBI has been associated with increased inpatient mortality following injury and worse long-term neurologic outcome in survivors of TBI (Bratton and Davis 1997; Holland et al. 2003). Post-TBI medical interventions including induced systemic hypertension and mechanical ventilation can result in nonneurogenic ALI (Contant et al. 2001; Lou et al. 2013). Development of neurogenic pulmonary edema (NPE) occurs minutes to hours following TBI and typically resolves within days (Bratton and Davis 1997). The possible underlying factors in NPE are the severity of injury leading to increased intracranial pressure and the subsequent increased circulating catecholamines (Demling and Riessen 1990). TBI also is associated with greater incidence of pulmonary infections than that seen following major surgeries, burn injury, and polytrauma (Dziedzic et al. 2004). Clinical reports indicate that over 40 percent of TBI patients with artificial ventilation develop pneumonia and are four times more likely to die from pneumonia (Harrison-Felix et al. 2006). The increased risk of developing pneumonia post-TBI is potentially attributed in part to a systemic immune response syndrome (SIRS) characterized by increased circulating pro-inflammatory cytokines (TNF- α and IL-6) (Keel and Trentz 2005; Kossmann et al. 1995).

The combined impact of alcohol and TBI on pulmonary infections has been minimally investigated. Although, epidemiological studies have shown that in trauma patients, chronic alcohol abuse can independently increase the risk of ALI and ARDS two- to four-fold (Guidot and Hart 2005). In a prospective study of traumatic injury patients with evidence of acute alcohol intoxication or chronic alcohol abuse, chronic alcohol was associated with increased incidence of pneumonia or respiratory failure as a result of its immunosuppressive effects. However, no significant increase in incidence of pneumonia or respiratory failure and

mortality was observed in patients with acute alcohol intoxication with BAC above 100mg/dL (De Guise et al. 2009; Jurkovich et al. 1993). The importance of length and amount of pre-existing alcohol intake and TBI severity may be the key factors in determining a patient's risk for pneumonia. Taken together, the potential effects of chronic alcohol abuse and TBI could potentiate and further increase immunosuppression or immune dysfunction, thus leading to greater susceptibility for pneumonia, ARDS, and ultimately death.

Neuroendocrine System

TBI can lead to a variety of neuroendocrine abnormalities, such as gonadotropin deficiency, growth hormone deficiency, corticotrophin deficiency, and vasopressin alterations (Behan and Agha 2007; Powner and Boccalandro 2008). As a result of the mechanical compression to the pituitary gland or disruption of the pituitary stalk, hypopituitarism can occur and corticotrophin insufficiency is commonly observed after TBI (Agha et al. 2004; Cohan et al. 2005). Excessive alcohol use also has been reported to be associated with neuroendocrine dysfunction, notably in the form of altered regulation of hypothalamic–pituitary–adrenal axis (HPA), resulting in a decreased corticotrophin release (Behan and Agha 2007; Helms et al. 2014). Therefore, it is possible that the combination of alcohol and TBI-induced HPA dysfunction can lead to a dampened cortisol release, which may have an impact on the immune system. Interestingly, a hyperadrenergic state marked by elevated levels of catecholamines can occur after TBI, and alcohol intoxication at the time of TBI has been shown to blunt the sympatho-adrenal activation in a dose-dependent manner (Woolf et al. 1990). Vasopressin has been suggested to play a role in blood brain barrier disruption, edema formation, and the production of pro-inflammatory mediators after TBI (Szmydynger-

Chodobska et al. 2010). Vasopressin abnormalities leading to diabetes insipidus or the syndrome of inappropriate anti-diuretic hormone (SIADH) frequently are observed after TBI (Behan and Agha 2007), and acute alcohol intoxication is known to alter AVP release (Taivainen et al. 1995). Whether alcohol intoxication at the time of TBI or during the recovery period from TBI further dysregulates these neuroendocrine mechanisms remains to be examined.

Cardiovascular System

Cardiovascular complications including slow heart rate (i.e., bradycardia), hypotension, electrocardiographic changes, arrhythmias, and increased circulating cardiac enzymes have been reported following TBI (Bourdages et al. 2010; Wittebole et al. 2005). Chronic alcohol abuse alone can lead to alcoholic cardiomyopathy and potentially heart failure (Skotzko et al. 2009), and the underlying etiology has been reviewed (Lang et al. 2005). Several studies by Zink and colleagues (1998*a,b*, 2006) focused on the combined effects of acute alcohol intoxication on hemorrhagic shock and TBI in swine, showing decreased survival time, lowered MABP, and reduced cerebral perfusion pressure, which may worsen secondary brain injury. These studies did not investigate alterations in immune function or expression and levels of immune modulators or their actions on cardiovascular function. Overall, the post-TBI cardiovascular complications, including vascular function, have been understudied in both clinical and experimental models of TBI. More specifically, the combined impact of alcohol, TBI, and immune alterations on cardiovascular dysfunction and disease progression has not been examined. A possible prediction is that chronic alcohol-induced immunosuppression would worsen post-TBI cardiovascular complications; and in chronic alcoholics, dilated cardiomyopathy may compound TBI-related cardiovascular complica-

tions increasing morbidity and mortality.

Summary

The deleterious effects of alcohol on the immune system in three traumatic injuries are discussed in this review and are summarized in figures 1, 2, and 3. It is evident that, independently, acute or chronic alcohol consumption and traumatic injury negatively modulate the immune system, and the end result is an uncontrolled release of inflammatory mediators. The most important message of this review is the accumulation of evidence that alcohol combined with traumatic injury can significantly affect morbidity and mortality through disruption in host immune responses. Following burn injury, for instance, the risk for infection is greatly increased because of increased gut permeability and increased pro-inflammatory cytokine expression in the lungs (figure 1). Alcohol use following hemorrhage can increase inflammation and oxidative stress in the gut while decreasing lung barrier function and subsequently increasing susceptibility to infection (figure 2). In the central nervous system, alcohol use following TBI can increase neuroinflammation and prolong the recovery period (figure 3). Overall this information is important, because it provides a wealth of evidence that alcohol combined with trauma is a dramatic and preventable cause of increased morbidity and mortality following injury. Mechanistically, two common pro-inflammatory cytokines that are consistently upregulated in all burn injury, hemorrhagic shock, and TBI are TNF- α and IL-6. A fuller understanding of their temporal pattern of expression and downstream effects requires further investigation. Although the studies described in this review have generated important information on the impact of alcohol combined with different types of traumatic injury, and the resultant adverse effects on the immune system, further preclinical

and clinical studies to dissect the complex cascade of immunomodulation following injury are necessary. Specifically, further investigation is warranted to determine the underlying mechanisms involved in immune modulation by acute or chronic alcohol intake and the effects on (1) metabolism and the cardiovascular system following burn, (2) the neuroendocrine system following hemorrhagic shock, and (3) neuroinflammation and the neuroendocrine system following traumatic injury. The responses of the immune system to these inflammatory stimuli are variable and appear to be dependent on the severity of the injury, comorbidities, and the level of alcohol intoxication. Thus, it is necessary to systemically address these variables for translational research to identify potential therapeutic strategies. Furthermore, therapeutic targets for immunomodulation and attenuation of tissue injury in intoxicated and injured patients are likely to reduce morbidity and mortality and improve post-injury quality of life among these patients.

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Macrophages and Alcohol-Related Liver Inflammation

Cynthia Ju, Ph.D., and Pranoti Mandrekar, Ph.D.

Recent studies have suggested that macrophages have a critical role in the development of alcohol-induced inflammation in the liver. To define the precise pathogenic function of these cells during alcoholic liver disease (ALD), it is extremely important to conduct extensive studies in clinical settings that further elucidate the phenotypic diversity of macrophages in the context of ALD. Research to date already has identified several characteristics of macrophages that underlie the cells' actions, including macrophage polarization and their phenotypic diversity. Other analyses have focused on the contributions of resident versus infiltrating macrophages/monocytes, as well as on the roles of macrophage mediators, in the development of ALD. Findings point to the potential of macrophages as a therapeutic target in alcoholic liver injury. Future studies directed toward understanding how alcohol affects macrophage phenotypic switch in the liver and other tissues, whether the liver microenvironment determines macrophage function in ALD, and if targeting of macrophages alleviates alcoholic liver injury, will provide promising strategies to manage patients with alcoholic hepatitis.

Key words: Alcohol consumption; alcoholic liver disease; alcoholic liver injury; alcoholic hepatitis; alcohol-related liver inflammation; liver; immunity; innate immune response; adaptive immune response; macrophage; macrophage phenotypic switch; Kupffer cell

Alcoholic liver disease (ALD) is a complex disease that affects millions of people worldwide and eventually can lead to liver cirrhosis and liver cancer (i.e., hepatocellular carcinoma). Aside from the direct cytotoxic and the oxidative-stress-mediated effects that alcohol and its metabolite, acetaldehyde, exert on hepatocytes, alcohol ingestion activates both the innate and adaptive immune responses in the liver. These responses involve multiple hepatic cell types, including resident macrophages, natural killer cells, natural killer T cells, lymphocytes, and neutrophils. In particular, resident macrophages in the liver, also known as Kupffer cells, are important for clearing pathogens, including bacteria, viruses, immune complexes, bacterial products called endotoxin or lipopolysaccharide (LPS), and tumor cells, from the liver (Jenne and Kubes 2013; Thomson and Knolle 2010). Research tools such as fate mapping, multifocal

microscopy, transgenic/reporter mouse models, and next-generation sequencing recently have led to a better understanding of the origins, heterogeneity, and plasticity in the phenotypes and functions of macrophages and their circulating precursor cells (i.e., monocytes).

The activation of circulating monocytes and accumulation of macrophages in the liver are important pathophysiological features in patients with ALD. However, the role of hepatic macrophages in the pathogenesis of ALD has not been fully elucidated. This review will discuss some of the new findings in monocyte/macrophage biology, provide an update of the current studies on the involvement of liver macrophages in ALD, and identify remaining questions to be addressed in order to develop macrophage-targeted therapy for ALD.

Phenotypic and Functional Heterogeneity of Monocytes and Macrophages

Macrophages, which play an important role in the initial innate immune response to infection with pathogens or other insults, fall into two main categories—infiltrating macrophages and tissue-resident macrophages. Infiltrating macrophages are derived from precursor cells called monocytes that circulate throughout the body and are recruited into the tissues when an inflammatory reaction occurs. Tissue-resident macrophages, in contrast, always remain localized within one tissue, serving as sentries and first line of defense against any infection or injury in that tissue.

Monocytes are circulating innate immune cells formed from progenitor cells in the bone marrow; the monocytes then differentiate into numerous subsets of macrophages (Fogg et al. 2006). In both humans and mice, monocytes can be divided into two major subsets—classical and nonclassical—depending on the marker proteins that they exhibit on their surface (Ingersoll et al. 2011; Sunderkotter et al. 2004; Ziegler-Heitbrock 2007) (see table 1). In humans, monocytes (all of which express CD115⁺) are divided into the two major subsets based on their CD14 and CD16 expression, as well as on their expression of markers called CCR2 and CX3/CR1:

- The predominant subset, representing 90 percent of circulating monocytes, is the classical subset characterized by the marker combination CD14^{hi}CD16⁻ and CCR2⁺/CX3CR1^{lo}.

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- The less abundant nonclassical monocyte subset further can be divided into two groups characterized by the marker combinations CD14^{dim}CD16⁺ and CCR2⁻/CX3CR1^{hi}/CCR5^{hi} (nonclassical monocytes) or CD14^{hi}CD16⁺ and CCR2⁻/CX3CR1^{hi}/CCR5^{hi} (intermediate monocytes).

Analogous to their human counterparts, murine subsets include classical Ly6C^{hi} monocytes, which are similar to the human CD14^{hi}CD16⁻ monocytes, and nonclassical Ly6C^{lo} monocytes, which are similar to human CD14^{dim}CD16⁺ monocytes. These human and murine cells patrol in the blood vessels until they are recruited to the organs in case of an injury or insult. Although the gene-expression profiles related to activation and trafficking have been well conserved between murine and human monocytes, the ratios of various subsets can differ between mice and humans. Therefore, it is important to be careful and take these differences into consideration when extending experimental murine monocyte studies to human disease.¹

Resident macrophages can be found in a variety of tissues, such as the brain, skin, lungs, liver, and spleen. Tissue-resident macrophages exhibit a large diversity of phenotypes and functions, based on their tissue of residence, raising the question of the origin of these cells (Davies et al. 2013). Recent fate-mapping studies have revealed that embryonic yolk sac and/or fetal liver progenitor cells are the source of many tissue-resident macrophages, such as those in the liver (i.e., Kupffer cells), skin, and central nervous system (i.e., microglia) (Gomez Perdiguero and Geissmann 2013). Tissue-resident macrophages are defined as a heterogeneous population of immune cells important for maintaining the homeostatic function of the specific tissue (Davies et al. 2013). Whether tissue macrophages are self-renewing or continuously replenished from the bone marrow still is a matter of debate. However, overwhelming evidence suggests that bone-marrow–derived circulating monocytes can be recruited to the site of injury early during inflammation in tissues, where they differentiate into macrophages. Classical and nonclassical monocytes are recruited in a sequential fashion, depending on the nature of insult (e.g., infection or infarction) and the injured tissue. Additionally, both resident macrophages and recruited monocytes reportedly are capable of self-renewal induced by certain cytokines, such as interleukin (IL)-4 (Jenkins et al. 2011).

The Kupffer cells in the liver are the largest population of tissue-resident macrophages and largely contribute to inflammatory reactions in the liver. The innate immune function of Kupffer cells not only is critical in the body's response to liver injury but also is crucial in tolerogenic responses to antigens in the liver. Kupffer cells are located in the hepatic sinusoids and fall into two major subsets (Klein et al. 2007):

Table 1 Monocyte Populations of Human and Mouse Origin

Monocytes		Markers	Function
Human	Classical	CD14 ^{hi} CD16 ⁻ CCR2 ⁺ CX3CR1 ^{lo}	Phagocytosis and inflammatory effectors
	Intermediate	CD14 ^{hi} CD16 ⁺ CCR2 ⁻ CX3CR1 ^{hi}	Inflammatory effectors
	Nonclassical	CD14 ^{dim} CD16 ⁺ CCR2 ⁻ CX3CR1 ^{hi}	Patrolling, antiviral role
Mouse	Classical	CD11b ⁺ Ly6C ^{hi} CCR2 ⁺ CX3CR1 ⁻	Inflammatory effectors
	Nonclassical	CD11b ⁺ Ly6C ^{lo} CCR2 ⁻ CX3CR1 ⁺	Patrolling, tissue repair

- Radiosensitive macrophages that are replaced rapidly by hematopoietic precursors and are important in inflammatory reactions; and
- Radioresistant, long-lived Kupffer cells that do not participate in inflammatory foci.

Mouse models frequently are used to investigate various aspects of macrophage function. However, as with the monocyte precursors, differences in the characteristics of murine and human macrophages exist that must be taken into account when using mice as preclinical models of disease (Mestas and Hughes 2004). For example, murine and human macrophages can differ in the expression of surface molecules called Toll-like receptors (TLRs) that are involved in macrophage activation, in their responses to immune activators, and in their production of nitric oxide.

Macrophage Polarization

Macrophages have a unique ability to alter their phenotypes and, thus, their functions, depending on tissue microenvironmental cues, such as the presence of cytokines, growth factors, pathogen-associated molecular pattern molecules (PAMPs), and damage-associated molecular pattern molecules (DAMPs). This process is known as polarization and results in the emergence of two macrophage phenotypes labeled M1 and M2 macrophages. M1 macrophages primarily have proinflammatory effects. For example, classically activated M1 macrophages help mediate the initial defense against intracellular bacteria and viruses; in addition, they are important for the response to a tissue injury. The M1 macrophages produce proinflammatory and stress mediators and cytokines, such as IL-1, tumor necrosis factor alpha (TNF α), interferon γ , IL-12, IL-18, nitric oxide, and reactive oxygen species (ROS), and can activate adaptive immune responses (Jouanguy et al. 1999; Shaughnessy and Swanson 2007). Once the infection or injury is

¹ Comprehensive transcriptomic analysis on unique human and murine immune-cell gene expression during differentiation, activation, and tissue-specific localization is available from project consortia such as ImmGen (www.immgen.org) and InnateDB (www.innatedb.ca).

controlled, macrophages convert to an anti-inflammatory, tissue-restorative phenotype in order to reign in excessive tissue-damaging inflammatory responses (Benoit et al. 2008; Noel et al. 2004). These cells usually are referred to as alternatively activated macrophages (M2) and help promote the resolution of inflammation as well as tissue repair (Sica and Mantovani 2012) (see figure 1). They can be distinguished from the M1 macrophages by the presence of high levels of several marker proteins (e.g., Fizz1, Mrc1, Ym1, and Arg1) (Gordon 2003; Mantovani et al. 2002).

The functional heterogeneity of macrophages is reflected in their differential, sometimes opposing, roles in various diseases (Sica and Mantovani 2012). For example, whereas M1 cells are essential for eliminating bacteria and viruses during acute infection, a dysregulated M1 response can result in collateral tissue damage. Thus, the proinflammatory function of M1 macrophages contributes to conditions such as autoimmune diseases (e.g., arthritis and multiple sclerosis) and metabolic diseases (e.g., insulin resistance, diabetes, and atherosclerosis). Similarly, although M2 macrophages often are associated with tissue repair and immune regulation, excessive M2 responses can contribute to chronic diseases such as atopic dermatitis, asthma, and tissue fibrosis. Additionally, diseases characterized by changes in the phenotype of the cells over time resulting from changes in the tissue environment also may be accompanied

by a switch in macrophage phenotype (i.e., macrophage plasticity). For example, during early stages of cancer, tumor-associated macrophages resemble the classically activated M1 cells, which promote anti-tumor immune responses. As the tumor progresses, however, these tumor-associated macrophages switch to a regulatory phenotype that suppresses anti-tumor immunity and facilitates tumor growth (Allavena et al. 2008). As another example, adipose-tissue macrophages in nonobese individuals primarily exhibit a wound-healing phenotype, with little production of proinflammatory cytokines. In obese patients, however, the adipose-tissue macrophages switch to a proinflammatory M1-like phenotype characterized by cytokine production that leads to insulin resistance (Zeyda and Stulnig 2007).

Although it is convenient to divide macrophages into M1 and M2 cells, it is important to note that this division is oversimplified. The M1 and M2 macrophages only represent the two extremes of a full spectrum of phenotypes, and within either category there are subpopulations with different phenotypes and functions. For example, the M2 cells can be classified into at least two subtypes, wound-healing and immune-regulatory macrophages (Edwards et al. 2006). The wound-healing macrophages develop in response to the cytokines IL-4 and IL-13 that are released by various types of leukocytes. Compared with M1 macrophages, these cells produce much lower levels of proinflammatory cytokines,

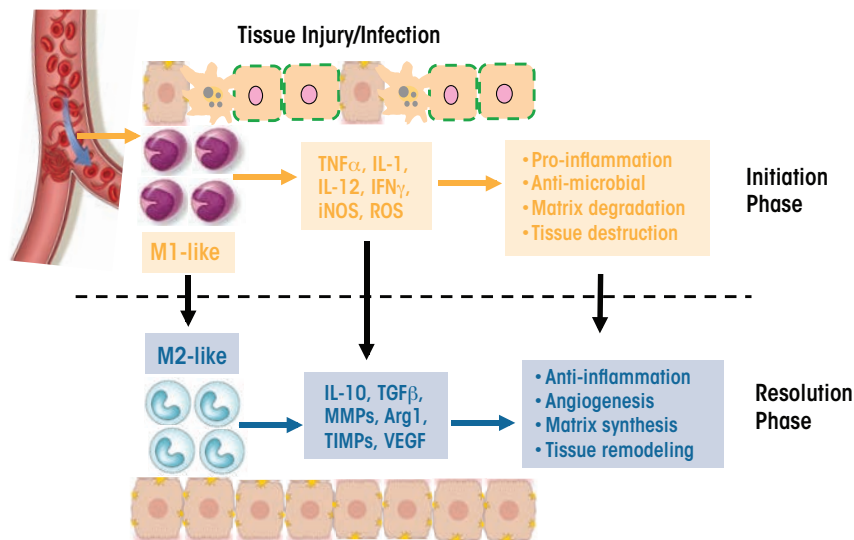


Figure 1 Schematic representation of macrophage plasticity and its involvement in tissue injury. Macrophages recruited to the site of an injury or infection during the initiation phase of the inflammatory reaction have an M1 phenotype. They produce proinflammatory and stress mediators and cytokines, such as tumor necrosis factor α (TNF α), interleukin (IL)-1 and -12, interferon γ (IFN γ), an enzyme generating nitric oxide (iNOS), and reactive oxygen species (ROS). These macrophages have proinflammatory and antimicrobial effects and lead to matrix degradation and tissue destruction. During the resolution phase of the injury, these M1 macrophages are converted into an M2 phenotype with a different cytokine and chemokine repertoire, including IL-10, transforming growth factor β (TGF- β), matrix metalloproteinases (MMPs), arginase 1 (Arg1), tissue inhibitors of metalloproteinases (TIMPs), and vascular epithelial growth factor (VEGF). These M2 macrophages have anti-inflammatory effects and promote blood-vessel formation (angiogenesis), matrix synthesis, and tissue remodeling.

ROS, and nitric oxide but higher levels of molecules that promote tissue regeneration and wound healing (e.g., mannose receptors, extracellular matrix components, and factors regulating matrix remodeling). Conversely, immune-regulatory macrophages arise during late stages of the adaptive immune response or in response to stress-induced upregulation of glucocorticoids. These macrophages are characterized by the production of high levels of IL-10. Factors that induce the generation of immune-regulatory macrophages include immune complexes, prostaglandins, apoptotic cells, adenosine, histamine, and adiponectin. Unlike the wound-healing macrophages, the regulatory macrophages do not induce extracellular matrix remodeling.

The plasticity of macrophage phenotypes is controlled by various intracellular molecular mechanisms, including signaling proteins, transcription factors, and epigenetic events. For example, activation of macrophages via TLRs and interferon receptors, which induces a signaling mechanism involving a molecule called STAT1,² steers their polarization toward the M1 phenotype (Qin et al. 2012). Conversely, alternative activation via IL-4/IL-13 and STAT6-mediated mechanisms generates the M2 phenotype (Daley et al. 2010; Moreno et al. 2003; Stolfi et al. 2011). Other M2-like phenotypes are induced via IL-10/STAT3 and IL-3/STAT5 signaling mechanisms (Sica and Mantovani 2012). Another important regulator of macrophage polarization is the enzyme JNK, which phosphorylates STAT6 (Shirakawa et al. 2011). Obese mice deficient in a JNK activator called MLK3 lack M1 macrophage polarization, suggesting a role for JNK in activation of the M1 phenotype (Gadang et al. 2013). IRF proteins, which modulate the transcription of certain genes, also are important regulators of macrophage polarization. For example, IRF5 activity promotes IL-12 gene transcription and is associated with an M1 phenotype, whereas repression of IRF5 induces IL-10, resulting in an M2 phenotype (Krausgruber et al. 2011). Similarly, activation of a regulatory protein complex called Notch/IRF8 leads to M1 polarization (Xu et al. 2012), whereas activation of M-CSF/IRF4 leads to M2 polarization. Another family of proteins called SOCSs also serves as essential regulators of macrophage polarization, with the specific cytokine stimulus and SOCS isoform involved determining whether the cells attain an M1 or M2 phenotype. Thus, the presence of IL-4 acting on SOCS1/STAT1 induces an M1 phenotype (Whyte et al. 2011), whereas interferon γ acting in concert with TLR can induce SOCS3/STAT3 and result in M2 macrophage polarization (Arnold et al. 2014). Various receptors located in the cells' nucleus, such as molecules called PPAR γ , PPAR δ , Kruppel like factor-4, and c-myc also contribute to macrophage polarization downstream of the IRF/STAT-SOCS pathway (Zhou et al. 2014). Finally, regulatory processes that affect DNA structure and gene expression without altering the DNA sequence (i.e., epigenetic mechanisms) promote the induction of an M2 phenotype and inhibit M1-characteristic

genes (Banerjee et al. 2013; Satoh et al. 2010). These epigenetic regulators include such factors as histone demethylase, Jumonji D3, and microRNA let-7c.

In ALD, macrophage imprinting and polarization to M1 or M2 phenotypes is influenced by cytokine mediators in the liver. The detailed investigation of pathways activated by cytokines and stress proteins in the liver during ALD will provide insights into the polarization of resident versus infiltrating liver macrophages.

Macrophages in ALD

Significance of Macrophages in Clinical ALD

Macrophages seem to play a central role in ALD. In fact, recent findings suggest the coexistence and complex inter-

Table 2 Complete Names of Enzymes and Other Molecules Mentioned in This Article and Their Abbreviations

Abbreviation	Complete Name
CCR2	C-C chemokine receptor 2
CD	Cluster of differentiation
CX3/CR1	C-X3-C motif chemokine receptor 1
ERK	Extracellular-signal-regulated kinase
IKK	Inhibitor of nuclear factor kappa-B kinase
IL	Interleukin
IRAK	Interleukin-1 receptor-associated kinase
IRF	Interferon regulatory factor
JNK	C-jun N-terminal kinase
LPS	Lipopolysaccharide
MCP	Monocyte chemoattractant protein
M-CSF	Macrophage colony-stimulating factor
MIP	Macrophage inflammatory protein
MLK	Mixed lineage kinase
PD-1	Programmed cell death protein 1
PPAR	Peroxisome proliferator-activated receptor
SOCS	Suppressor of cytokine signaling
STAT	Signal transducers and activators of transcription
TGF	Transforming growth factor
TIM-3	T-cell immunoglobulin mucin-3
TLR	Toll-like receptor
TNF α	Tumor necrosis factor alpha

² For a list of the complete names of this and other molecules mentioned in this article, see table 2.

actions of different types of macrophages in ALD (Lee et al. 2014). Thus, immunohistochemical analyses of liver samples from patients with alcoholic steatohepatitis identified macrophages that express receptors and cytokines commonly associated with M1 cells, as well as markers associated with M2 cells. Numerous other analyses have indicated that macrophage function may be clinically correlated with disease state in patients with alcoholic hepatitis and fibrosis, as follows:

- Increased macrophage numbers have been reported in both early (i.e., fatty liver) and late (i.e., hepatitis and cirrhosis) stages of ALD (Karakucuk et al. 1989), although no clear correlation exists between macrophage numbers and disease severity.
- The levels of chemokines involved in monocyte recruitment, particularly MCP-1, MIP-1 α , and MIP-1 β , were increased in the liver of patients with ALD (Afford et al. 1998).
- In analyses of gene-expression profiles, the expression of inflammatory genes was higher in macrophages from patients with alcohol-related cirrhosis than in macrophages from patients with Hepatitis C virus–related cirrhosis (Tapia-Abellan et al. 2012).
- Factors that imply monocyte activation, such as neopterin and leukocyte-function–associated antigen 3, were elevated in ALD patients (Luna-Casado et al. 1997).
- Circulating monocytes from ALD patients express TNF α receptors and spontaneously produce TNF α . When stimulated by LPS, they release even higher levels of TNF α (Gobejishvili et al. 2006; Zhang et al. 2001). Highly elevated TNF α levels in the blood, in turn, are associated with poorer outcomes in patients with acute alcoholic hepatitis (Bird et al. 1990). In some cases, normal levels of the anti-inflammatory cytokine IL-10 were linked to a failure to inhibit the excessive production of TNF α (Le Moine et al. 1995).
- Patients with alcoholic hepatitis and/or cirrhosis exhibit elevated levels of other cytokines (e.g., IL-6, IL-8, and IL-18) and chemokines produced by circulating monocytes and liver macrophages (Afford et al. 1998; Fisher et al. 1999). These increased cytokine levels are correlated with clinical outcomes (Khoruts et al. 1991; McClain and Cohen 1989).
- Global gene-expression profiling of liver samples from patients with alcohol-related cirrhosis demonstrated unique gene-expression patterns that differed between early and late stages of cirrhosis. Genes expressed at much higher levels in early than late stage of cirrhosis included those related to macrophage activation, proliferation, and migration (Lederer et al. 2006), emphasizing the role of macrophages in the progression of ALD.

Additional clinical studies evaluating macrophages and circulating monocytes from human patients at different stages of ALD are needed to understand the precise functional contributions of monocytes/macrophages to disease progression.

Role of Kupffer Cells in ALD

Kupffer cells are liver-resident macrophages that are activated through the CD14/TLR4 receptor complex in response to increased intestinal translocation of LPS during prolonged alcohol consumption and which may contribute to alcohol-induced liver injury. Animal studies have revealed that acute and chronic ethanol administration are associated with signs of CD14/TLR4 activation of macrophages in the liver, including upregulation of CD14 as well as increased production of TNF α , MCP-1, and ROS (Enomoto et al. 2001). Furthermore, depletion of liver macrophages through various approaches prevented alcohol-induced liver inflammation (Koop et al. 1997; Petrasek et al. 2012), confirming that the cells are needed to induce liver injury.

Researchers have investigated how alcohol consumption may trigger Kupffer-cell activation. ROS production may be one of the mechanisms contributing to increased sensitization of Kupffer cells to LPS in the alcoholic liver (Thakur et al. 2006). During prolonged alcohol exposure, Kupffer cells produce ROS, likely mediated by induction of an enzyme involved in alcohol metabolism in the liver (i.e., cytochrome P450 2E1) (Kono et al. 2000). The crucial role of ROS production in Kupffer-cell activation also was demonstrated in studies in which rats were pretreated with an agent that inhibits an enzyme essential for ROS production (i.e., NADPH oxidase). This pretreatment normalized ROS production in alcohol-fed rats as well as reduced phosphorylation of the signaling molecule ERK1/2 and inhibited production of the proinflammatory cytokine TNF α in Kupffer cells (Kono et al. 2000; Thakur et al. 2006).

Another essential component in alcohol-mediated Kupffer-cell activation is the CD14/TLR4 receptor complex. LPS-induced activation of this receptor complex on Kupffer cells triggers downstream signaling kinases (i.e., IRAK and IKK), ultimately leading to the induction of the proinflammatory cytokines TNF α , IL-6, and MCP-1. Consistent with this model, Kupffer cells from alcohol-fed mice are sensitized to LPS and exhibit increased LPS responses, leading to higher levels of TNF α (Nagy 2003) and MCP-1 (Mandrekar et al. 2011). Enhanced expression of multiple TLRs also can contribute to ROS-mediated Kupffer-cell sensitization in the alcoholic liver (Gustot et al. 2006). Hritz and colleagues (2008) and Inokuchi and colleagues (2011) confirmed the importance of TLR4 expression on Kupffer cells and bone-marrow–derived immune cells in ALD. However, it is unclear whether liver-resident Kupffer-cell–specific TLR4 is the only TLR

contributing to alcohol-mediated pathogenesis, and this issue requires further investigation using mice deficient in macrophage-specific TLR4. Nevertheless, the findings to date suggest that both alcohol-induced ROS and increased Kupffer-cell sensitization to endotoxin, which lead to enhanced proinflammatory responses, are major players in Kupffer-cell activation in ALD.

Inhibition of Kupffer-cell activation and reduction of proinflammatory cytokines—particularly inhibition of proinflammatory cytokine production by Kupffer cells—has been a major focus of efforts to alleviate ALD. For example, it may be possible to reverse Kupffer-cell sensitization by treating alcohol-exposed Kupffer-cell primary cultures with adiponectin, an anti-inflammatory adipokine (Thakur et al. 2006). Treatment with globular adiponectin prevents LPS-stimulated TNF α expression in Kupffer cells by activating the IL-10/STAT3/hemoxygenase-1 pathway and inducing M2 macrophages (Mandal et al. 2010, 2011). M2 macrophages, in turn, seem to be associated with reduced or limited liver injury, because in current drinkers with mild liver injury and steatosis, M2 macrophages are predominant, whereas patients with severe liver injury exhibit M1 macrophages (Wan et al. 2014). Another possible approach to ALD treatment may involve the desensitization of alcohol-exposed Kupffer cells by increasing IL-10 levels. The alcohol-induced decrease in IL-10 has been shown to contribute to the sensitization of macrophages, and studies in IL-10–deficient mice found increased alcohol-mediated proinflammatory cytokine production (Hill et al. 2002). Recent studies also have indicated an IL-10–mediated protective effect via activation of TLR3 in alcoholic liver (Byun et al. 2013).³ Selective targeting of TLR signaling pathways in Kupffer cells likely will provide better insights into the contribution of the balance between pro- and anti-inflammatory cytokine production in ALD.

Hepatic Infiltrating Macrophages in ALD

Tissue-resident macrophages, such as Kupffer cells in the liver, not only protect against pathogens but also help nourish and maintain the cells (i.e., exert trophic functions) and ensure tissue homeostasis. However, under stress conditions caused by infection or by inflammation in the absence of infection (i.e., sterile inflammation), additional monocytes infiltrate the damaged tissue and differentiate into macrophages that help clear the pathogens, remove dead cells and cell debris, and restore tissue homeostasis. In fact, in many disease models (e.g., peritoneal inflammation) the tissue macrophages that have been described actually are derived from such infiltrating monocytes (Ghosh et al. 2010).

Studies of acute and chronic liver injuries also have demonstrated the hepatic recruitment of monocytes. For example, acute treatment of mice with carbon tetrachloride (CCl₄), which causes liver damage, results in an influx of

infiltrating macrophages that can increase the total number of hepatic macrophages tenfold (Karlmark et al. 2009). A recent study in mice with chronic CCl₄-induced liver fibrosis demonstrated that infiltrating macrophages played an important role in the progression and regression of the fibrosis (Ramachandran et al. 2012). Similarly, in a mouse model of acetaminophen-induced liver injury, infiltrating macrophages recruited during the recovery phase contributed substantially to tissue repair (Holt et al. 2008).

Chronic alcohol-induced liver disease also is mediated and likely propagated by infiltrating immune cells, because chronic ethanol administration can cause accumulation of infiltrating macrophages in the liver of mice (Wang et al. 2014). The infiltrating macrophages consist of two subsets—Ly-6C^{hi} and Ly-6C^{low} cells—with distinct genetic profiles. The Ly-6C^{low} cells exhibit an anti-inflammatory and tissue-protective phenotype, expressing low levels of proinflammatory cytokines and high levels of anti-inflammatory molecules that may be involved in tissue repair (Arnold et al. 2007; Nahrendorf et al. 2007). Conversely, the Ly-6C^{hi} cells exhibit a proinflammatory tissue-damaging phenotype; however, upon phagocytosis of apoptotic hepatocytes, they seem to switch to a Ly-6C^{low} phenotype (Wang et al. 2014). The two subsets of infiltrating macrophages coexist and exhibit distinct, and sometimes opposite, functions in many models of inflammatory tissue injury. In a model of kidney injury, bone marrow Ly-6C^{hi} monocytes were recruited to the injured kidney, where they differentiated into functionally distinct Ly-6C^{low} cells (Lin et al. 2009). In the livers of animals with CCl₄-induced fibrosis, Ly-6C^{low} infiltrating macrophages, which were derived from the Ly-6C^{hi} cells, were important for resolving inflammation and fibrosis and restoring tissue homeostasis (Ramachandran et al. 2012). Studies of the contribution of infiltrating-macrophage subsets in myocardial infarction also have demonstrated sequential recruitment of Ly-6C^{hi} and Ly-6C^{low} cells into the tissue. The proinflammatory Ly-6C^{hi} cells, which infiltrate the tissue during the early phase of injury, have proteolytic and phagocytic functions. At a later phase of the myocardial infarction, Ly-6C^{low} cells are recruited that possess attenuated inflammatory properties and are involved in tissue repair by promoting blood-vessel formation (i.e., angiogenesis) and activation of heart muscle cells (i.e., myofibroblasts) (Nahrendorf et al. 2007).

In humans, an increase in the number (i.e., expansion) of the nonclassical CD14⁺CD16⁺ monocytes, which correspond to the Ly-6C^{low} infiltrating macrophages, occurs in a variety of inflammatory diseases, including rheumatoid arthritis, atherosclerosis, asthma, atopic eczema, pancreatitis, and alveolar proteinosis. Nonclassical CD14⁺CD16⁺ monocytes also expand in the circulation and liver of patients with chronic liver disease, suggesting their involvement in the progression of liver inflammation and fibrogenesis (Ziegler-Heitbrock 2007).

³ In addition to TLR3 and TLR4, increased expression of TLR2 and TLR8 has been identified by immunohistochemistry in liver biopsies from alcoholic hepatitis patients (Lee et al. 2014).

Macrophage Mediators in ALD

The heterogeneous populations of both resident and infiltrating macrophages present in the liver have multiple functions that are relevant to ALD (see figure 2):

- They can serve as antigen-presenting cells that display foreign molecules on their surface, thereby triggering adaptive immune responses.
- They may exhibit liver proteins that have been modified by malondialdehyde-acetaldehyde (i.e., malondialdehyde-acetaldehyde adducts) (Willis et al. 2002). This modification can change or impair the protein's functions. In patients with ALD, these adducts also may be associated with the presence of autoantibodies.
- They normally produce antimicrobial peptides and mediators and have microbial killing activities; however, these functions may be compromised during ALD.
- Through activation of TLR-mediated signaling, they may lead to increased expression of immunoinhibitory receptors called PD-1 and TIM-3 on T cells, thereby impairing antimicrobial activity in patients with alcoholic hepatitis (Markwick et al. 2015).
- Certain subpopulations (e.g. Ly6C^{hi} infiltrating macrophages) produce a variety of proinflammatory mediators,

including ROS, reactive nitrogen species, proinflammatory cytokines, and chemokines, thereby causing tissue damage.

Among the mediators identified, the cytokine TNF α has been extensively studied not only in patients with alcoholic hepatitis but also in animal models of ALD (Bird et al. 1990). The analyses found that mice lacking the TNF α receptor were protected from ALD (Yin et al. 1999); moreover, antibodies against TNF α were able to ameliorate alcohol-induced liver injury (Iimuro et al. 1997). Both of these findings indicate that TNF α is crucial in the pathophysiology of ALD. The role of IL-6 in ALD also has been widely investigated. Alcohol-fed, IL-6-deficient mice showed increased liver injury, suggesting a protective role for IL-6 (El-Assal et al. 2004). Additional analyses demonstrated that IL-6 reduces or increases inflammation in ALD in a cell-type-specific manner and exerts its effects via the STAT3 signaling molecule (Horiguchi et al. 2008), confirming the significant contribution of the IL-6/STAT3 axis in the development of ALD.

Other macrophage mediators involved in ALD include the chemokines IL-8, MCP-1, and MIF, which either inhibit leukocytes or help recruit them to the sites of injury and inflammation (Barnes et al. 2013; Mandrekar et al. 2011). Whereas IL-8 induces neutrophil infiltration, MCP-1 and MIF, which primarily are produced by Kupffer cells and infiltrating macrophages, facilitate the recruitment of additional monocytes/macrophages in ALD. Chemokines also induce the activation of stellate cells, which helps promote disease progression to liver fibrosis. Thus, the recruitment

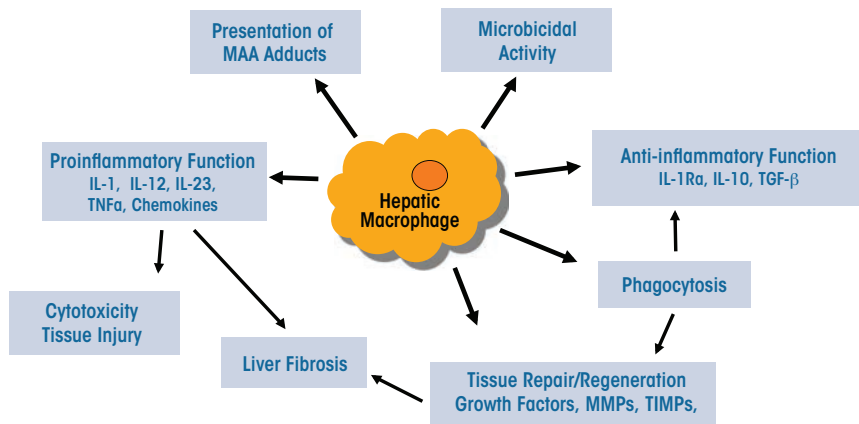


Figure 2 Macrophage functions in alcoholic liver disease. Macrophages fulfill a variety of functions in the context of alcoholic liver disease, including both proinflammatory and anti-inflammatory functions, depending on the state of the disease. These activities include the production of proinflammatory cytokines (e.g., interleukin [IL]-1, -12, and -23; tumor necrosis factor alpha [TNF α]) and chemokines, as well as of anti-inflammatory cytokines (e.g., IL-10, IL-1 receptor alpha [IL-1Ra]), and transforming growth factor beta [TGF- β]). Other relevant activities include presentation of malondialdehyde-acetaldehyde (MAA) adducts and microbicidal and phagocytotic activity, as well as tissue repair and regeneration through the production of growth factors, matrix metalloproteinases (MMPs), and tissue inhibitors of metalloproteinases (TIMPs).

of inflammatory cells sets off a vicious cycle in which inflammatory and stellate cells stimulate one another, leading to fibrosis and cirrhosis (Karlmark et al. 2009). Given the central role that MCP-1 and MIF seem to play in ALD, chemokines can be considered likely therapeutic targets for this condition (Mandrekar et al. 2011; Seki et al. 2009).

Other macrophage mediators in addition to cytokines and chemokines include the complement system and adipokines such as adiponectin and leptin. The C3 and C5 complement systems are activated in macrophages during early phases of murine ALD and contribute to disease initiation and progression (Roychowdhury et al. 2009). Adipokines, in contrast, seem to negatively regulate macrophage function in murine ALD. Identification of novel macrophage mediators that can regulate polarization and thus influence development and progression in ALD is needed.

As liver injury progresses, macrophages also are needed to clear dead cells or cellular debris by phagocytosis, which is a critical step for successful resolution of inflammation and promotion of tissue repair. As a result of phagocytosis, macrophages begin to produce anti-inflammatory cytokines, such as IL-10 and TGF- β (Henson and Bratton 2013; Kornis et al. 2011; Xiao et al. 2008), as well as growth factors and tissue-remodeling mediators that have proinflammatory effects. Thus, a recent immunohistochemistry study observed robust TGF- β expression in macrophages of liver samples from alcoholic hepatitis patients (Lee et al. 2014). When liver injury persists, however, the chronic inflammation and tissue-repair processes can lead to tissue fibrosis. Insufficient oxygen supply to the tissue (i.e., hypoxia) may be a factor in this process, because liver tissue hypoxia has been observed after chronic ethanol feeding (Arteel et al. 1996). Hypoxia causes stabilization and activation of proteins called hypoxia-inducible factors (HIFs), which regulate multiple pathways that control cell survival, proliferation, and metabolism. Macrophages are known to accumulate in large numbers within hypoxic areas of injured tissues (Murdoch et al. 2005). In a mouse model of liver injury, chronic liver injury induced macrophage expression of HIF1 α , which promotes fibrosis by regulating the production of profibrogenic mediators (Copple et al. 2012; Mehal 2012).

Oxidative-stress-mediated activation of macrophages and subsequent production of cytokines that influence macrophage polarization are major contributors to inflammation in ALD.

ALD Therapy—Are Macrophages a Plausible Target?

Regardless of disease stage, abstinence from alcohol has been the most effective treatment in ALD. However, patients often lack motivation and compliance, leading to relapse. Another approach includes aggressive nutritional and anti-oxidant therapies using zinc (Kang and Zhou 2005), vitamins, and S-adenosylmethionine to restore nutritional status in alcoholic cirrhosis, albeit with limited beneficial outcomes. Alternative therapies using silymarin

and betaine also have been suggested for future clinical trials in ALD (Frazier et al. 2011). Anti-inflammatory treatments targeting macrophage function, such as treatment with corticosteroids, pentoxifylline, or anti-TNF α antibodies, also have been evaluated for ALD patients for more than 30 years. Success, however, has been limited to date. Clinical trials using glucocorticoids in patients with acute alcoholic hepatitis showed minor benefits but ultimately were terminated because of a heightened risk of sepsis and gastrointestinal bleeding (Maddrey et al. 1978). Subsequent studies evaluated the effects of therapy with specific anti-TNF α antibodies, again with limited success. Consequently, the need for the development of effective strategies for patients with alcoholic hepatitis and cirrhosis remains unfulfilled.

To address this need, researchers also are assessing a variety of strategies to target macrophages in preclinical murine ALD studies. These strategies often use cytokine inhibitors or intracellular mediators to regulate cytokine production, with some promising results:

- Approaches targeting alcohol-induced IL-1 β signaling in macrophages using an IL-1 receptor antagonist (e.g., anakinra) have yielded a reduction in alcohol-induced inflammatory responses in murine liver (Petrasek et al. 2012).
- Studies using globular adiponectin to induce IL-10 production in Kupffer cells via the enzyme heme oxygenase-1 alleviated murine ALD (Mandal et al. 2010). Induction of this enzyme in liver macrophages by modulating carbon monoxide availability in the liver also had beneficial effects in mouse models of ALD (Bakhautdin et al. 2014).
- Efforts centering on the MCP-1 and MIF produced by Kupffer cells and infiltrating macrophages in the mouse alcoholic liver identified these chemokines as effective targets (Barnes et al. 2013; Mandrekar et al. 2011).
- Strategies targeting stress-induced heat-shock protein 90 with specific inhibitors—an approach currently assessed in clinical trials for cancer—helped ameliorate ALD by inhibiting macrophage inflammatory responses in murine liver (Ambade et al. 2014).

These studies collectively support clinical evaluations of macrophage-targeting therapies in alcoholic-hepatitis patients. Clinical research combining biologics, small-molecule drugs, and antioxidant therapies targeting macrophage function and phenotype may provide lasting therapeutic efficacy in alcoholic hepatitis and cirrhosis.

Conclusion and Perspectives

As in many chronic inflammatory diseases, macrophages have emerged as critical players and perhaps a therapeutic target in ALD. However, depleting all hepatic macrophages will not be an effective approach because of the heterogeneity

and phenotype diversity of these cells; the specific populations to be targeted for maximum benefit remain to be determined.

There are many questions that still need to be addressed with regard to the role of hepatic macrophages in ALD development. For example, how do infiltrating monocytes differentiate within the liver during ALD? What are the tissue-environmental cues and molecular-signaling pathways that drive the reprogramming of infiltrating macrophages in the alcoholic liver? Another important question is how the

number of infiltrating macrophages is controlled after tissue homeostasis is reestablished. Do excess cells undergo apoptosis or do they emigrate? Moreover, in-depth knowledge of the molecules and pathways that control and regulate the phenotype and functions of hepatic macrophages is critical for developing therapeutic strategies to treat ALD. For example, the functions of Ly-6C^{low} infiltrating macrophages in tissue repair and wound healing can be utilized to prevent chronic liver inflammation during the early phase

Glossary

Adipokine: A bioactive factor produced and secreted by fat (adipose) tissue that can modulate the function of other tissues.

Alveolar proteinosis: A chronic lung disease characterized by the filling of the *alveoli* with a protein-like material that prevents ventilation of the affected area; results in shortness of breath, coughing, chest pain, weight loss, and spitting up of blood.

Alveoli: Sac-like structures in the lungs where the gas exchange between the inhaled air and blood takes place.

Autoantibody: An immune molecule (i.e., antibody) formed in response to, and acting against, one of the individual's own normal tissue constituents.

Chemokine: Any of a family of small *cytokines* that induce the movement of *leukocytes* (e.g., to the site of an infection).

Complement system: A complex system of about 20 distinct proteins, their receptors, and related regulatory proteins that induce the destruction (i.e., lysis) of cells during an immune response as well as regulate various other biologic functions (e.g., *phagocytosis*).

Cytokine: Any of the non-antibody proteins released by one type of immune cell on contact with a specific antigen that acts as a mediator between cells (e.g., in the generation of an immune response).

Damage-associated molecular pattern molecules (DAMPs): Molecules that

can initiate an immune response in response to cell or tissue damage (i.e., as part of a noninfectious inflammatory response).

Endotoxin: Toxic molecule associated with the membranes of certain bacteria that are released when the cells are disrupted and have numerous biologic effects (e.g., fever, altered resistance to bacterial infection, shock); endotoxins are composed of lipopolysaccharides (LPS).

Epigenetic: Pertaining to mechanisms that alter the activity of genes without changing their DNA sequences (e.g., by chemically modifying the DNA or altering the accessibility of the DNA for regulatory proteins).

Fate mapping: An experimental approach to determine the origins of various tissues in the adult organism from the embryonic structures and to track the development of specific cells through several developmental stages.

Leukocytes: Any of variety of white blood cells, such as monocytes or lymphocytes.

Macrophage polarization: Process during which macrophages acquire specific characteristics and functions in response to external signals (e.g., certain *cytokines*); the two main types of polarized macrophages are M1 (classically activated) and M2 (alternatively activated) macrophages, each of which produces specific *cytokines* and induces specific immune responses.

Malondialdehyde: An organic compound formed during the degradation of lipids by reactive oxygen species (e.g., during alcohol metabolism, which results in formation of reactive oxygen species); malondialdehyde can interact with certain DNA building blocks, forming DNA adducts, which can induce mutations in the DNA.

Pathogen-associated molecular pattern molecules (PAMPs): Molecules that can initiate an immune response in response to infection with a pathogen (i.e., as part of an infectious inflammatory response).

Phagocytosis: Process by which a cell (e.g., a macrophage) takes up microorganisms or cell fragments in membrane-enclosed vesicles in which the engulfed material is killed and digested.

Steatohepatitis: Condition in which fat droplets accumulate in the liver (e.g., as a consequence of alcohol misuse) with simultaneous inflammation of the liver.

Stellate cell: Cell type found in the liver with a characteristic star-like shape that is mainly responsible for fat storage in the liver as well as for collagen production; source of excess collagen produced during hepatitis.

Tolerogenic: Capable of inducing immunologic tolerance (i.e., lack of a reaction to a molecule that would normally trigger an immune response).

of ALD. The conversion of the proinflammatory tissue-damaging Ly-6C^{hi} infiltrating macrophages to anti-inflammatory tissue restorative Ly-6C^{low} cells can serve as a target for treatment of advanced stages of ALD, such as alcoholic steatohepatitis, and has been suggested in human and mouse studies (Singal et al. 2013). Inhibition of macrophage-mediated inflammation is already being used as a therapeutic option in other conditions; for example, agents such as statins, thiazolidinedione, and n-3 fatty acids, which can prevent macrophage-mediated inflammation, are a preferred strategy in diabetes treatment (Ji et al. 2009; Methe et al. 2005; Ramirez et al. 2008; Yeop Han et al. 2010). These therapies also warrant evaluation for their effects in attenuating liver injury and inflammation in alcoholic steatohepatitis.

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The Role of Innate Immunity in Alcoholic Liver Disease

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The innate immune system represents the first-line response to invading microbes, tissue damage, or aberrant cell growth. Many of the proteins and cells involved in innate immunity are produced by, and reside in, the liver. This abundance in immune cells and proteins reflects the liver's adaptation to various immune challenges but also makes the organ particularly vulnerable to alcohol's effects. Heavy alcohol consumption may produce leakage of microbes and microbial products from the gastrointestinal tract, which quickly reach the liver via the portal vein. Exposure to these immune challenges and to alcohol and its breakdown products dysregulates the liver's normally fine-tuned immune signaling pathways, leading to activation of various cellular sensors of pathogen- or damage-associated molecular patterns. The ensuing expression of pro-inflammatory cytokines (e.g., tumor necrosis factor α [TNF α], interleukin [IL]-8, and IL-1 β) results in cellular dysfunction that contributes to alcoholic liver disease (ALD). Investigations into the roles of the various components of liver innate immunity in ALD have begun to uncover the molecular basis of this disease. Further progress in this area may help inform the development of interventions targeting the innate system to augment current treatments of ALD. These treatments could include antibodies against pro-inflammatory cytokines, use of anti-inflammatory cytokines, or suppression of alcohol-induced epigenetic regulators of innate immunity.

Key words: Alcohol use, abuse and dependence; heavy alcohol drinking; alcohol effects and consequences; alcoholic liver disease; liver; gastrointestinal tract; immunity; innate immune system; immune cells; cytokines; chemokines; inflammation

Heavy consumption of alcohol poses a well-known health risk worldwide. Alcohol's effects on health and well-being are numerous and include injuries and fatalities resulting from alcohol-induced incapacitation. Moreover, chronic and heavy alcohol consumption affects the integrity and function of vital tissues and organs, causing slow but significant structural and functional damage over time. One of alcohol's principal actions is damage to the liver, the primary organ for its metabolism. As a result, some 90 percent of heavy drinkers (i.e., those drinking 60 g or more of alcohol per day)—and even some who drink less—develop fatty liver (i.e., steatosis) (O'Shea et al. 2009). Up to one-third of heavy drinkers may incur more

extensive liver injury, including alcoholic hepatitis, scarring (i.e., fibrosis), cirrhosis, or liver cancer (Gao et al. 2011). Moreover, about 70 percent of individuals who develop alcoholic hepatitis will progress to cirrhosis (Schwartz and Reinus 2012). The spectrum of alcohol-induced liver injuries ranging from steatosis to cirrhosis, defined here as alcoholic liver disease (ALD), is therefore a major cause of liver impairment worldwide (Gao et al. 2011).

A major contributor to ALD is alcohol-induced activation of liver innate immunity, precipitating disorders ranging from localized and transient inflammation to widespread hepatocellular injury and tissue damage (Cohen and Nagy 2011; Gao et al.

2011; Orman et al. 2013; Seki and Schnabl 2012; Wang et al. 2012). Given the pivotal role of the innate immune system in protecting the liver against foreign agents, it may seem surprising that some of the worst outcomes of alcohol-induced liver disease are the result of activation of innate immune cells. But, in fact, recent studies have revealed that alcohol induces immune activation, which drives the progression of ALD.

Innate immunity comprises chemical-physical barriers (e.g., epidermal cells, mucous membranes, and pH), as well as cellular defenses against any invading microbe or agent the immune system perceives as dangerous to the body's cells and tissues (Gao et al. 2011). These cellular defenses, which

include both immune cells (e.g., macrophages and dendritic cells) and proteins (e.g., cytokines), normally are well balanced to sense and respond to harmful agents while avoiding unnecessary immune activation. Alcohol disrupts this balance, triggering immune responses that result in inflammation (Gao et al. 2011; Seki and Schnabl 2012; Szabo et al. 2011; Wang et al. 2012). Continued high alcohol intake fuels a multistage process in which alcohol-induced liver damage advances along a continuum of steatosis, inflammation, and fibrosis, to the final stage, cirrhosis, marked by widespread tissue deformation and damage (Gao et al. 2011; Orman et al. 2013; Seki and Schnabl 2012; Wang et al. 2012).

It has been known for some time that alcohol consumption triggers inflammation of the liver, but how alcohol brings about this disease state has long remained unclear. More recently, researchers have uncovered key roles of Toll-like receptors (TLRs), whose activation during alcohol exposure results in upregulation of pro-inflammatory cytokines (e.g., tumor necrosis factor α [TNF α] and interleukin [IL]-1 β) and chemokines (e.g., monocyte chemoattractant protein [MCP]-1). Moreover, these immune responses result in production of reactive oxygen species (ROS), epigenetic changes, and infiltration of tissues with circulating monocytes and neutrophils (Gao et al. 2011; Petrasek et al. 2013; Seki and Schnabl 2012; Szabo et al. 2011; Wang et al. 2012).

Although the exact molecular mechanisms through which alcohol activates innate immune cells are not entirely understood, there is increasing evidence for the close relationship between the effects of alcohol on the gastrointestinal (GI) tract and injury to the liver. Heavy alcohol consumption changes the composition of microbial communities in the GI system, tipping the balance toward more pathogenic species. Recent observations in animal models suggest that these changes are involved in promoting ALD (Yan and Schnabl 2012). Alcohol also seems to

disrupt the structural integrity of the gut, causing release of bacteria and bacterial products into the circulation, which activates innate immune responses (Rao 2009; Seki and Schnabl 2012; Yan and Schnabl 2012). Because the GI tract is closely connected to the liver via the portal vein, the liver is a focal point for these alcohol-induced, gut-derived immune challenges.

Receptors located on resident immune cells in the liver (i.e., Kupffer cells; see sidebar, “Liver Cell Types and Their Roles in ALD”) sense and transmit these immune challenges. These receptors are specifically adapted to the high-challenge environment of the liver, and this adaptation contributes to the decreased responsiveness to immune challenges (i.e., liver tolerance) in healthy individuals (Petrasek et al. 2013; Seki and Schnabl 2012). However, alcohol’s effects on the gut and on immune cells, such as Kupffer cells, reduce liver tolerance and thus increase the potential for persistent inflammation. For example, microbial metabolites and cellular products released in response to the damage caused by alcohol and its metabolites activate cell surface (e.g., TLR4) and intracellular (e.g., nucleotide-binding oligomerization domain [NOD]-like) receptors (Cohen et al. 2011; Petrasek et al. 2013). This activation triggers the expression of pro-inflammatory genes, secretion of cytokines, and recruitment of various immune cells.

Additional findings suggest that alcohol exposure leads to heritable changes in how genes are expressed (e.g., epigenetic regulation) (Curtis et al. 2013). These long-lasting changes in gene expression may shift production of immune cells from anti- to pro-inflammatory cells and may induce other cellular changes that promote inflammation and ALD. Alcohol consumption also destabilizes reduction and oxidation processes (i.e., the redox balance) in the liver (Cohen et al. 2011), leading to increased production of destructive ROS that damage tissues and thus activate innate immune cells in the organ.

The goal of this review is to highlight recent advances in efforts to unravel the role of innate immunity in ALD. The following sections will focus on knowledge gleaned from recent studies of the roles of innate immune cells, proteins, and pathways in the development and progression of ALD. Although ALD is a human disease, much of the current knowledge of the role of innate immunity in ALD has been inferred from animal and in vitro cellular models of alcohol exposure. The significant degree of conservation in innate immune pathways from mouse to human bolsters the idea that many, if not most, findings in these animal and cellular models can be extrapolated to people. However, most of the information from the animal and cellular models discussed in this review awaits confirmation in studies with human subjects. The article will also explore how this knowledge may be used for treating and managing this disease.

The Natural History of ALD

Approximately 30 percent of people who regularly consume large amounts of alcohol have a significantly increased risk for developing ALD (Lucey et al. 2009; O’Shea et al. 2010), which becomes chronic and progressively worse if alcohol consumption continues unchecked (Gao et al. 2011). The disease typically commences with the development of fatty liver (i.e., hepatic steatosis); with continued heavy alcohol consumption, steatosis may transition to inflammation, resulting in tissue damage and fibrosis (see figure 1). Ultimately, chronic ALD results in extensive organ damage and disease characterized by necrosis (i.e., cirrhosis), and in about 2 percent of cases, cancer (i.e., hepatocellular carcinoma) may develop (Orman et al. 2013; Schwartz and Reinus 2012). Alcoholic hepatitis—an acute manifestation of ALD that may coincide with clinical signs of fatty liver (in which case it is termed

alcoholic steatohepatitis) (Lucey et al. 2009)—may occur at any stage of the disease process and significantly predisposes patients to developing cirrhosis.

The first stage in ALD, hepatic steatosis, involves several processes. Alcohol's metabolism generates an overabundance of the metabolic intermediate nicotinamide adenine dinucleotide in its reduced form (NADH), which stimulates the synthesis of excess

fatty acids in the liver (Lieber 2004). In addition, recent evidence has shown significant involvement of innate immune pathways in steatosis (Mandrekar et al. 2011). This evidence points to substantial crosstalk between metabolic and immune pathways and highlights the multifactorial nature of this initial stage. Steatosis typically resolves with abstinence from alcohol in people who have no other conditions (e.g., obesity) that promote steatosis.

However, continued alcohol use may lead to alcoholic hepatitis, a moderate to severe disorder arising from acute alcohol-induced inflammation for which no highly effective treatment currently is available.

Chronic alcohol use may also lead to the development of fibrosis (Hernandez-Gea and Friedman 2011), characterized by the generation of scar tissue composed of extracellular matrix proteins, such as collagens. As

Liver Cell Types and Their Roles in ALD

Kupffer Cells

Kupffer cells are macrophages located in the liver sinusoids. They usually are among the first cells exposed to alcohol-induced, microbe-derived immunogenic challenges originating from the gut, including lipopolysaccharides (LPSs) and peptidoglycans. Kupffer cells have a dual role in mediating pro-inflammatory responses and moderating these responses through expression of anti-inflammatory cytokines. They express Toll-like receptors (TLRs), including TLR4, TLR2, TLR3, and TLR9, which, on contact with LPS, lipoteichoic acid (a component of the cell walls in Gram-positive bacteria), viral RNA, and CpG-island DNA, respectively, trigger pro-inflammatory response pathways. For example, in response to LPS stimulation, Kupffer cells produce inflammatory cytokines (e.g., tumor necrosis factor α [TNF α], interleukin [IL]-1 β , IL-6, and IL-10) and several chemokines through the central regulator of inflammation, nuclear factor κ B (NF- κ B). Secretion of IL-12 and IL-18 activates production of interferon γ (IFN- γ) in natural killers cells, and production of transforming growth factor β 1 (TGF- β 1) and ROS contribute to alcohol-induced fibrogenesis in liver tissues. Kupffer cells contribute to liver tolerance by expressing anti-inflammatory IL-10 on exposure to LPS.

Hepatic Stellate Cells

Hepatic stellate cells (HSCs) are activated by liver damage and express TLR2, TLR4, and TLR9, which respond to lipoteichoic acid, LPS, and CpG DNA, respectively. TLR stimulation in HSCs results in expression of IL-6, TGF- β 1, and monocyte chemotactic protein (MCP-1). On activation, HSCs differentiate into myofibroblasts,

representing the major producers of extracellular matrix, which contributes to fibrosis.

Hepatocytes

Hepatocytes are epithelial cells and the major cell type of the liver. They significantly contribute to elimination of inflammation-inducing LPS from circulating blood. LPS uptake by hepatocytes requires activity of the TLR4–CD14–MD-2 complex. Hepatocytes are the target for TNF α released by Kupffer cells in response to alcohol and LPS exposure and may undergo apoptosis or necrosis in response to TNF α receptor activation.

Hepatic Dendritic Cells

Activation of TLR9 and TLR7 on or in specialized plasmacytoid dendritic cells results in production of IFN- α . Conventional dendritic cells of the liver respond to LPS or lipoteichoic acid via activation of TLR4 or TLR2 by producing TNF α , IL-12, or IL-6.

Biliary Epithelial Cells

Biliary epithelial cells express TLRs 1 through 10 and exhibit activation of NF- κ B expression and TNF α expression after stimulation with high doses of alcohol-induced LPS.

Sinusoidal Endothelial Cells

Sinusoidal epithelial cells (SECs) line the hepatic sinusoids and express TLR4–CD14 along with TLR9. Exposing SECs to LPS downregulates NF- κ B activation, CD54 expression, and leukocyte adhesion. In these cells, LPS tolerance is not controlled via TLR4 expression, and the role of SECs in uptake of LPS in the liver is unclear.

in steatosis, both aberrant metabolic processes and activation of immune responses play roles in the development and progression of fibrosis. Acetaldehyde generated during the oxidative breakdown of alcohol inhibits certain immune cells (i.e., natural killer cells) that normally moderate fibrosis by inducing apoptosis in activated hepatic stellate cells (HSCs) (Hernandez-Gea and Friedman 2011; Orman et al. 2013). In addition, cytokines secreted by Kupffer cells, as well as inflammatory scar-associated macrophages recruited from the periphery (Ramachandran and Iredale 2012), activate quiescent HSCs, resulting in the development and proliferation of extracellular matrix-producing myofibroblasts, whose activity precipitates fibrosis.

About 10 to 20 percent of patients with fibrosis who continue to heavily consume alcohol progress to the final stage of ALD, cirrhosis (Orman et al. 2013). This disease stage is characterized by widespread damage to the liver, including fibrotic deformation of tissues and blood vessels, as well as necrosis of cells. The main features of cirrhosis are the formation of nodules of varying sizes, which signify localized regeneration of lost tissues, and the obstruction of blood vessels, which causes portal hypertension. Release of immunogenic cellular debris from necrotic liver cells and the loss of the liver's ability to clear microbial and other pro-inflammatory metabolites from the circulation results in unremitting stimulation of innate immune pathways. As a result, cirrhosis generally is associated with a poor prognosis, with a median survival time of about 10 years. Further, liver cancer (i.e., hepatocellular carcinoma) is seen in about 2 percent of patients with cirrhosis (Orman et al. 2013).

Innate Immunity and ALD

As mentioned above, various innate immune cells and their actions play prominent and complex roles in the

initiation and progression of ALD. The oxidative breakdown of alcohol by dedicated alcohol dehydrogenases and by cytochrome P450 monooxygenases generates ROS that may damage proteins, lipids, and other cellular structures. In addition, alcohol's breakdown metabolite, acetaldehyde, exerts toxic effects on cellular structures and DNA (Wang et al. 2012). The ROS- and acetaldehyde-induced cell damage activates innate immune cells, triggering an inflammatory reaction even in the absence of invading pathogens (i.e., sterile inflammation)

(Kubes and Mehal 2012). Sterile inflammation results from activation of pro-inflammatory pathways in immune and other cells carrying receptors for detecting damage-associated molecular patterns (DAMPs; also called alarmins). These molecules are released by stressed or necrotic cells, such as hepatocytes damaged by alcohol or its breakdown products. These immune pathways are essential for clearing damaged cells and cellular debris from tissues; however, their persistent activation by alcohol leads to repeated cycles of cell damage and

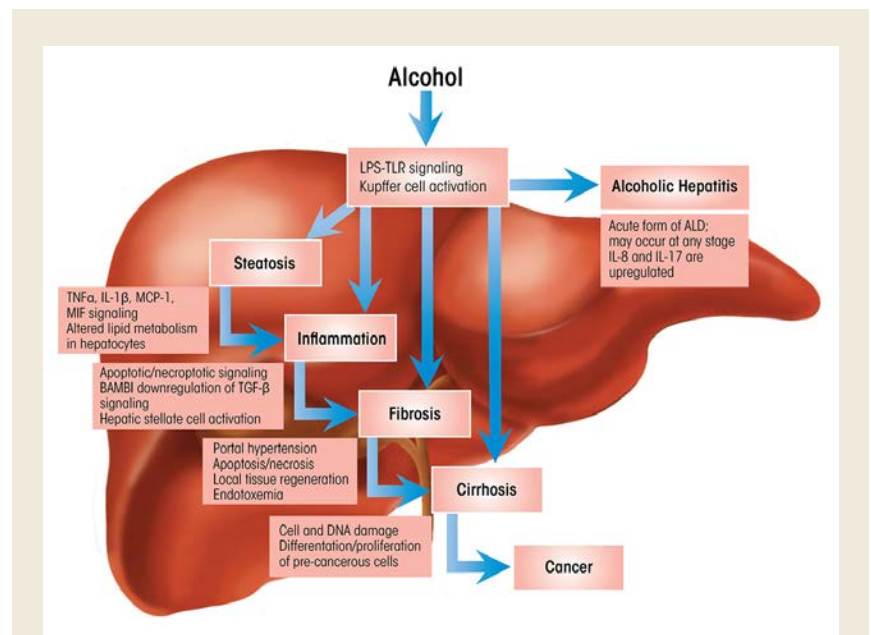


Figure 1 The role of innate immunity in the natural history of alcoholic liver disease (ALD). Heavy alcohol consumption causes release of bacterial products (i.e., lipopolysaccharides [LPSs]) from the gut into the bloodstream. These LPSs lead to activation of liver innate immunity by stimulating Toll-like receptor 4 (TLR 4) signaling on Kupffer cells and hepatocytes. The damaging effects of alcohol and its metabolism on cells trigger additional immune responses. Steatosis and inflammation in hepatocytes represent the early stages of ALD; continued alcohol-induced inflammation leads to apoptosis/necroptosis in hepatocytes. Downregulation of BMP and activin membrane-bound inhibitor (BAMBI) and increased transforming growth factor β (TGF- β) signaling activate hepatic stellate cells, which differentiate into myofibroblasts causing fibrosis. About 10 to 20 percent of patients with ALD (about 70 percent of patients with alcoholic hepatitis) progress to cirrhosis. Differentiation and proliferation of precancerous liver cells present in cirrhosis lead to cancer in about 10 percent of cirrhosis patients. Acute alcohol-induced inflammation (i.e., alcoholic hepatitis), characterized by high levels of pro-inflammatory cytokines (e.g., interleukin [IL]-17 and IL-8), may occur at any stage of ALD and, in severe cases, may cause death in about 50 percent of patients.

resultant stimulation of innate immune cells, causing chronic inflammation of the liver.

Chronic alcohol intake also has more indirect effects that play a major role in ALD. Excessive alcohol consumption changes the composition of microbes found in the gut (i.e., the gut microbiome) and seems to contribute to loss of tight cellular connections in the small intestine (Rao 2009). This alcohol-induced breach of the gut barrier causes release of immunogenic compounds, primarily bacterial lipopolysaccharide (LPS, also known as endotoxin) (Bode et al. 1987) and other cell-wall constituents like peptidoglycans and microbial DNA, into the circulation. LPS is a major trigger of pro-inflammatory pathways, and its role in inflammation of the liver and stimulation of innate immunity is well established (Petrasek et al. 2013; Rao 2009; Seki and Schnabl 2012). Unmethylated CpG-containing DNAs released from bacterial cells have also emerged as a significant activator of liver innate immunity (Petrasek et al. 2013; Seki and Schnabl 2012).

In addition, alcohol depletes the levels of *S*-adenosylmethionine (SAM) (Lieber 2000) a universal methyl donor important for epigenetic regulation of transcription. Intragastric feeding of SAM in rats diminished the activity of alcohol-activated innate immune pathways (Oliva et al. 2011), highlighting the potential role of SAM in moderating innate immune responses.

Effects on Kupffer Cells and the Complement System

Kupffer cells are the resident macrophages of the liver and have key functions in innate immunity. Because of the crucial role Kupffer cells play in defending the liver against pathogens, they are among the first immune cells to respond to alcohol-induced surges of microbial metabolites, such as LPS. These bacterial products engage with the TLR4 receptors on the Kupffer cells. LPS-induced TLR4 activation stimulates the production of cytokines,

including TNF α , IL-6, and IL-1 β , and of chemokines, such as KC (CXCL1), MIP-2 (CXCL2), MCP-1 (CCL2), and RANTES (Gao et al. 2011; Mandrekar and Szabo 2009; Petrasek et al. 2013; Szabo et al. 2011). Secretion of these molecules from Kupffer cells, in turn, activates a pro-inflammatory cascade affecting processes in other liver cells. For example, tumor necrosis factor α (TNF α) secreted by activated Kupffer cells interacts with TNF α receptors on hepatocytes (see figures 2 and 3). TNF α receptor activation, in turn, contributes to steatosis and, ultimately, to necrosis and apoptosis of the hepatocytes that normally clear LPS and other xenobiotic compounds from the liver (Gao et al. 2011). Alcohol also affects macrophage plasticity—the environmentally determined activation to either classical pro-inflammatory (i.e., M1) or alternative anti-inflammatory (i.e., M2) macrophages. Alcohol represses activation to the M2 phenotype and thus skews macrophage distributions toward the pro-inflammatory M1 state (Louvet et al. 2011; Mandal et al. 2011).

The complement system is a major antimicrobial defense pathway that straddles both innate and adaptive immunity. Most of the proteins in this system are produced in the liver. Alcohol activates complement pathways (Cohen et al. 2010) and, in heavy drinkers, can also compromise complement action by impairing liver function. Complement activation results in the production of C3a and C5a anaphylatoxins—short peptides of the complement system. Through interactions with C3a and C5a receptors, these anaphylatoxins trigger the production of pro-inflammatory innate immune proteins, such as cytokines, in leukocytes and thus contribute to inflammation (Cohen et al. 2011) (see figures 2 and 4).

Expression and Activation of TLRs

TLRs are expressed on many cells of the liver, including Kupffer cells, endothelial cells, dendritic cells, biliary epithelial cells, HSCs, and hepato-

cytes. The expression of the TLRs and their level of responsiveness on these different cells normally are adjusted to promote appropriate reactions to immune challenges and prevent misplaced and potentially damaging responses (Petrasek et al. 2013). Alcohol exposure turns up the dial of this finely tuned TLR network, heightening TLR responses to external and internal triggers, such as pathogen-associated molecular patterns (PAMPs) and DAMPs, respectively (Petrasek et al. 2013; Seki and Schnabl 2012).

TLR4 plays a very prominent role in alcohol-induced inflammation, activating two distinct signaling pathways—the myeloid differentiation primary response gene 88 (MyD88)-dependent pathway and the MyD88-independent pathway (Petrasek et al. 2013; Seki and Schnabl 2012; Wang et al. 2012). Engagement of the MyD88-dependent TLR pathway triggers expression of nuclear factor kappa B (NF- κ B), a central transcriptional regulator of immune responses and pro-inflammatory pathways. TLR4-mediated, MyD88-dependent signaling also promotes mitogen-activated protein kinase (MAPK)-induced production of cytokines, including TNF α . The MyD88-independent pathway proceeds via a different major adaptor protein, TIR domain-containing adapter-inducing interferon- β (TRIF), and results in production of interferon regulatory factor 3 (IRF3), type 1 interferons (IFNs), and pro-inflammatory cytokines.

Experiments in rodent models of ALD have demonstrated that TLR4–TRIF signaling plays an essential role in alcohol-induced activation of TLR4 in Kupffer cells (Hritz et al. 2008; Mandal et al. 2010*b*) (see figure 2). Moreover, TLR4 signaling in both immune cells (i.e., Kupffer cells) and nonimmune cells involved in tissue repair (i.e., HSCs) is required for the development of alcoholic hepatitis and fibrosis (Inokuchi et al. 2011). Co-receptor proteins (e.g., cluster of differentiation [CD] 14 and myeloid differentiation [MD] 2) influence the

responsiveness of TLR4 to receptor ligands, such as LPS.

Cytokines and Chemokines in ALD

Cytokines are relatively small proteins (i.e., less than 30 kDa in size), many of which are produced by various cells in response to injury or contact with pathogens. Patients with ALD often have elevated levels of various cytokines (see sidebar, “Key Cytokines and Hormonal Peptides in ALD”) (Szabo et al. 2011). The cytokines are key components of the innate immune system, facilitating cell-to-cell communication and regulating proliferation and maturation of cell populations in response to immune challenges and environmental changes. Cytokines engage with cells via specific receptors on the cellular surfaces, sometimes triggering their own (i.e., autocrine) production in the cells or amplifying or inhibiting the activities of other cytokines. These interactive cytokine networks play an indispensable role in mediating innate immune responses, and their relative contributions define the different types and outcomes of these responses. A subset of cytokines, the chemokines, recruit immune cells, such as neutrophils and lymphocytes, to sites of injury or infection; thus, chemokines are often involved in pro-inflammatory signaling pathways.

As can be expected from alcohol's effect on the innate immune cells of the liver, such as Kupffer cells, and on LPS-activated TLR signaling, heavy alcohol consumption stimulates the production of many cytokines. One of the major cytokines in ALD and one of the first to be associated with the condition is TNF α (McClain and Cohen 1989). TNF α is expressed early in response to alcohol exposure, and its production coincides with liver damage; moreover, abolishing its expression in animal models of ALD mitigates liver injury (Gao 2012; Wang et al. 2012). These observations underscore that TNF α 's prominent pro-inflammatory role in ALD and its

activity significantly contributes to alcohol-induced liver damage.

Interleukins are also among the pro-inflammatory cytokines implicated in ALD. For example, TNF α , along with other NF- κ B–induced agents, stimulates the expression of IL-8, whose levels are greatly increased

in alcoholic hepatitis (Sheron et al. 1993). IL-17 is also upregulated in ALD, and although its activity is lower than that of TNF α , it seems to play a role in both inflammation and fibrosis of the liver (Lemmers et al. 2009).

Alcohol exposure also induces expression of anti-inflammatory cyto-

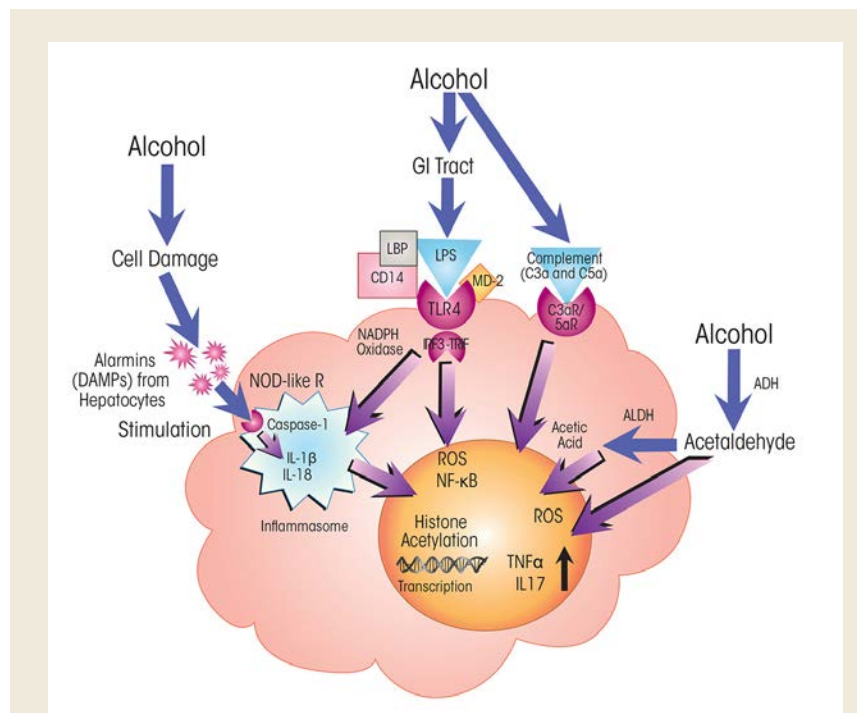


Figure 2 Alcohol's effects on pro-inflammatory pathways in liver macrophages (i.e., Kupffer cells). Excessive alcohol consumption increases the permeability of the gastrointestinal (GI) tract, exposing Kupffer cells to bacterial endotoxin (i.e., lipopolysaccharide [LPS]). LPS is bound by LPS-binding protein (LBP), enabling engagement with Toll-like receptor 4 (TLR 4) and activating the myeloid differentiation primary response (MyD) 88–independent signaling pathway involving interferon regulatory factor 3 (IRF3) and TIR domain–containing adapter-inducing interferon- β (TRIF). IRF3–TRIF signaling induces production of reactive oxygen species (ROS) by nicotinamide adenine dinucleotide phosphate (NADPH) oxidases and activates nuclear factor κ B (NF- κ B) and histone acetylation, which trigger transcription of genes for several pro-inflammatory cytokines (i.e., tumor necrosis factor α [TNF α] and interleukin [IL]-17). Alcohol's breakdown to acetaldehyde and acetate also stimulates ROS signaling and cytokine production. In addition, IRF3–TRIF signaling and detection of damage-associated molecular patterns (DAMPs or alarmins) released from hepatocytes after alcohol exposure stimulate the inflammasome, a multiprotein complex containing caspase 1, which cleaves and thus activates another pro-inflammatory cytokine, IL-1 β . Alcohol activates complement, generating anaphylatoxins C3a and C5a, which dock with their cognate receptor on Kupffer cells, further stimulating cytokine production.

NOTES: ADH = alcohol dehydrogenase; ALDH = acetaldehyde dehydrogenase; C3a/C5a R = C3a/C5a receptor; CD14 = cluster of differentiation 14 protein; MD-2 = myeloid differentiation 2 protein; NOD-like R = nucleotide-binding oligomerization domain–like receptor.

kines. For example, three interleukins—IL-6, IL-10, and IL-22—activate signal transducer and activator of transcription 3 (STAT3), a transcriptional regulator of an array of genes involved in immunity and cellular defenses and differentiation (Gao 2012; Wang et al. 2012). IL-22 binds to specific receptors on epithelial cells and on hepatocytes and triggers the expression of anti-apoptotic and anti-oxidative stress genes while repressing genes involved

in lipid production (Gao 2012; Wang et al. 2012). It often remains unclear whether the expression of anti-inflammatory cytokines reflects a compensatory response of the immune system to the alcohol-induced upregulation of the pro-inflammatory cytokines or to the cell damage alcohol produces.

Interestingly, although IL-6, IL-10, and IL-22 all stimulate STAT3, only IL-22 seems to have solely anti-inflammatory effects

protecting against acute and chronic liver damage (Park et al. 2011). IL-6 and IL-10, in contrast, have dual roles as both pro- and anti-inflammatory proteins in ALD (Gao 2012; Wang et al. 2012). Their specific effects seem to be determined by the cell type affected and the stage of ALD. For example, IL-6 increases the expression of pro-inflammatory cytokines in Kupffer cells (Gao 2012), but its activity also protects hepatocytes. Recent findings suggest that alcohol-induced oxidative stress stimulates the expression of IL-6, promoting senescence in hepatocytes, which, in turn, makes cells more resistant to steatosis and apoptosis (Wan et al. 2014). IL-10 blocks the activation of TNF α and complement, thus reducing expression of pro-inflammatory pathways; but it also checks expression of IL-6, thus limiting liver regeneration afforded by IL-6-induced upregulation of expression of liver-protective genes (Gao 2012).

Chemokines also play critical roles in alcohol-induced inflammation. For example, the levels of MCP-1 (also known as CCL2) are elevated in patients with ALD, and upregulated MCP-1 expression is also observed in Kupffer cells and hepatocytes of alcohol-fed mice (Mandrekar et al. 2011). Feeding alcohol to MCP-1-deficient mice results in less steatosis, lower expression of pro-inflammatory cytokines (i.e., of TNF α , IL-1 β , and IL-6), and lower levels of oxidative stress than in wild-type mice (Mandrekar et al. 2011). Moreover, MCP-1 is required for activating cytokine expression in response to LPS (Mandrekar et al. 2011). In patients with ALD, increased MCP-1 expression is associated with increased disease severity and elevated levels of the pro-inflammatory cytokine IL-8 (Dégre et al. 2012). One major role of MCP-1 in ALD is to recruit neutrophils to inflamed liver tissues. However, because circulating neutrophils in the ALD patients lack MCP-1 receptors (Dégre et al. 2012), the exact mechanisms by which MCP-1 exerts its control over neutrophil movement remain to be elucidated.

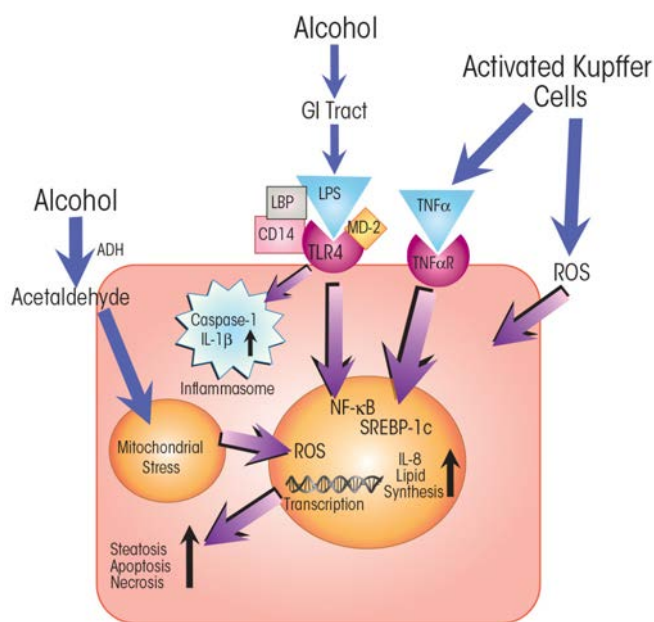


Figure 3 Alcohol's direct effects on activity and viability of parenchymal liver cells (i.e., hepatocytes) and on immune-cell signaling to hepatocytes. Excessive alcohol consumption increases the permeability of the gastrointestinal (GI) tract, exposing hepatocytes to bacterial endotoxin (i.e., lipopolysaccharide [LPS]). LPS is bound by LPS-binding protein (LBP), enabling engagement with Toll-like receptor 4 (TLR4) and activation of pro-inflammatory signaling pathways. TLR4 signaling activates expression of nuclear factor κ B (NF- κ B), which, along with reactive oxygen species (ROS) generated in mitochondria (as a result of exposure to the toxic alcohol-breakdown product acetaldehyde) and Kupffer cells, activates transcription of pro-inflammatory cytokines (i.e., IL-8). Tumor necrosis factor α (TNF α) produced by activated Kupffer cells stimulates sterol regulatory element-binding protein 1c (SREBP-1c), which triggers expression of genes in lipid synthesis, in turn initiating the development of abnormal fat deposition (i.e., steatosis). The combined action of lipid synthesis and upregulated expression of pro-inflammatory cytokines may spur programmed cell death (i.e., apoptosis) and necrosis, resulting in alcohol-induced loss of hepatocytes from tissues

NOTES: ADH = alcohol dehydrogenase; CD14 = cluster of differentiation 14 protein; MD-2 = myeloid differentiation 2 protein; TNF α R = TNF α receptor.

Nevertheless, these results connect chemokines to lipid metabolism in the liver and suggest that MCP-1 plays a major role in alcohol-induced liver inflammation by activating several pro-inflammatory cytokines in response to common triggers of ALD and by promoting neutrophil infiltration into liver tissues.

Barnes and colleagues (2013) recently demonstrated that macrophage migration inhibitory factor (MIF)—a multifunctional pro-inflammatory cytokine and chemokine, which also has some hormonal features—has a critical role in both the early and chronic stages of liver injury in a mouse model of ALD. MIF-deficient mice are protected

against several of alcohol's effects on the innate immune system, including inflammation, and also against hepatocyte damage and apoptosis. Similar to MCP-1, MIF plays a role in alcohol-induced lipid accumulation in liver cells (Barnes et al. 2013), suggesting a role for both chemokines in regulating lipid metabolism directly or indirectly. The findings lend further support to existing evidence that links innate immune pathways and proteins to the regulation of fundamental metabolic processes.

The effect of the hormonal peptide adiponectin on innate immunity, specifically on anti-inflammatory cytokine production and activity, is also

worth noting. Adiponectin is secreted by fat cells (i.e., adipocytes) and has been shown to alleviate steatosis, inflammation, and liver damage in animal models (Xu et al. 2003). Recent evidence suggests that adiponectin moderates alcohol-induced production of pro-inflammatory TNF α and promotes expression of IL-10 (Mandal et al. 2010a). Because IL-10 activates STAT3, its activation by adiponectin lowers inflammation by stimulating STAT3-induced expression of anti-inflammatory genes in myeloid cells, such as Kupffer cells. In addition, adiponectin stimulates heme oxygenase 1 (HO-1), which suppresses the pro-inflammatory TLR4-dependent/

Key Cytokines and Hormonal Peptides in ALD

Tumor Necrosis Factor α

Tumor necrosis factor α (TNF α) is a major pro-inflammatory cytokine whose levels are increased in the blood and liver of individuals with alcoholic liver disease (ALD). TNF α expression is regulated by the transcription factor nuclear factor kappa B (NF- κ B). It is upregulated in macrophages (i.e., Kupffer cells) as well as in circulating monocytes in response to Toll-like receptor 4 (TLR4) activation by bacterial endotoxin (i.e., lipopolysaccharide [LPS]) and by the breakdown products of alcohol, acetaldehyde and acetic acid. TNF α induces necrosis and apoptosis in hepatocytes, thus contributing to inflammation in ALD. TNF α repression by the phosphodiester-inhibitor pentoxifylline and by treatment with TNF α antibody alleviates TNF α -induced liver damage in mice and improves the short-term survival of ALD patients, respectively, but increases the risk for infections in ALD patients.

Interleukin 1 β

Interleukin-1 β (IL-1 β) along with type I IL-1 receptor (IL-1R1), and IL-1 receptor antagonist (IL-1Ra), is an important regulator of the IL-1 signaling complex. This complex plays a critical role in alcohol-induced hepatic steatosis, inflammation, and damage. IL-1 β activation is mediated through the inflammasome, a multiprotein complex in macrophages that senses and transduces endogenous danger signals via IL-1 β cleavage by caspase-1.

IL-1 β increases the activity of pro-inflammatory monocyte chemotactic protein (MCP-1) in hepatocytes and contributes to increased TLR4-dependent pro-inflammatory signaling in macrophages.

IL-6

IL-6 has both pro- and anti-inflammatory activities. It increases expression of pro-inflammatory cytokines in macrophages and decreases necrosis-associated inflammation in hepatocytes, which aids recovery from injury and facilitates tissue regeneration. Along with IL-10 and IL-22, IL-6 activates signal transducer and activator of transcription 3 (STAT3), which controls expression of a set of genes involved in innate immunity and in cell survival and differentiation. IL-6 release from M2 macrophages induces senescence and blocks apoptosis and steatosis in hepatocytes in the early stage of alcohol-induced liver injury in mice. IL-6 activates STAT3 in sinusoidal endothelial cells of the liver, thereby increasing cell survival. IL-6 levels, along with those of IL-8 and IL-10, are increased in patients with ALD who have no clinical signs of liver disease.

IL-8

IL-8 is released from injured hepatocytes and has important pro-inflammatory roles as a chemokine that recruits neutrophils to sites of inflammation. Its expression is

MyD88-independent pathway in Kupffer cells (Mandal et al. 2010*b*). Alcohol-induced oxidative stress decreases secretion of adiponectin by adipocytes (Tang et al. 2012), which links oxidative stress to decreased levels of liver-protective, anti-inflammatory hormones, representing yet another mechanism by which alcohol perturbs liver innate immunity.

Another small peptide, ghrelin, which is produced mainly in the gut but also in the liver, has been shown to promote antifibrotic and hepatoprotective effects in both animals and humans with hepatic fibrosis (Moreno et al. 2010). Ghrelin decreases activation of NF- κ B in hepatocytes, which attenuates apoptotic signaling in these

cells. It also limits expression of collagen- α 1 and TGF- β 1 but not of NF- κ B and IL-8 in HSCs, indicating that ghrelin protects liver tissues mainly by suppressing fibrogenic activities in liver cells (Moreno et al. 2010).

Activation of the Inflammasome and IL-1 β Expression

Recent observations by Petrasek and colleagues (2012) in a mouse model of alcoholic hepatitis support significant involvement of another important cytokine, IL-1 β —which has roles in rheumatoid arthritis and autoimmune disorders—in alcohol-induced inflammation. The researchers show that the IL-1 β signaling

complex is essential for the initiation of alcohol-induced inflammation and progression to liver fibrosis. IL-1 β is activated through the inflammasome, a large protein assembly composed of NOD-like receptor proteins that sense alarmins, the caspase-1 (Casp-1) protein (an enzyme that cleaves other proteins to activate them), and an apoptosis-associated speck-like CARD-domain-containing (ASC) protein (Szabo and Csak 2012) (see figures 2 and 3). The inflammasome is activated in Kupffer cells of alcohol-fed mice (Petrasek et al. 2012) as well as in hepatocytes exposed to LPS and fatty acids (Csak et al. 2011). It is stimulated by intracellular signals such as alarmins, which engage with

Key Cytokines and Hormonal Peptides in ALD (continued)

induced by TNF α via activation through NF- κ B. IL-8 levels are greatly increased in people with acute alcoholic hepatitis but are only moderately upregulated in those with cirrhosis. IL-8 levels, along with those for IL-6 and IL-10, are elevated in individuals with alcoholism who have no signs of liver disease.

IL-10

IL-10 is a strong suppressor of inflammation by preventing production of the pro-inflammatory cytokines TNF α , IL-1 β , and IL-6 in macrophages. However, its anti-inflammatory, hepatoprotective effects are contingent on the expression of other cytokines, and its inhibitory effect on IL-6 expression can delay liver regeneration and increase steatosis. IL-10 expression is moderately to highly increased in ALD and, along with that of IL-6 and IL-8, is also upregulated in alcoholic patients without signs of liver disease. IL-10 acts only on immune cells expressing its cognate receptors and facilitates sustained activation of the transcription factor STAT3 in Kupffer cells, thus inhibiting inflammation. IL-10 also inhibits fibrosis.

IL-17

IL-17 is a recently discovered, pro-inflammatory chemokine whose levels are increased in people with ALD. It is produced by monocytes and T cells and plays an important role in recruiting neutrophils to inflamed liver tissues. It may act in concert with TNF α to activate NF- κ B,

thereby inducing expression of other pro-inflammatory cytokines. IL-17's main targets are hepatic stellate cells (HSCs), in which it induces production of pro-inflammatory IL-8. IL-17 is also thought to be involved in the development of fibrosis.

IL-22

IL-22 is an anti-inflammatory cytokine whose expression limits steatosis and liver damage. The IL-22 targets are hepatocytes in which it activates the transcription factor STAT3. The antioxidant, antiapoptotic, and antisteatotic actions of IL-22 make it a promising target of interventions for treating ALD with minimal side effects because of the restricted distribution of IL-22 receptors.

Adiponectin

Adiponectin is an adipokine, a peptide hormone, whose secretion from fat cells (i.e., adipocytes) is inhibited by alcohol. Adiponectin increases fatty acid oxidation and thus suppresses steatosis. It also decreases expression of the pro-inflammatory cytokine TNF- α in macrophages (i.e., Kupffer cells) by inducing expression of heme oxygenase 1 (HO-1), which decreases TLR4/MyD88-independent signaling, and by increasing polarization to anti-inflammatory M2 macrophages. In addition, adiponectin upregulates expression of anti-inflammatory IL-10.

pattern-recognition domains of the NOD-like receptors.

Inflammasome activity in Kupffer cells promotes Casp-1-mediated cleavage, and thus activation, of IL-1 β (Petrasek et al. 2012). Blocking IL-1 β activity strongly decreases liver inflammation and damage (Petrasek et al. 2012). These findings further amplify the view that IL-1 β acts as a pro-inflammatory cytokine in immune cells in ALD and that IL-1 β signaling through the inflammasome is required for alcohol-induced liver injury. Coupled with the observations of Mandrekar and colleagues (2011) on MCP-1, these findings also establish a link between innate immune activity and steatosis. This association is further supported by the observation that steatosis can be prevented when innate immune responses are experimentally abrogated by checking IL-1 β or MCP-1 activity. Additional investigations into pro-inflammatory signaling in the methyl-choline deficiency model of non-alcoholic steatohepatitis have indicated that activation of the inflammasome and generation of IL-1 β are independent of TLR4 but contingent on the MyD88-dependent pathway (Csak et al. 2014).

Cytokine Effects on Hepatocytes and HSCs

Alcohol-induced increases in LPS stimulate TNF α production and its release primarily from Kupffer cells (McClain and Cohen 1989; Wang et al. 2012). In addition, alcohol sensitizes other liver cells to TNF α 's actions (An et al. 2012). One distinct role of TNF α is to induce programmed cell-death pathways (i.e., apoptosis and necroptosis) by binding to and activating death receptors on cells. Activation of these receptors results in the expression of pro-apoptotic or necroptotic mediators (e.g., caspases and receptor-interacting proteins). Alcohol thus has profound effects on cell viability by activating the expression of cytotoxic cytokines and increasing cell sensitivity to the actions of

these cytokines. For instance, whereas hepatocytes in healthy people are not highly sensitive to TNF α activation of death receptor pathways, alcohol primes cells for the TNF α -mediated stimulation of cell-death pathways (Pastorino and Hoek 2000).

TNF α binds to and activates two receptors—TNF-R1 and TNF-R2—and the outcome of their activation hinges on the relative levels of stimulation of three main pathways: a pro-apoptotic pathway, a pro-necroptotic pathway, and a cell-survival pathway (Vanden Berghe et al. 2014). Binding to TNF-R1 activates apoptotic and necrotic pathways through a signaling cascade involving multiple proteins, including TNF-R1-associated death domain protein (TRADD); TNF receptor-associated factor 2 (TRAF2); and caspases 8, 3, and 7. TNF-R1 activation also may result in the stimulation of a cell-survival pathway involving NF- κ B. In contrast, activation of TNF-R2 stimulates only cell survival (Malhi et al. 2010).

Apoptosis is an important regulatory mechanism for controlling the size of cell populations (e.g., neutrophils) or to avoid unchecked proliferation of abnormal cells. However, apoptosis triggered via the action of alcohol-induced TNF α on, for example, hepatocytes can severely impair liver function. Recent evidence also indicates that chronic ethanol feeding can activate necroptotic cell-death pathways in hepatocytes. Mice in which an important necroptotic regulator—receptor-interacting protein kinase (RIP)-3—is inactivated are protected from alcohol-induced liver injury (Roychowdhury et al. 2013). Hepatocytes are the major cell type in the liver and, along with Kupffer cells, eliminate most of the LPS from circulation. Widespread apoptosis and necrosis of hepatocytes therefore increase levels of circulating LPS, which in turn fuels further inflammation through activation of TLR4 on Kupffer cells and ultimately escalates the release of pro-apoptotic TNF α . This helps explain why

patients with severe liver disease (i.e., cirrhosis) often show high levels of LPS in the blood (i.e., endotoxemia), resulting in sepsis (Bode et al. 1987).

Persistent alcohol-induced activation of cytokines in immune cells such as Kupffer cells also promotes fibrosis. Production and secretion of transforming growth factor (TGF)- β and of platelet-derived growth factor (PDGF) from Kupffer cells or from inflammatory scar-associated macrophages activates HSCs (see figure 4), triggering them to develop into myofibroblasts. Moreover, HSCs may be further activated by engulfing apoptotic hepatocytes. HSCs express a number of receptors involved in innate immunity, including the C5a receptor, whose ligand, C5a, is a potent mitogen that may stimulate HSC migration (Das et al. 2014). HSCs also express TLR4, which senses LPS. Although LPS alone cannot activate HSCs, its action via TLR4 downregulates expression of BMP and activin membrane-bound inhibitor (BAMBI), thereby sensitizing the cells to activation by TGF- β (Liu et al. 2014; Seki et al. 2007). BAMBI is a pseudoreceptor, which in quiescent HSCs diminishes TGF- β 's activity by binding to it without triggering intracellular TGF- β signaling. As shown by Seki and colleagues (2007), decreased BAMBI expression sensitizes HSCs to TGF- β activation, and LPS-induced TLR4 activation stimulates chemokine secretion along with recruitment and activation of Kupffer cells. Moreover, LPS activates TLR4 signaling in HSCs through the MyD88-dependent NF- κ B pathway, demonstrating involvement of this key innate immune pathway in fibrosis and recruitment of immune cells. NF- κ B directly binds the BAMBI promoter, along with histone deacetylase (HDAC) 1, thus repressing BAMBI expression and promoting TGF- β activation (Liu et al. 2014).

Activated HSCs produce and secrete extracellular matrix proteins (i.e., collagens), resulting in fibrogenesis (Hernandez-Gea and Friedman 2011;

Seki and Schnabl 2012). Although fibrogenesis is essential for normal tissue repair, its dysregulation by recurrent activation of cytokines acting on HSCs precipitates inflammatory fibrosis. This stage of ALD involves the formation of scar tissue,

which interferes with normal tissue function and often results in portal hypertension (Hernandez-Gea and Friedman 2011). Another cytokine pathway involving HSCs and leading to fibrosis in ALD patients involves IL-17. This cytokine, which is produced

by peripheral blood cells (i.e., Th17 lymphocytes) in patients with alcoholic hepatitis and cirrhosis, stimulates its cognate receptors on HSCs (Lemmers et al. 2009). In response, the cells secrete IL-8 and growth-related oncogene (GRO)- α , recruiting neutrophils to their tissue, resulting in localized pro-inflammatory immune-cell infiltrates and fibrosis scores closely correlated with IL-17 levels.

Together, these findings indicate that cytokines produced by immune cells, such as TNF α produced by Kupffer cells and IL-17 produced by Th-17 lymphocytes, play a major role in ALD by affecting the viability and function of hepatocytes and by activating quiescent HSCs to produce excess extracellular proteins. These observations thus provide critical insight into the molecular processes and mechanisms that produce liver damage and fibrosis in ALD.

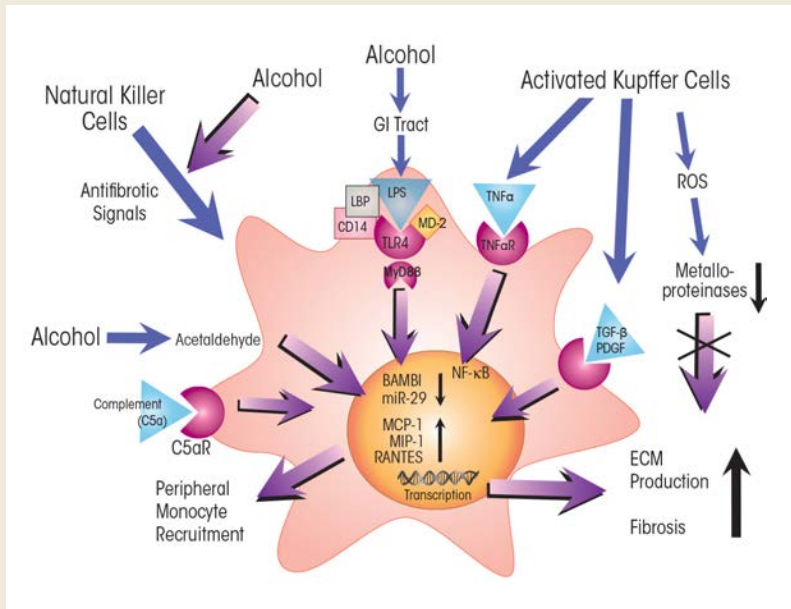


Figure 4 Alcohol's effects on fibrogenic pathways in hepatic stellate cells (HSCs). HSCs are quiescent liver cells that, on stimulation by pro-inflammatory proteins and other agents, differentiate into myofibroblasts to repair damaged tissues. Excessive alcohol consumption increases the permeability of the gastrointestinal (GI) tract, exposing HSCs to endotoxin (i.e., lipopolysaccharide [LPS]). LPS is bound by LPS-binding protein (LBP), enabling engagement with Toll-like receptor 4 (TLR 4) and activating the myeloid differentiation primary response (MyD88)-dependent pathway. MyD88 signaling decreases expression of BMP and activin membrane-bound inhibitor (BAMBI), a pseudoreceptor that suppresses responses to transforming growth factor β (TGF- β ; secreted by activated Kupffer cells). Thus, alcohol-induced TLR4-MyD88 signaling increases the HSCs' responsiveness to TGF- β . microRNA 29 (miR-29) inhibits the production of extracellular matrix (ECM), and its downregulation by MyD88 signaling therefore increases ECM deposition. TLR4-MyD88 signaling in HSCs—along with complement 5a and exposure to the alcohol-breakdown product acetaldehyde and platelet-derived growth factor (PDGF) and tumor necrosis factor α (TNF α) secreted from activated Kupffer cells—upregulates the expression of various chemokines (i.e., monocyte chemoattractant protein [MCP-1], macrophage inflammatory protein 1 [MIP-1], and regulated on activation, normal T cell expressed and secreted [RANTES]). These chemokines recruit macrophages (i.e., Kupffer cells and scar-associated macrophages) and other immune cells to the site where HSCs reside (i.e., the liver perisinusoidal space or space of Disse). These signals spur the differentiation of HSCs into myelofibroblasts that produce and secrete ECM, leading to liver fibrosis. In addition, Kupffer cell-produced ROS inhibit activities of metalloproteinases, which normally degrade ECM and thus inhibit fibrosis.

NOTES: C5aR = C5a receptor; CD14 = cluster of differentiation 14 protein; MD-2 = myeloid differentiation 2 protein; TNF α R = TNF α receptor.

Epigenetic Effects

Alcohol exerts additional effects on the innate immune system, for example, by producing epigenetic changes in the expression of genes for pro- and anti-inflammatory pathways (Curtis et al. 2013). Epigenetic changes affect the activity at gene promoters or entire gene regions and can have long-term and even heritable effects on gene expression without altering the underlying DNA sequence. Three main mechanisms operate in epigenetics:

- DNA methyltransferases, using SAM as methyl donor, methylate a cytosine nucleotide at CpG-rich regions (i.e., CpG islands) in the DNA of gene promoters, which decreases expression of the downstream genes.
- Methylation, acetylation, phosphorylation, ubiquitination, or sumoylation of the proteins around which DNA is coiled (i.e., histones) alters accessibility

of the transcriptional proteins to the DNA.

- Some investigators are now extending the concept of epigenetics to include transcriptional regulation by microRNAs (miRNAs). These molecules regulate the expression of mRNAs with which they share similar sequences (Curtis et al. 2013).

The study of alcohol's effects on epigenetic regulation and of the mechanisms by which alcohol exerts these effects has been a rapidly emerging field over the past decade. Insight gleaned from initial studies has shown that alcohol can interfere with the fundamental processes of epigenetic regulation in people with ALD as well as in animal models of the disease or in cultured human cells exposed to alcohol or its metabolic byproducts (reviewed by Kruman and Fowler 2014; Mandrekar 2011; Shukla and Lim 2013).

Studies in animals and in human cells lines have demonstrated that alcohol and LPS increase the expression of microRNA-34a, which helps alleviate alcohol-induced apoptosis in hepatocytes and biliary epithelial cells by targeting caspase 2 and sirtuin 1. The elevated expression of miRNA-34a is the result of an alcohol-induced decrease in methylation (i.e., hypomethylation) at a CpG island in the miRNA-34a promoter (Meng et al. 2012).

Alcohol also alters the cellular levels of SAM and of histone acetyltransferases (HATs) and deacetylases (HDACs), whose activities make DNA more or less accessible, respectively, to gene transcription. For example, the histone deacetylase HDAC1 has been shown to play a critical role in the silenced expression in HSCs of the fibrosis-attenuating protein BAMBI (see figure 4) (Liu et al. 2014). In addition, chemical inhibition of HDAC activity seems to reduce inflammation by reversing an alcohol-induced perturbation in macrophage polarization that results in a greater proportion of

pro-inflammatory (M1) macrophages (Curtis et al. 2013). Oxidative stress caused by alcohol metabolism also triggers epigenetic changes, and alcohol-induced release of LPS and activation of TLR4 affects both HAT and HDAC activities, resulting in epigenetic changes in DNA regions containing genes for pro-inflammatory cytokines (Curtis et al. 2013).

Approaches for Resolving Alcohol-Induced Liver Inflammation

Standard interventions for treating ALD depend on the stage and severity of the disease and typically include counseling abstinence from alcohol use; administration of corticosteroids (to inhibit alcohol-induced, pro-inflammatory pathways) and nutritional support for alcoholic hepatitis; and, in advanced cases, liver transplantation (Gao and Bataller 2011; Orman et al. 2013). Although rates of disability and death caused by ALD remain high despite these interventions, such treatments can significantly improve quality of life and avert early death caused by ALD. Overturning earlier assumptions about the persistence of alcohol-induced liver damage, recent studies have reported that some of the tissue injuries present even in the advanced stages of ALD, such as fibrosis, are reversible (Hernandez-Gea and Friedman 2011). This makes the discovery of new treatments that can augment existing ones even more urgent.

Recognition of the central role of innate immunity in ALD has spurred research into modulating the activity of key immune cells and cytokines. To this end, the discovery of the central role of TNF α in promoting inflammation in ALD prompted studies in which antibodies against TNF α were used to alleviate alcohol-induced inflammation. TNF α antibodies indeed significantly dampen liver inflammation (Gao 2012), but because TNF α is critical to fighting microbial pathogens,

this approach often increases the risk for serious infections in ALD patients.

The limitations of the above approach highlight that cytokine-based interventions will need to be carefully calibrated according to the activity profile and radius of action of each cytokine to minimize or prevent adverse effects. For instance, exploitation of the hepatoprotective properties of IL-6 is limited by the abundance of IL-6 receptors in many tissues, potentially resulting in off-target effects. However, as proposed by Gao (2012), using IL-6 in ex vivo treatment of donor livers to reverse minor organ damage (e.g., steatosis) before transplantation into ALD patients could have some utility.

Current approaches for interventions in vivo focus on those immune regulators that target only a few cells or tissues. As discussed previously, IL-22 has an array of hepatoprotective activities, including antioxidant, antimicrobial, and antiapoptotic effects. Moreover, expression of the IL-22 receptor, IL-22R, is confined to epithelial cells, such as hepatocytes. This has led to the proposition that combining the use of IL-22 with anti-inflammatory corticosteroids and TNF α inhibitors could offset the immunosuppressing effects of these two agents and promote recovery of liver tissues (Gao 2012). However, because evidence from animal models suggests that IL-22 may play a role in the development of hepatic carcinoma (Park et al. 2011), such use would be restricted to ALD patients who do not have cirrhosis (which may contain precancerous cells) or liver cancer (Gao 2012).

The discovery of the role of epigenetic factors in the development of ALD and their effects on immune cells and responses opens the way to possible interventions that target key epigenetic regulators and processes in ALD. For example, because HDAC1 seems to make HSCs more receptive to the fibrosis-inducing action of TGF β (Liu et al. 2014), inactivation of HDAC1 via antibodies or chemical agents may

augment current treatments for halting or reversing fibrosis in patients with ALD. In addition, chemical inactivation of HDACs involved in alcohol's effects on macrophage polarization to pro-inflammatory M1 macrophages may help reduce inflammation, steatosis, and fibrosis in tissues. Thus, HDAC inhibitors or activators of HATs that prevent or reverse the effects of HDACs may someday prove useful in the treatment of ALD.

Finally, technological advances to sequence and analyze DNA of patients has helped identify key genetic variants such as single-nucleotide polymorphisms (SNPs) in genes involved in liver diseases (Guo et al. 2009; Singal et al. 2014). Although in its early stages and not yet fully extended to the specific etiology of ALD, genetic profiling of ALD patients for SNP variants in genes involved in innate immune pathways could help identify patients vulnerable to advanced stages of ALD (e.g., cirrhosis) (Guo et al. 2009). Such personalized-medicine approaches could significantly improve the success and cost-effectiveness of current treatments and spur development of new interventions for ALD.

Conclusions

Innate immunity plays a central role in ALD, and recent studies have uncovered several pivotal molecular mechanisms underlying alcohol's effects on the immune system of the liver. Excessive consumption of alcohol alters the characteristics and composition of the microbiome in the GI tract and increases translocation of bacteria and bacterial products, such as LPS and peptidoglycans, from the gut via the portal system to the liver. This increased influx of LPS, along with the direct effects of alcohol on immune cells and liver tissues, activates innate immune pathways. This activation occurs via stimulation of TLRs and through sensors of cell damage on or in the immune cells of the liver, such as Kupffer cells. These processes

lead to the production of several pro-inflammatory cytokines (e.g., of TNF α , IL-1 β , IL-8, and IL-17), triggering steatosis in hepatocytes and inducing fibrogenic pathways in HSCs. Moreover, production of chemokines, such as MCP-1 and MIF, leads to the infiltration of liver tissues by monocytes, neutrophils, and dendritic cells whose activities can further increase inflammation and impede recovery. Alcohol and its metabolic breakdown products acetaldehyde and acetate, along with ROS produced during alcohol metabolism, generate oxidative stress and affect epigenetic regulation that trigger activation of pro-inflammatory pathways such as polarization to M1 macrophages.

The insight gleaned from these complex interactions and pathways may provide the impetus for devising treatments using pro- or anti-inflammatory cytokines that act on defined cell types or employing agents that control epigenetic regulators to expand currently available interventions for treating ALD.

Financial Disclosure

The author declares that she has no competing financial interests.

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The Gastrointestinal Microbiome

Alcohol Effects on the Composition of Intestinal Microbiota

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The excessive use of alcohol is a global problem causing many adverse pathological health effects and a significant financial health care burden. This review addresses the effect of alcohol consumption on the microbiota in the gastrointestinal tract (GIT). Although data are limited in humans, studies highlight the importance of changes in the intestinal microbiota in alcohol-related disorders. Alcohol-induced changes in the GIT microbiota composition and metabolic function may contribute to the well-established link between alcohol-induced oxidative stress, intestinal hyperpermeability to luminal bacterial products, and the subsequent development of alcoholic liver disease (ALD), as well as other diseases. In addition, clinical and preclinical data suggest that alcohol-related disorders are associated with quantitative and qualitative dysbiotic changes in the intestinal microbiota and may be associated with increased GIT inflammation, intestinal hyperpermeability resulting in endotoxemia, systemic inflammation, and tissue damage/organ pathologies including ALD. Thus, gut-directed interventions, such as probiotic and synbiotic modulation of the intestinal microbiota, should be considered and evaluated for prevention and treatment of alcohol-associated pathologies.

Key words: Alcohol consumption; alcohol use, abuse, and dependence; alcohol use disorder (AUD); alcoholic liver disease (ALD); microbiota; intestinal microbiota; microbiota analyses; gastrointestinal microbiome; dysbiosis; probiotics; synbiotics

It has been estimated that approximately 2 billion people worldwide drink alcohol on a daily basis, with more than 70 million people having a diagnosed alcohol use disorder (World Health Organization 2004). Globally, alcohol use is the fifth leading risk factor for premature death and disability among people between the ages of 15 and 49 (Lim et al. 2012). Excessive alcohol consumption in the United States accounts for 80,000 deaths yearly (Centers for Disease Control and Prevention 2004) and is the third leading preventable cause of death in the United States (Mokdad et al. 2004). In addition, the Centers for Disease Control and Prevention (CDC) found that in 2006, excessive drinking cost the United States more than \$224 billion (Bouchery et al. 2011). In a subgroup of alcoholics, alcohol consumption

is linked with tissue injury and organ dysfunction, including alcoholic liver disease (ALD) (Purohit et al. 2008), increased risk of developing cancer (Seitz and Stickel 2007), abnormal function of the immune system that increases the risk of acute and chronic infections (Szabo and Mandrekar 2009), pancreatitis (Chowdhury and Gupta 2006), heart disease (Liedtke and DeMuth 1975), and disruption of the circadian clock (Spanagel et al. 2005). The observation that only some alcoholics develop alcohol-induced pathology indicates that, although alcohol is necessary, it is not sufficient to cause organ dysfunction. Consequently, factors other than the toxicity of alcohol are involved in generating health complications, one of which may be alcohol-induced changes in intestinal microbiota composition and/or function.

The intestinal microbiota is classified as the total collection of microbial organisms (bacteria and microbes) within the gastrointestinal tract (GIT). It contains tens of trillions of microorganisms, including at least 1,000 different species of known bacteria, the vast majority of which belong to the phyla *Firmicutes* and *Bacteroidetes* (Ley et al. 2008). The metagenome is the collection of all the different genes found within the gut microbiome; the GIT microbiome contains more than 3 million unique genes, outnumbering the number of human genes 150 to 1 (Proctor 2011). The GIT and the intestinal microbiota display a symbiotic relationship. The microbiota contributes to the extraction of energy from food and synthesis of vitamins and amino acids, and helps form barriers against pathogens (Tappenden and Deutsch 2007). Disruption of intestinal microbiota homeostasis—called dysbiosis—has been associated with inflammatory bowel disease (IBD) (Hold et al. 2014), irritable bowel syndrome (IBS) (Kassinen et al. 2007), celiac disease (Nadal et al. 2007), food allergies (Kuvaeva et al. 1984), type 1

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diabetes (Wen et al. 2008), type 2 diabetes (Larsen et al. 2010), cancer (Schwabe and Jobin 2013), obesity (Turnbaugh et al. 2006), and cardiovascular disease (Harris et al. 2012). Although it is unclear whether dysbiosis is the cause or the result of these diseases, factors that contribute to the development and progression of many of these diseases are known to influence the GIT microbiota.

Dysbiosis can be caused by environmental factors commonly encountered in Western societies, including diet (David et al. 2014), disruption of circadian rhythms (Voigt et al. 2014), and alcoholic beverage consumption (Mutlu et al. 2009; Yan et al. 2011) (figure 1). It is well-established that diet influences intestinal microbiota composition and diversity (David et al. 2014) (figure 1). Diets high in fat alter intestinal microbiota (Cani et al. 2007), as do “Western” diets, comprising high fat and high sugar (Turnbaugh et al. 2008). The consequence of diets high in fat or sugar may contribute to the development of obesity and liver injury (Frazier et al. 2011), as well as IBD, IBS, celiac disease, type 1 and type 2 diabetes, food allergies, and cardiovascular disease (Brown et al. 2012; Manzel et al. 2014), at least in genetically susceptible individuals. Alcohol is another

dietary disruptor of the intestinal microbiota. A limited number of studies have examined the effects of alcohol on the microbiota in rodents (Mutlu et al. 2009; Yan et al. 2011) and humans (Bode et al. 1984; Chen et al. 2011; Mutlu et al. 2012; Queipo-Ortuno et al. 2012). These changes seem to be relevant for alcohol-associated pathologies because interventions known to alter the intestinal microbiota diminish some alcohol-associated pathologies such as liver disease (Bull-Otterson et al. 2013; Liu et al. 2004; Mutlu et al. 2009).

In this review, we examine alcohol-induced effects on microbiota and how interventions targeted at normalizing alcohol-induced dysbiosis may mitigate some of the detrimental effects of alcohol.

Analyzing the Intestinal Microbial Community

Before we can understand the influence of alcohol on the GIT microbiota, we need to understand a bit about how researchers measure these microorganisms and evaluate changes in their populations. In fact, it is difficult to directly

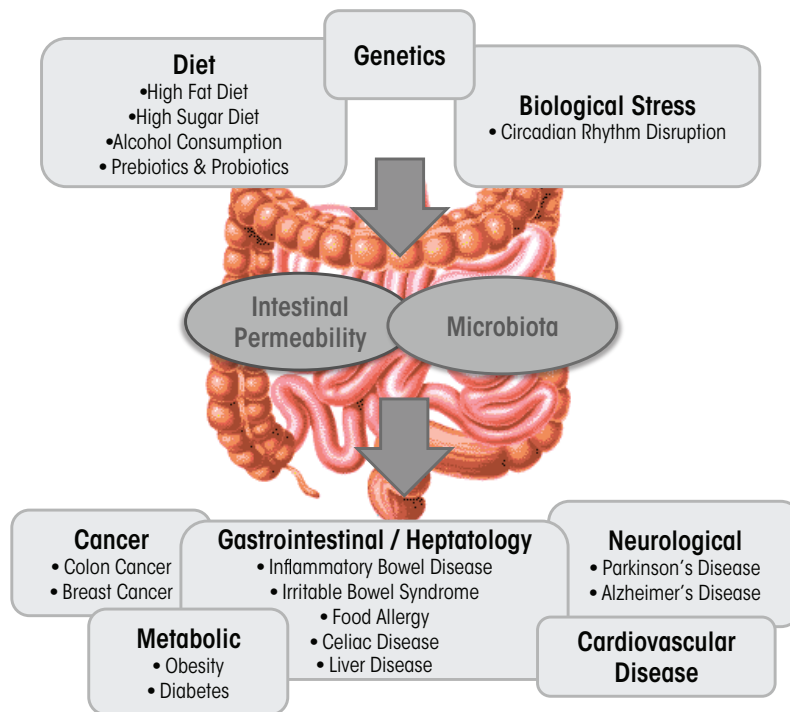


Figure 1 Disruption of intestinal microbiota homeostasis (dysbiosis) has been associated with these diseases (shown above). In addition, dysbiosis can be caused by environmental factors commonly encountered in Western societies, including diet, genetics, disruption of circadian rhythms, and alcoholic beverage consumption. Dysbiosis also can be prevented or treated with probiotics and prebiotics.

measure microbial communities such as those within the GIT because of a number of confounding factors. For one, microorganisms maintain incredible genetic diversity but house this diversity in an extraordinarily limited array of cellular morphologies (Woese 1987). In addition, microorganisms have redundant functional capabilities, share divergent functional capabilities with closely related microorganisms, have the potential for high metabolic diversity within single microbial lineages, and are extraordinarily difficult to isolate under laboratory conditions. Taken together, these confounding factors compel researchers to use molecular tools—tools that examine DNA and RNA—to analyze these complex communities. These tools fall into two broad categories: polymerase chain reaction (PCR)–based targeted approaches and shotgun sequencing approaches (figure 2), which we explain in detail in the sidebar.

Because it is exceedingly difficult to obtain microbial samples from different locations in the GIT, researchers overwhelmingly extract the genomic DNA they need to analyze the GIT microbiota from mucosa-associated colonic tissue biopsies and from fecal samples. However, using these samples assumes that the colonic tissue and feces are a suitable proxy for the GIT. A study (Stearns et al. 2011) addressed this issue in an analysis of microbiota community structure in mouth, stomach, duodenum, colon, and stool, via gastroscopy and colonoscopy from four healthy individuals. When examined in the context of the entire GIT, colonic tissue and fecal samples were most similar to each other in all individuals. However, the community composition was substantially altered in colon and fecal samples from the same individual: three of four individuals had a much reduced level of microorganisms from the phylum *Bacteroidetes* in fecal samples. This led to a substantially altered ratio of *Firmicutes* to *Bacteroidetes*, a ratio that has been used as a diagnostic parameter in studies of disease (see sidebar). Eckburg and colleagues (2005) also found a similar divergence between GIT colonic tissue and fecal microbiota. Thus, although colonic tissue and fecal samples will continue to serve as common, imperfect proxies for GIT microbiota, they should not be considered a perfect representation of the entire GIT microbial community, which undergoes dramatic changes from the stomach to colon (Stearns et al. 2011). No obvious solution is available, leaving only highly invasive sampling techniques as a mechanism to collect samples from multiple locations of the GIT.

Alcohol-Induced Effects and Implications on the Intestinal Microbiota

The study of alcohol's effects on the structure and activity of GIT microbiota still is in its infancy, particularly compared with other alcohol-induced effects. The literature reviewed below demonstrates that alcohol consumption leads to quantitative and qualitative dysbiosis in the intestinal microbiota of rodents and humans (table 1). These studies

demonstrate alterations in the dominant bacterial taxa from the phyla *Bacteroidetes* and *Firmicutes* and, in several studies, an increase in bacteria from the phylum *Proteobacteria*.

Rodent Models

Studies in mice and rats find both alcohol-induced bacterial overgrowth and dysbiosis. In one study, C57BL/6 mice were intragastrically fed alcohol (30.9 g/kg per day; 40 percent of their total daily calories from alcohol) for 3 weeks and compared with control mice intragastrically fed an isocaloric liquid diet. The alcohol-fed mice developed ALD, which was associated with small intestinal bacterial overgrowth and dysbiosis in the cecum—the beginning of the large intestine (Yan et al. 2011). In particular, the GIT microbiota of alcohol-treated mice showed a decrease in *Firmicutes* and an increase in the relative abundance of *Bacteroidetes* and *Verrucomicrobia*, among other bacteria (table 1). In comparison, the GIT microbiota of control-fed mice showed a relative predominance of bacteria from the phylum *Firmicutes*. In a separate study, Sprague-Dawley rats intragastrically fed alcohol daily (8 g/kg per day) for 10 weeks showed altered colonic mucosa-associated bacterial microbiota composition leading to ileal and colonic dysbiosis (Mutlu et al. 2009). In prior studies, Sprague-Dawley rats developed intestinal oxidative stress, intestinal hyperpermeability, endotoxemia, and steatohepatitis by the 10th week of alcohol treatment (Keshavarzian et al. 2009), suggesting that changes in the microbiota may be contributing to the alcohol-induced effects on the intestine and liver. Intestinal dysbiosis may potentially contribute to the pathogenesis of liver disease by altering intestinal barrier integrity, resulting in intestinal hyperpermeability, as well as increased production of proinflammatory factors that could both promote liver pathology.

Humans

Chronic alcohol consumption in humans also causes bacterial overgrowth and dysbiosis. One study using culture-based methods, for example, found alcohol-induced alterations, including small intestine bacterial overgrowth of both aerobic and anaerobic bacteria in the jejunum (Bode et al. 1984). Another study showed that alcohol consumption alters the composition of mucosa-associated microbiota in human sigmoid biopsies taken from alcoholics with and without ALD as well as healthy control subjects (Mutlu et al. 2012). In this study, the researchers used 16S rRNA gene sequencing to assess the microbiota. They found that the microbial community was significantly altered—containing a lower abundance of *Bacteroidetes* and a higher abundance of *Proteobacteria*—in a subgroup of alcoholics with and without liver disease (table 1). Other studies show that dysbiotic microbiota in alcoholics also correlates with a high level of endotoxin in the blood, indicating that dysbiosis may contribute to intestinal hyperpermeability and/or the increased translocation of gram-negative microbial bacterial products

Methods for Analyzing the Gastrointestinal Microbiota

To understand the results of microbiota analyses, it can help to understand a bit about the methods researchers use. As mentioned in the main article, researchers tend to use techniques that look for DNA and RNA related to specific microorganisms. To do that, they typically use one of two techniques: polymerase chain reaction (PCR) and shotgun sequencing. Here, we explain in general terms how each method is used to analyze GIT microbiota.

PCR

To successfully use PCR, researchers needed to find an appropriate gene target that would be common enough among microorganisms so

they could use a known segment for searching but different enough so that they could individuate among microorganisms. They quickly selected ribosomal RNA (rRNA) genes (Pace 1986; Woese 1987). Ribosomal RNAs are essential for protein synthesis within all cells and therefore their genes have many features that make them desirable for determining the makeup of complex microbial communities. In particular, the genes contain regions of DNA that are highly variable among species and so can serve as a kind of identifier; but they also contain regions that are highly conserved, or the same among many species, and are therefore suitable for the development of broad-range PCR primers

that use snippets of known DNA to search for specific genes. As a result of these features, rRNA genes have become the “gold standard” for molecular analyses, and they are typically analyzed using PCR-based techniques coupled with indirect fingerprinting or direct sequencing, including with next-generation sequencing (NGS). To profile GIT microbial communities using rRNA gene analysis, researchers typically extract genomic DNA from mucosa-associated colonic biopsies and fecal matter. They then use PCR to amplify the DNA, creating what are called “amplicons,” using primers targeting conserved regions of the small subunit (SSU or 16S) rRNA gene from all bacteria and some-

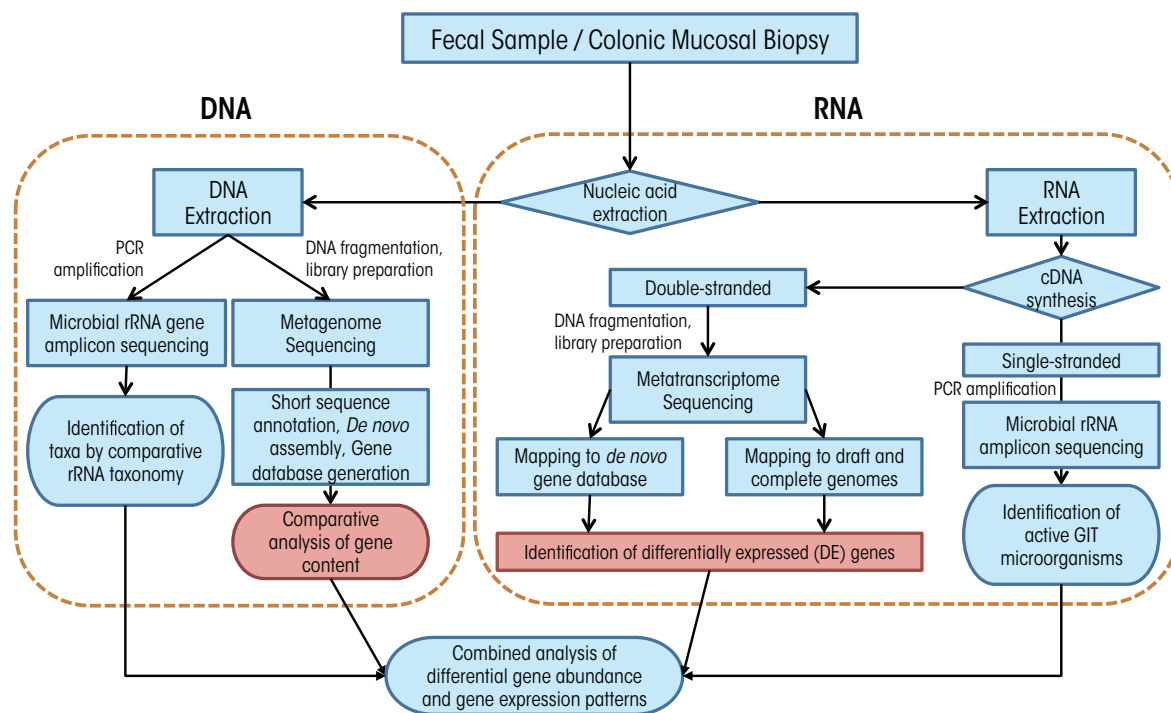


Figure 2 Basic pipeline for amplicon-based and shotgun sequencing approaches to the interrogation of GIT microbial communities. Nucleic acids can be interrogated independently to characterize the community structure and gene content of total (DNA) and active (RNA) microbial communities or combined to examine how shifts in microbiota are correlated with changes in community gene expression patterns.

Methods for Analyzing the Gastrointestinal Microbiota (*continued*)

times archaea. The researchers then sequence these PCR amplicons after suitable preparation for the chosen sequencing platform (Langille et al. 2013). Whereas it was previously common to have clone libraries on the order of 100 sequences per sample, it is more typical with NGS approaches to have sequence libraries of 10,000 to 100,000 sequences per sample. A suite of bioinformatics tools has been developed to process this high-throughput data such as RDP (Cole et al. 2005), mothur (Schloss et al. 2009), and QIIME (Caporaso et al. 2010).

Because of limitations inherent in the analysis of a structural gene, such as the rRNA gene that is common to all organisms, this method should be viewed as the first step in a multi-tiered approach to the analysis of microbial communities. The following are some limitations: (1) rRNA gene sequencing does not provide definitive physiological information about an organism; (2) for DNA-based methods, the presence of an organism's rRNA gene does not guarantee that the organism is active in the studied system at the time of sampling; (3) variation in the number of rRNA genes among bacterial lineages distorts the true diversity of microorganisms in an environmental sample; and (4) difficulty in species- and strain-level phylogenetic resolution among some taxa, depending upon the region of rRNA gene analyzed. Nonetheless, for large studies with many samples, a preliminary screen using this method is often suitable for identifying large-scale shifts in microbial community structure and for identifying statistically significant changes in the relative abundance of organisms between groups or treatments.

That said, the interpretation of results from the analysis of microbial community composition using DNA-based methods can be confounded by the presence of DNA from dead, dormant, or weakly active organisms contributing little to overall microbial community function. To circumvent these limitations, researchers can directly target rRNAs instead of rRNA genes. In such an approach, researchers extract total RNA from an environmental sample and reverse transcribe this RNA using either a random primer mix or a gene-specific "reverse" primer matching the rRNA (figure 2). This process generates single-stranded complementary DNA (cDNA), which is then used as a template for PCR and sequencing with domain-level primer sets as is done with genomic DNA. As microbial RNA is labile and degrades rapidly if not continually produced, rRNA analysis reflects only active microorganisms, and the relative abundance of rRNAs represents the relative activity of organisms in the system. Although rRNA analysis still does not provide an explicit link to physiology for most organisms, such analyses may find stronger correlation to measured functions at the time of sampling. Microbial RNA degrades rapidly, and for GIT colonic tissue and fecal samples, the time delay until RNA can be extracted may result in a serious distortion of active organisms and gene expression patterns from *in situ*. Thus, animal model systems in which animals are killed for sampling may be more suitable for RNA studies as mRNAs and ribosomes can be preserved rapidly for downstream analyses.

Shotgun Metagenomic and Metatranscriptomic Sequencing

Although amplicon sequencing approaches are extremely useful for GIT microbiota community characterization, they are limited by the need to have some known DNA sequences to look for. Therefore, to detect novel genes and gene variants, it is necessary to have sequencing approaches that do not depend on such information. Researchers use so-called "shotgun" sequencing approaches (figure 2) to circumvent the need for a priori sequence information through the use of molecular manipulations of nucleic acids to attach known sequences for priming of sequencing reactions to unknown sequences. Shotgun sequencing approaches, in which no a priori selection of a region or gene of interest is performed, provides a holistic view of microbial communities, gene content, and expression patterns. However, low-abundance taxa or those with small genomes, like viruses, may be swamped out by high-abundance or large genome organisms and may benefit from targeted amplification approaches.

Two techniques are used for more detailed assessments of GIT microbiota functional capabilities: In shotgun metagenomics, total genomic DNA is fragmented and sequenced directly (Qin et al. 2010), and in shotgun metatranscriptomics, fragmented messenger RNAs are sequenced directly (Perez-Cobas et al. 2013). These techniques can provide data to identify active organisms and metabolic activities at the time of sampling (metatranscriptome) and to directly link community function to specific microbial lineages, even at the species or subspecies level (metagenome and metatranscriptome). Such in-depth analyses can

Methods for Analyzing the Gastrointestinal Microbiota (*continued*)

identify key GIT microbiota community members, identify essential genes associated with the GIT microbiota, and improve metabolic modeling to predict the physiology of dominant organisms in environments undergoing global changes (Greenblum et al. 2012; Karlsson et al. 2013; Qin et al. 2010). Metagenome sequencing can provide much more detailed taxonomy of communities based on genes other than rRNAs, particularly at the species and strain level (Morowitz et al. 2011; Poretsky et al. 2014). In particular, GIT microbiota analyses of disease states and obesity have found widespread application (Greenblum et al. 2012; Karlsson et al. 2012, 2013; Manichanh et al. 2006; Qin et al. 2012). A full survey of the methods for analysis of metagenomic data is beyond this review; however, many recent articles provide deeper overviews (Cho and Blaser 2012) and describe suitable pipelines (Huson et al. 2007; Meyer et al. 2008; Treangen et al. 2013; Zakrzewski et al. 2013).

Although powerful, these approaches are limited by many factors:

- High cost attributed to heavy sequence demand;
- Insufficiently robust reference databases to provide suitable annotation to all recovered gene fragments;
- High microbial diversity in the GIT, which leads to limited coverage of most organisms aside from highly abundant organisms;
- High transcript abundance of housekeeping genes; and
- High computer memory and computational demand for analysis.

Because of the relatively high cost of shotgun sequencing approaches relative to amplicon sequencing approaches (typically about 20 to 30 times higher cost), researchers must carefully tailor their project goals to the appropriate molecular methodology. In a tiered sequencing approach, researchers perform amplicon sequencing on all samples and use their analysis of amplicon data to select critical or representative samples for deeper sequence analysis.

Considerations for Nucleic Acid Extraction

Analysis of gastrointestinal tract (GIT) microbiota communities presents several features worthy of consideration. In particular, researchers take the majority of samples from feces and mucosa-associated colonic tissue biopsies. Traditionally, extraction of nucleic acids from mammalian feces generated nucleic acid templates of poor purity. However, new extraction protocols and commercial kits have largely removed nucleic acid purity as a limitation to downstream molecular analyses (Claassen et al. 2013; Ó Cuív et al. 2011). Indeed, many manufacturers produce kits specifically for GIT colonic tissue and fecal DNA extraction (e.g., Mo Bio PowerFecal® DNA Isolation Kit; Qiagen QIAamp DNA Stool Mini Kit; Zymo ZR Fecal DNA MiniPrep kit; Epicentre ExtractMaster™ Fecal DNA Extraction Kit). Although many of these extraction kits have similar chemistry, other features of the kits may be critical to the maximum recovery of genomic DNA from GIT colonic tissue and feces and to minimize distortion of the GIT microbiota community as a result of differential lysis of different types of microbial cells.

Mammalian GIT microbiota communities are dominated by bacteria from two phyla: *Bacteroidetes* and *Firmicutes* (Ley et al. 2008), and researchers have used the ratio of these phyla as a diagnostic parameter. For example, Mariat and colleagues (2009) observed dramatic age-related changes in the ratio of *Firmicutes* and *Bacteroidetes* (F/B) in feces from healthy individuals, and the ratio has been broadly utilized in studies of obesity, with greater numbers of *Firmicutes* in obese patients (Ley et al. 2006). That said, sampling processing procedures can affect this ratio because the phylum *Firmicutes* consists of mostly gram-positive bacteria with thick cell walls that can make them difficult to lyse, thus high-energy lysis steps (e.g., bead-beating) are important in extraction protocols. In addition, lytic enzymes such as lysozyme, mutanolysin, and lysostaphin can be used individually or in combination to enhance lysis of difficult-to-lyse organisms (Yuan et al. 2012). One study (Bahl et al. 2012) demonstrated that freezing of fecal samples prior to DNA extraction can alter the F/B ratio, with enhanced relative abundance of *Firmicutes* after freezing. As a result of these issues, it may be difficult to easily compare directly between studies of fecal samples processed under different conditions. Likewise, protocols should be carefully considered and rigorously adhered to in order to provide reproducible handling for each sample.

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from the intestinal lumen into systemic circulation (Mutlu et al. 2009; Rimola 1991). Similarly, 16S rRNA gene analysis of fecal microbiota from human subjects with hepatitis B or alcohol-related cirrhosis shows a reduction in *Bacteroidetes* and an increase in *Proteobacteria* and *Fusobacteria*, compared with healthy control subjects (table 1) (Chen et al. 2011). At a finer taxonomic resolution, this study also shows a significant increase in potentially dangerous bacteria from the families *Prevotellaceae*, *Enterobacteriaceae*, *Veillonellaceae*, and *Streptococcaceae* in subjects with alcoholic cirrhosis, compared with subjects with hepatitis B cirrhosis and with control subjects. The prevalence of potentially pathogenic bacteria in patients with cirrhosis may affect prognosis, something supported by previous research (Guarner et al. 1997; Liu et al. 2004). Other lower resolution studies find that the relative abundance of bacteria from the phylum *Bacteroidetes* decreases as those from the phylum *Proteobacteria* increase and that individuals with cirrhosis exhibit a unique increase in *Fusobacteria* (Chen et al. 2011; Mutlu et al. 2012). Overall, alcoholics and cirrhosis patients demonstrate microbial communities enriched in *Proteobacteria* of the

class *Gammaproteobacteria* and *Firmicute* of the class *Bacilli*. In contrast, *Firmicutes* of the class *Clostridia* are depleted in alcoholics but are not significantly changed in the cirrhosis group, with the exception of *Veillonellaceae*, which is increased and *Lachnospira*, which is decreased (table 1). These findings suggest that microbiota community differences between alcoholics and alcoholics with cirrhosis (e.g., *Fusobacteria*, *Clostridia*) may contribute to the development of liver disease or may be a biomarker indicating liver disease (figure 3). Future studies will need to determine the cause-and-effect relationship of the microbiota community structure and liver disease.

Although alcohol can cause intestinal dysbiosis, some alcoholic beverages contain compounds that may favorably alter the GIT microbiota community composition. A study showed the effects of dietary polyphenols on the human GIT microbiota in human healthy control subjects who consumed red wine (272 mL per day), de-alcoholized red wine (272 mL per day), or gin (100 mL per day) for 20 days and had their total fecal DNA assessed from stool collected at baseline and after treatment (Queipo-Ortuno

Table 1 Changes in the Intestinal Microbiome Associated With Alcohol in Rodent Models and Humans

Reference	Tested Organism	Experimental Condition	Methodology	Major Taxa Altered in Presence of Alcohol ^{a,b}	Major Finding
Yan et al. 2011	Mouse	3-week alcohol-fed mice/control isocaloric liquid	<ul style="list-style-type: none"> •16S rRNA gene amplicon sequencing (pyro-sequencing) •Mouse cecum 	<ul style="list-style-type: none"> ↑<i>Verrucomicrobia</i> phylum: ↑<i>Akkermansia</i> genus ↑<i>Bacteroidetes</i> phylum: ↑<i>Bacteroidetes</i> class, ↑<i>Bacteroidales</i> order, ↑<i>Bacteroides</i> genus, ↑<i>Porphyromonadaceae</i> family ↓<i>Firmicutes</i> phylum: ↓<i>Lactococcus</i>, ↓<i>Pediococcus</i>, ↓<i>Lactobacillus</i>, and ↓<i>Leuconostoc</i> genus 	Alcohol-fed mice have GIT microbial community composition significantly altered from control mice indicating dysbiosis.
Mutlu et al. 2009	Rat	10-week alcohol-fed rats/control isocaloric dextrose	<ul style="list-style-type: none"> •Length heterogeneity PCR (LH-PCR) •Ileal and colonic rat mucosa tissue 		Alcohol-fed rats have GIT microbial community composition significantly altered from control rats. Dysbiosis may be an important mechanism of alcohol-induced endotoxemia.
Mutlu et al. 2012	Human	•Alcoholics with and without alcoholic liver disease/healthy patients	<ul style="list-style-type: none"> •16S rRNA gene amplicon sequencing (pyro-sequencing) •Mucosa sigmoid biopsies 	<ul style="list-style-type: none"> ↑<i>Proteobacteria</i> phylum: ↑<i>Gammaproteobacteria</i> class ↑<i>Firmicutes</i> phylum: ↑<i>Bacilli</i> & ↓<i>Clostridia</i> class ↓<i>Bacteroidetes</i> phylum: ↓<i>Bacteroidetes</i> class ↓<i>Verrucomicrobia</i> phylum: ↓<i>Verrucomicrobiae</i> class 	Human chronic alcohol use is associated with changes in the mucosa-associated colonic bacterial composition in a subset of alcoholics from healthy controls. Dysbiotic microbial community alteration correlated with high level of serum endotoxin.
Chen et al. 2011	Human	•Cirrhotic/healthy patients •Alcoholic cirrhotic/healthy patients •Hepatitis B virus cirrhosis/alcoholic cirrhotic patients	<ul style="list-style-type: none"> •16S rRNA gene amplicon sequencing (pyro-sequencing) •Fecal samples 	<ul style="list-style-type: none"> ↑<i>Proteobacteria</i> phylum: ↑<i>Gammaproteobacteria</i> class: ↑<i>Enterobacteriaceae</i> family ↑<i>Firmicutes</i> phylum: ↑<i>Bacilli</i> class: ↑<i>Streptococcaceae</i> family; <i>Clostridia</i> class: ↑<i>Veillonellaceae</i> and ↓<i>Lachnospiraceae</i> family ↑<i>Fusobacteria</i> phylum: ↑<i>Fusobacteria</i> class ↓<i>Bacteroidetes</i> phylum: ↓<i>Bacteroidetes</i> class *<i>Bacteroidetes</i> phylum: ↑<i>Prevotellaceae</i> family 	Fecal GIT microbial community composition significantly altered in patients with cirrhosis compared with healthy individuals. * <i>Prevotellaceae</i> was enriched in alcoholic cirrhosis patients when compared with HBV cirrhosis patients and healthy controls.
Queipo-Ortuno et al. 2012	Human	Healthy patients 20-day intake of either red wine, de-alcoholized red wine, or gin	<ul style="list-style-type: none"> •Quantitative real-time PCR •Fecal samples 	<p>Red wine</p> <ul style="list-style-type: none"> ↑<i>Proteobacteria</i> phylum: (↓Gin) ↑<i>Fusobacteria</i> phylum: (↑De-Alcoholized) (↓Gin) ↑<i>Firmicutes</i> phylum: (↓Gin) ↑<i>Bacteroidetes</i> phylum: (↓Gin) <p>Red wine</p> <ul style="list-style-type: none"> ↑<i>Enterococcus</i> genus (↑De-Alcoholized) (↓Gin) ↑<i>Prevotella</i> genus (↑De-Alcoholized) (↓Gin) ↑<i>Bacteroides</i> genus (↑De-Alcoholized) (↓Gin) ↑<i>Bifidobacterium</i> genus (↑De-Alcoholized) (↓Gin) ↑<i>Bacteroides</i> uniformis species: (↑De-Alcoholized) (↓Gin) ↑<i>Eggerthella lenta</i> species (↑De-alcoholized) (↓Gin) ↑<i>Blautia coccooides-Eubacterium rectale</i> species (↑De-alcoholized) (↓Gin) ↓<i>Clostridium</i> genus (↓De-Alcoholized) (↑Gin) ↓<i>Clostridium histolyticum</i> species (↓De-alcoholized) (↑Gin) 	Red wine consumption, compared to de-alcoholized red wine and gin, significantly altered the growth of select GIT microbiota in healthy patients. This microbial community composition could influence the host's metabolism. Also, polyphenol consumption suggests possible prebiotic benefits, due to the increase growth of <i>Bifidobacterium</i> .
Bode et al. 1984	Human	Alcoholic/hospitalized control patients	<ul style="list-style-type: none"> •Aerobic and anaerobic bacterial culture incubation •Jejunum aspirates 	<ul style="list-style-type: none"> ↑Gram-negative anaerobic bacteria ↑Endospore-forming rods ↑Coliform microorganisms 	Chronic alcohol abuse leads to small intestinal bacterial overgrowth, suggesting dysbiosis may contribute to functional and morphological abnormalities in the GIT.

NOTES: ^a A comparison of bacterial Taxa either ↑, increased or ↓, decreased relative to the presence of alcohol. ^b Taxonomy was updated using the NCBI Taxonomy Browser.

et al. 2012). Red wine polyphenol significantly increases the abundance of *Proteobacteria*, *Fusobacteria*, *Firmicutes* and *Bacteroidetes*, whereas gin consumption significantly decreases these same bacterial phyla (table 1). De-alcoholized red wine consumption significantly increases *Fusobacteria*, and gin consumption increases *Clostridium* abundance compared with de-alcoholized and red wine (table 1). Red wine and de-alcoholized red wine consumption increases the abundance of *Bifidobacterium*, a bacterium that has been shown to be beneficial in the GIT (Gibson et al. 1995). Thus, it seems that polyphenol consumption is associated with an increase in bacteria that are known to promote GIT health, whereas alcohol consumption alone may be damaging to the microbiota balance. The significant decrease of *Clostridium* associated with the consumption of red wine polyphenols suggests that polyphenols may have an inhibitory effect on the growth of *Clostridium*, which has been linked to the progression of colonic cancer and the onset of IBD (Guarner and Malagelada 2003). These results indicate that polyphenol consumption may be used as a dietary intervention to alter the microbiota in a specific way. In addition, daily moderate consumption of red wine polyphenols increases the growth of *Bifidobacterium*, which could be associated with positive prebiotic effects of GIT microbiota, production of beneficial organic acids, and the growth inhibition of pathogenic bacteria (Gibson et al. 1995). Also, as an important consideration to evaluating alcohol-induced effects on the GIT microbiota, differences attributed to the type of alcohol consumption may be contributing to intra- and interstudy variability.

Whether alcohol-induced dysbiosis contributes to the pathogenesis of diseases, such as ALD or alcohol-related cirrhosis, is undetermined. Future studies will need to determine the biological, functional, and clinical significance of the dysbiotic intestinal microbiota composition in alcohol-related disorders.

From Dysbiosis to Disease

Once alcohol disrupts the intestinal microbiota, both the microbiota and microbiome may increase susceptibility to pathological changes (Lozupone et al. 2012). The majority of the reviewed studies indicate an association between alcohol-induced intestinal bacterial overgrowth and dysbiosis and the development/progression of ALD and cirrhosis. Indeed, disrupted intestinal barrier function, which is associated with alcohol consumption, in combination with alcohol-induced bacterial overgrowth and dysbiosis, could be highly relevant for the development of alcohol-induced liver pathology, including nonalcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH), and ALD. Studies show that alcohol consumption disrupts the intestinal barrier (Keshavarzian et al. 1999) via increasing oxidative stress burden in the intestine, which in turn disrupts tight junctions and promotes intestinal hyperpermeability (Rao et al. 2004). Increased intestinal hyperpermeability allows

proinflammatory/pathogenic microbial products, including endotoxin (e.g., lipopolysaccharide [LPS] and peptidoglycan), to translocate from the intestinal lumen to the liver via the portal vein (Frazier et al. 2011). Exposure to these bacterial products causes inflammation in the liver, which may work in conjunction with the direct effects of alcohol to cause ALD (Schnabl and Brenner 2014). This translocation of viable bacterial products during bacterial overgrowth or alcohol-induced dysbiosis may significantly contribute to end-stage liver disease observed in alcohol cirrhosis patients and may therefore contribute to the mortality of cirrhotic patients by inducing infection (Schnabl and Brenner 2014).

Interventions to Normalize Alcohol-Induced Intestinal Dysbiosis

Research in rodents and humans has begun to investigate whether alcohol-induced intestinal dysbiosis and its consequences may be reversible with probiotic and synbiotic interventions (table 2). Probiotics are live microorganisms that, when taken by the host, have beneficial effects on the host beyond their simple nutritive value (Ewaschuk and Dieleman 2006). Synbiotics are a combination of probiotics and prebiotics—nondigestible fibrous compounds, such as oats, that stimulate the growth and activity of advantageous bacteria in the large intestine.

Probiotics, especially *Lactobacillus rhamnosus* GG (LGG), have several beneficial effects on intestinal function, including stimulating intestinal development and mucosal immunity, ameliorating diarrhea, prolonging remission in ulcerative colitis and pouchitis, reducing intestinal oxidative stress, and maintaining or improving intestinal barrier function (Bruzze et al. 2004; Ewaschuk and Dieleman 2006; O'Hara and Shanahan 2006; Resta-Lenert and Barrett 2003; Sartor 2004; Tao et al. 2006; Versalovic 2007). Synbiotics have been demonstrated to favorably alter liver metabolism in alcohol-fed animals (Martin et al. 2009).

Studies in rodents demonstrate that both probiotics and prebiotics prevent alcohol-induced dysbiosis. A study in Sprague-Dawley rats that had consumed alcohol (8 g/kg per day) daily for 10 weeks showed that intragastrically feeding them probiotic LGG (2.5×10^7 live once daily) or prebiotic oats (10 g/kg) prevented alcohol-induced GIT dysbiosis (Mutlu et al. 2009). The rats given the interventions had microbiota composition profiles similar to that of control rats that were intragastrically fed an isocaloric dextrose diet for 10 weeks. This finding corresponds to results obtained in an ALD rodent model demonstrating that LGG attenuates endoxemia and alcoholic steatohepatitis (Nanji et al. 1994). Furthermore, LGG and oat supplementation ameliorates alcohol-induced intestinal oxidative stress, intestinal hyperpermeability, and liver injury in rodent models of alcohol steatohepatitis (Forsyth et al. 2009; Tang et al. 2009). In another study, researchers orally fed C57BL/6 mice the Lieber-DeCarli diet with or without alcohol

Table 2 Changes in the Intestinal Microbiota Associated With Alcohol and Probiotic or Synbiotic Intervention in Rodent Models and Humans

Reference	Tested Organism	Experimental Condition	Methodology	Major Taxa Altered in Presence of Alcohol ^{a,b}	Major Finding
Mutlu et al. 2009	Rat	10 week: Control isocaloric dextrose-fed rats/alcohol-fed rats 1 week (at week 10): Alcohol + LGG-fed rats/alcohol + oat-fed rats/dextrose + oat-fed rats	<ul style="list-style-type: none"> Length heterogeneity PCR (LH-PCR) Colonic rat mucosa tissue 		Alcohol-fed rats have GIT microbial community composition significantly altered from control rats. Both probiotic (LGG) and prebiotic (oats) intervention prevented alcohol-induced dysbiosis, at week 10 in the colonic mucosa tissue of rats.
Bull-Otterson et al. 2013	Mice	6 week: Alcohol-fed mice/control isocaloric maltose dextrin-fed mice 3 week (at weeks 6–8): Alcohol + LGG-fed mice	<ul style="list-style-type: none"> 16S rRNA gene amplicon sequencing (pyro-sequencing) Fecal mice samples 	<p>Alcohol induced: ↑ <i>Proteobacteria</i> phylum: ↑ <i>Alcaligenes</i> genus ↑ <i>Artinobacteria</i> phylum: ↑ <i>Corynebacterium</i> genus ↑ <i>Firmicutes</i>: ↑ <i>Aerococcus</i>, ↑ <i>Listeria</i>, ↑ <i>Acetivibrio</i>, ↑ <i>Clostridiales</i>, ↑ <i>Allobaculum</i>, ↑ <i>Lactobacillus</i> genus</p> <p>↓ <i>Bacteroidetes</i> phylum: ↓ <i>Bacteroides</i>, ↓ <i>Parabacteroides</i>, ↓ <i>Tannerella</i>, ↓ <i>Hallella</i> genus ↓ <i>Firmicutes</i> phylum: ↓ <i>Lachnospiraceae</i>, ↓ <i>Ruminococcaceae</i> genus</p> <p>Alcohol + LGG: ↓ <i>Proteobacteria</i> phylum: ↓ <i>Alcaligene</i> genus ↓ <i>Artinobacteria</i> phylum: ↓ <i>Corynebacterium</i> genus</p> <p>↑ <i>Bacteroidetes</i> phylum ↑↑↑ <i>Firmicutes</i> phylum: ↑ <i>Lactobacillus</i>, ↑ <i>Ruminococcaceae</i> genus</p>	Alcohol-fed mice have fecal GIT microbial community composition significantly altered from control mice. Probiotic (LGG) treatment prevented alcohol induced dysbiosis expansion. LGG reversed the expansion of the <i>Proteobacteria</i> and <i>Actinobacteria</i> phyla, which could play a pathogenic role in the development of alcoholic liver disease. <i>Firmicutes</i> expanded greatly in the alcohol + LGG-fed group.
Liu et al. 2004	Human	30-day treatment: <ul style="list-style-type: none"> Cirrhotic with MHE + synbiotic or prebiotic or placebo/control patients <p>Subgroup: <ul style="list-style-type: none"> Sober alcoholics 2 weeks & etiology is alcohol-cirrhosis </p>	<ul style="list-style-type: none"> Quantitative bacteriological culture Fecal samples 	<p>Cirrhotic with MHE: ↑ <i>Escherichia coli</i> species ↑ <i>Staphylococcus</i> genus</p> <p>Cirrhotic with MHE + synbiotic ↓ <i>Escherichia coli</i> species ↓ <i>Staphylococcus</i> genus ↓ <i>Fusobacterium</i> genus ↑ <i>Lactobacillus</i> genus</p> <p>Cirrhotic with MHE + prebiotic ↓ <i>Escherichia coli</i> species ↓ <i>Fusobacterium</i> genus ↑ <i>Bifidobacterium</i> genus</p>	Cirrhotic patients with MHE were found to have significant fecal overgrowth of potentially pathogenic gram-negative (<i>E. coli</i>) and gram-positive (<i>Staphylococcus</i>) aerobic microbiota. After 30 days of synbiotic or prebiotic treatment, supplementation reduced <i>E. coli</i> , <i>Staphylococcus</i> , and <i>Fusobacterium</i> and increased <i>Lactobacillus</i> (Synbiotic) and <i>Bifidobacterium</i> (prebiotic) organisms in feces of cirrhotic patients with MHE.

NOTES: ^a A comparison of bacterial Taxa either ↑, increased or ↓, decreased relative to the presence of alcohol. ^b Taxonomy was updated using the NCBI Taxonomy Browser.

(5% vol/vol) for 6 weeks and gave a subset of the mice 1 mL of LGG (bacterial density 1×10^9 cfu/mL) orally each day for 6 to 8 weeks (Bull-Otterson et al. 2013). Similar to other findings, the alcohol-fed mice demonstrated a decrease in the abundance of *Bacteroidetes* and *Firmicutes* and an increase in *Proteobacteria* and *Actinobacteria* (table 2). However, probiotic LGG supplementation prevented this alcohol-induced dysbiotic intestinal microbiota composition, especially increasing *Firmicutes*, including *Lactobacillus*. Other studies find that LGG prevents alcohol-induced intestinal hyperpermeability, endotoxemia, and liver injury (Wang et al. 2011, 2013), supporting the notion that LGG may be a therapeutic approach to decrease the development of ALD.

Studies in humans show similar results. One study examined Minimal Hepatic Encephalopathy (MHE) patients with cirrhosis who typically have substantial alterations in their GIT microbiota composition caused by the overgrowth of the potentially pathogenic *Escherichia coli* and *Staphylococcal* species (table 2). Following 30 days of synbiotic and prebiotic treatments, these patients had significantly reduced viable counts of potentially pathogenic GIT microbiota with a concurrent significant increase in fecal content of *Lactobacillus* species (table 2) (Liu et al. 2004). Half of the patients receiving synbiotic treatment also exhibited a

significant reduction in blood ammonia levels, endotoxemia, and reversal of MHE, when compared with control subjects. These improvements in MHE correlate with similar findings showing that probiotic supplementation improved hepatic encephalopathy (HE) in patients with cirrhosis (Macbeth et al. 1965). Interestingly, probiotic LGG supplementation prevents alcohol-induced dysbiosis of the intestinal microbial community, and leads to an increase in *Firmicutes*, particularly of the genus *Lactobacillus*. Furthermore, in an U.S. Food and Drug Administration phase I study, the administration of probiotic LGG to cirrhotic patients with MHE (most of whom had Hepatitis C–induced cirrhosis) found that LGG significantly reduces dysbiosis, tumor necrosis factor (TNF)- α , and endotoxemia in comparison to placebo (Bajaj et al. 2014). In addition, LGG shows beneficial changes in the stool microbial profiles and significant changes in metabolite/microbiota correlations associated with amino acid, vitamin, and secondary bile-acid metabolism in comparison to MHE cirrhotic patients randomly assigned to placebo. In a comparison of the synbiotic and prebiotic treatment to cirrhotic patients with MHE in the study above, probiotic LGG does promote beneficial microbiota; however, it does not increase *Lactobacillus* and does not improve cognitive function in the patients for this randomized clinical trial. Thus, taken together, probiotics

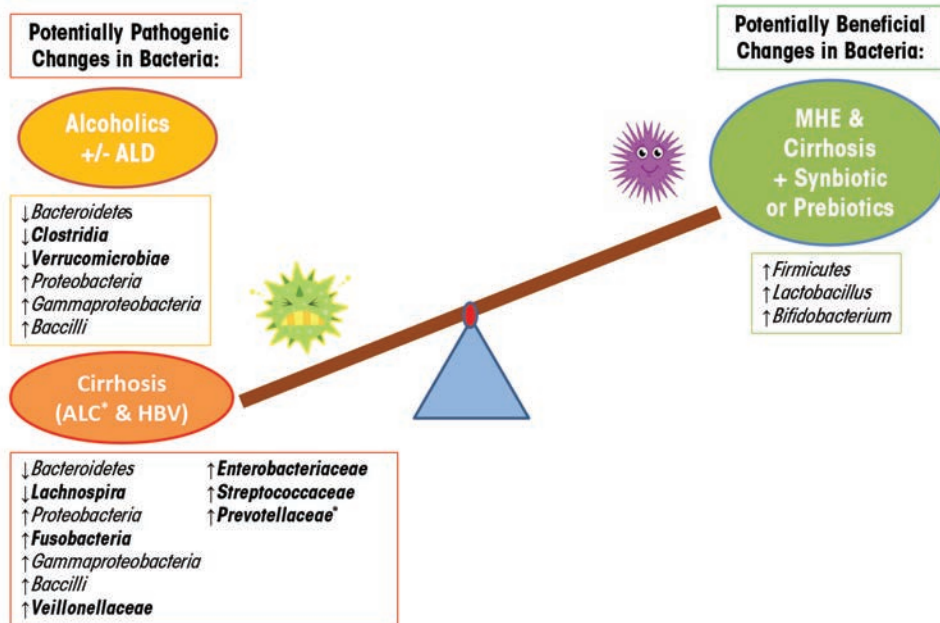


Figure 3 Alcohol-induced imbalances in the microbiome of the gastrointestinal tract (dysbiosis) have been associated with promoting potentially pathogenic changes in bacteria in alcoholics with and without liver disease and in patients with cirrhosis caused by hepatitis B or alcohol. Both alcoholic and cirrhosis patients demonstrate similar dysbiotic microbiota changes, except for the bacteria indicated, suggesting that these dysbiotic bacterial differences could contribute to liver disease or may be a biomarker indicating liver disease. Using synbiotics and prebiotics to treat Minimal Hepatic Encephalopathy patients with cirrhosis, significantly improved their GIT microbiota, suggesting that the same treatment may benefit patients with alcohol-induced dysbiosis.

and/or synbiotics may be a viable approach in humans to alter the GIT microbiota to a more favorable profile to improve clinical outcomes (figure 3).

Therapeutic Intervention for Treating Alcohol-Induced Intestinal Dysbiosis

The therapeutic intervention studies in this review indicate that in ALD rodent models and MHE alcohol-cirrhosis humans, probiotic and synbiotic intervention increases *Lactobacillus* and *Bifidobacterium* (table 2). These findings suggest that the intestinal microbiota play a role in attenuating alcohol-induced dysbiosis and liver injury. In addition, the modulation of intestinal microbiota could be a viable therapeutic strategy to prevent or normalize alcohol-induced dysbiosis and which would be expected to have beneficial effects on alcohol-induced liver injury as well as other inflammatory-mediated diseases resulting from chronic alcohol consumption.

Evidence suggests that probiotic and synbiotic interventions can not only reverse alcohol-induced dysbiosis but can improve the pathogenesis symptoms of the GIT and liver in ALD. Treatment with probiotics prevents or significantly decreases alcohol-induced intestinal permeability (Forsyth et al. 2009; Wang et al. 2012), intestinal oxidative stress and inflammation of the intestine and liver (Forsyth et al. 2009), TNF- α production (Wang et al. 2013), and expression of intestinal trefoil factor and its transcriptional regulator

hypoxia-inducible factor-2 α (HIF-2 α) (Wang et al. 2011) and attenuates endotoxemia and alcoholic steatoph hepatitis (Nanji et al. 1994) in rodent models and in humans with ALD. Probiotics also restore stool microbiota community structure and liver enzymes in ALD human patients (Kirpich et al. 2008). In addition, prebiotic oat supplementation prevents alcohol-induced gut leakiness in an ALD rat model by preventing alcohol-induced oxidative tissue damage (Tang et al. 2009). Thus, these studies suggest that probiotics (e.g., *Lactobacillus*) transform the intestinal microbiota community composition, which may prevent alcohol-induced dysbiosis, intestinal permeability, bacterial translocation, endotoxemia, and the development of ALD. Transformation of the intestinal microbiota may be a therapeutic target for the treatment of intestinal barrier dysfunction and the development of ALD.

Clinical studies suggest that probiotic consumption of *Lactobacilli*, *Bifidobacteria*, and *Lactococci* are effective for the prevention and treatment of a diverse range of disorders (Snydman 2008). History shows that probiotic consumption is safe in healthy people but must still be taken with caution in certain patient groups, including premature neonates, people with immune deficiency, people with short-bowel syndrome, people with central venous catheters, the elderly, and people with cardiac disease (Boyle et al. 2006; Snydman 2008). Clinical trials show that the effects of probiotics are variable depending on age, health, and disease state. Probiotic use also has its concerns. It presents a major risk of sepsis (Boyle et al. 2006) and has been associated with diseases such as bacteremia or endocarditis, toxic or metabolic effects on the GIT, and the transfer of antibiotic resistance in the gastrointestinal flora (Snydman 2008). In addition, the many properties of different probiotic species vary and can be strain specific. Therefore, the effect of new probiotic strains should be carefully analyzed in clinical trials before assuming they are safe to market as a potential therapeutic treatment.

Glossary

Dysbiosis: Dysbiosis is a term used to describe a microbial imbalance on or inside the body, commonly within the digestive tract where it has been associated with illness.

Endotoxemia: The presence of endotoxins in the blood, where endoxins are toxic substances bound to the cell wall of certain bacteria.

Polymerase Chain Reaction (PCR): A biochemical technology used to amplify a single or a few copies of a particular piece of DNA, generating millions of copies of that DNA sequence. Among other uses, the technique allows researchers to make enough copies of a piece of DNA to sequence it. PCR requires “primers” or small snippets of DNA that match a piece of the DNA researchers are attempting to replicate.

Tumor necrosis factor-alpha (TNF- α): A type of cytokine, or cell-signaling protein that can cause cell death.

Future Directions

Chronic alcohol consumption causes intestinal dysbiosis in both rodent models and humans. Dysbiosis in the intestinal microbiota may contribute to the pathogenesis of liver disease by altering intestinal barrier function leading, for example, to gut leakiness, the production of proinflammatory/pathogenic microbial products, and/or liver metabolic pathways. Further investigation into intestinal microbiota composition in alcoholism is necessary to identify new diagnostic as well as therapeutic targets to prevent alcohol-associated diseases, such as ALD. Such therapeutic avenues could include probiotics, prebiotics, synbiotics, or polyphenols to alleviate the symptoms associated with alcohol disorders. Thus, understanding the effect of alcohol on intestinal microbiota composition, may lead to a better understanding of its future functional activity, with the ultimate goal to restore intestinal microbiota homeostasis.

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The First Line of Defense

The Effects of Alcohol on Post-Burn Intestinal Barrier, Immune Cells, and Microbiome

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Alcohol (ethanol) is one of the most globally abused substances, and is one of the leading causes of premature death in the world. As a result of its complexity and direct contact with ingested alcohol, the intestine represents the primary source from which alcohol-associated pathologies stem. The gut is the largest reservoir of bacteria in the body, and under healthy conditions, it maintains a barrier preventing bacteria from translocating out of the intestinal lumen. The intestinal barrier is compromised following alcohol exposure, which can lead to life-threatening systemic complications including sepsis and multiple organ failure. Furthermore, alcohol is a major confounding factor in pathology associated with trauma. Experimental data from both human and animal studies suggest that alcohol perturbs the intestinal barrier and its function, which is exacerbated by a “second hit” from traumatic injury. This article highlights the role of alcohol-mediated alterations of the intestinal epithelia and its defense against bacteria within the gut, and the impact of alcohol on intestinal immunity, specifically on T cells and neutrophils. Finally, it discusses how the gut microbiome both contributes to and protects the intestines from dysbiosis after alcohol exposure and trauma.

Key words: Alcohol use, abuse, and dependence; alcohol consumption; alcohol exposure; alcohol effects and consequences; burns; immunity; immune cells; microbiome; intestine; gut; intestinal lumen; intestinal barrier; bacteria; sepsis; organ failure; trauma; T cells; neutrophils; dysbiosis; human studies; animal models

Each year 2.5 million people die from alcohol abuse and its related morbidities worldwide, making alcohol related deaths among the highest preventable causes of death, and the greatest cause of premature death and disability in men between ages 15 and 59 (World Health Organization 2011). Alcohol abuse predisposes individuals to life-threatening conditions such as alcoholic liver disease (ALD), acute respiratory distress syndrome (ARDS), sepsis, and multiple organ failure (MOF) (Bird and Kovacs 2008; Molina et al. 2003; Purohit et al. 2008). Further, studies show that intoxication often plays a role in physical injury (Pories et al. 1992). Data demonstrate that a majority of

patients admitted to the hospital for traumatic injury have detectable blood alcohol levels at the time of admittance (Grobmyer et al. 1996; Jones et al. 1991; Maier 2001; McGill et al. 1995; McGwin et al. 2000; Silver et al. 2008). These patients generally require more extensive care than patients who have not been drinking. They more frequently require surgical intervention, experience higher susceptibility to infection, and have longer hospital stays (Silver et al. 2008). Supporting these observations, experimental data suggest that alcohol at the time of trauma results in more severe pathology in animal models (Choudhry and Chaudry 2008; Messingham et al. 2002; Molina et al.

2003, 2013). As a result, researchers estimate that in the United States alone, trauma and alcohol-related expenses to society total \$185 billion annually (Li et al. 2004).

The disruptions to human biology that underlie the association between alcohol and these conditions bear exploring. The intestine, where alcohol first meets with digestive and immune mechanisms, is a primary source of alcohol-related pathologies. Here, alcohol and its metabolites encounter the physical barrier lining the gut that prevents invading pathogens from moving into the body. They also come into contact with a particularly complex frontier where the immune system

must distinguish between commensal bacteria that normally colonize human intestines, and foreign microbes that cause disease. Any disruption of these systems by alcohol certainly could contribute to inflammatory states in the body that may in turn lead to serious conditions such as sepsis and MOF.

In support of these possibilities, data have shown that acute alcohol exposure negatively affects the function of the intestines, and this is exacerbated by a second traumatic insult such as burn injury (Akhtar et al. 2009, 2011; Li et al. 2008a, 2009, 2011, 2012; Rendon et al. 2012, 2013, 2014). The consequences of disruptions to the intestinal barrier, immune cells, and microbiome (see Glossary) can be observed within 24 hours following injury, and likely contribute to the life-threatening complications mentioned above. Thus, understanding how both acute and chronic alcohol exposure disrupt the homeostatic gastrointestinal tract is paramount. This article will review relevant studies examining the role of gut epithelia in defense against pathogenic bacteria within the gut and the impact of alcohol on intestinal immunity, highlighting T cells and neutrophils. Finally, it will review how the gut microbiome plays a role in maintenance of gut barrier integrity following alcohol exposure and trauma.

Intestinal Anatomy and Histology

To fully understand the intricate relationships among the gut barrier, immune system, and microbiome, gastrointestinal (GI) anatomy requires review. The spatial relationships established between the lumen and barrier of the gut are essential for the proper function of the GI tract in digestion and nutrient absorption. The GI tract is a continuous tube that begins at the mouth and ends at the anus. The small and large intestines function mainly to absorb nutrients and water, and this review will focus on these organs.

The small intestine is divided into three regions: the duodenum, jejunum, and ileum, respectively. At the distal end of the ileum lies the cecum, which connects the small and large intestines. From the cecum, the large intestine (colon) is composed of four regions: the ascending, transverse, descending and sigmoid colon, respectively, terminating in the rectum and anus. The small and large intestines are held in place to prevent twisting by the

Data have shown that acute alcohol exposure negatively affects the function of the intestines, and this is exacerbated by a second traumatic insult such as burn injury.

mesentery, which also contains the mesenteric lymph nodes (MLNs). As shown in figure 1, the small and large intestines at the histological level contain a barrier of mucous and epithelial cells that block the translocation of bacteria in the lumen to sites in the body beyond the intestines. Just below the intestinal epithelia lies a layer of loose connective tissue called the lamina propria (LP), which connects the surface mucosal epithelium to the basement muscularis mucosae. The LP also contains a large number of intestinal immune cells. In addition, specialized regions within the small intestine called Peyer's patches (PPs) serve as lymphoid follicles, where naïve immune cells differentiate into a variety of mature immune cell subsets.

When a pathogen invades through the gut, the intestinal barrier and the immune cells in it mount a response to prevent infection. However, the picture gets more complex because of the gut microbiome, the mix of commensal bacterial species colonizing the lumen. The immediate proximity of the intestinal immune cells to the bacteria within

the lumen presents a major challenge for homeostatic regulation. Thus, the interactions between the immune cells, intestinal barrier, and luminal microbiome are of major interest in all areas related to pathology associated with the intestines. Alcohol modulates all of these components, and a disruption of any one can result in serious disease and/or infection that can affect all regions of the body.

The Homeostatic Intestinal Physical Barrier

Looking more closely at the meeting point of the lumen with the intestinal wall, the intestinal physical barrier consists of a layer of mucus and epithelial cells that line the lumen and provide a crucial first line of defense against pathogens. Starting from the lumen, the first component of the physical barrier is a mucus layer. Mucus offers protection from the luminal bacterial content and also lubricates the intestinal walls for passing bile (Bollinger et al. 2006; Groschwitz and Hogan 2009; Peterson and Artis 2014; Valatas and Kolios 2009). Immediately below the mucus layer, a single layer of epithelial cells forms a second barrier featuring tight junction protein complexes that adhere adjacent cells to each other (Peterson and Artis 2014; Ulluwishewa et al. 2011). The body maintains this barrier by regulating the proliferation and apoptosis of epithelial cells (Peterson and Artis 2014). Together, the mucus layer and epithelial cells of the intestinal barrier minimize interactions of inflammatory host immune cells with the luminal bacteria.

Mucus Layer

The mucus layer is a key component of the physical barrier and is formed by a glycoprotein, mucin (mainly mucin-2). Goblet cells found in the intestinal epithelial layer secrete mucin (Kim and Ho 2010). Mucin contains a glycosylated peptide backbone, which creates an incredibly viscous mucus layer effective at preventing pathogens

penetration (Hartmann et al. 2013). Recently, a study found that the small intestine has a porous mucus layer that allows uptake of mucin-2 (MUC2) by intestinal dendritic cells (DCs) (see “Primer on the Immune System” in this issue). DCs containing MUC2 were able to generate anti-inflammatory responses through β -catenin and NF κ B-mediated mechanisms, giving rise to a newly identified homeostatic role for the intestinal mucosa (Shan et al. 2013).

Epithelial Layer

The mucus layer is not impenetrable, however, and the tight junction complexes between the epithelial cells

below the mucus layer play a crucial role in providing a second level of protection. Tight junctions (figure 2) are multi-protein complexes consisting of transmembrane, scaffold, and adaptor proteins, which play an indispensable part in the maintenance of barrier function (Ivanov 2012). The proteins of tight junctions form a paracellular seal and function as a selectively permeable barrier between adjacent epithelial cells. They allow nutrients from food to pass out of the lumen while blocking passage of bacteria. Among the transmembrane proteins making up tight junctions are occludin, claudins, tricellulin, and junctional adhesions (Ulluwishewa et al.

2011). Although the function of occludin proteins is unknown, they are not essential for tight junction formation but appear instead to be instrumental in the regulation of the junctions (Balda and Matter 2008; Forster 2008; Groschwitz and Hogan 2009). Claudins are a family of both tissue- and cell-type-specific proteins considered to be the main structural components of the tight junctions. A third class of proteins found in tight junctions are junction-associated adhesion molecules (JAMs); however, little is known about their contribution to tight junction function and assembly (Balda and Matter 2008;

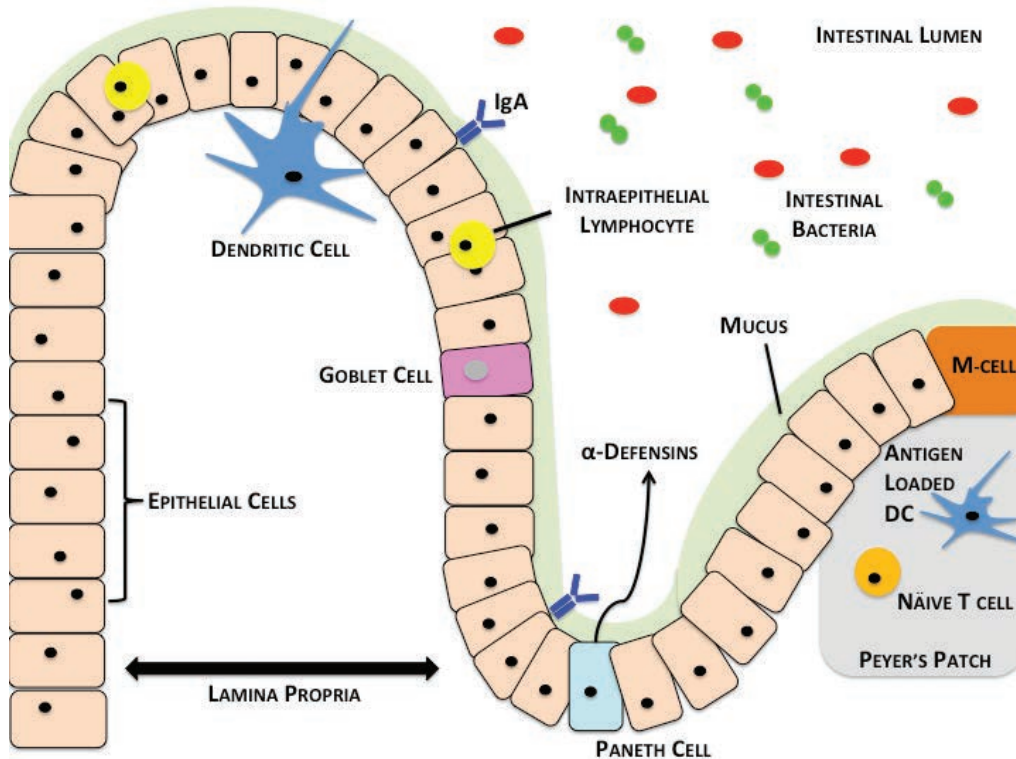


Figure 1 Overview of the intestinal barrier, immune cells, and microbiome. Luminal bacteria (red and green) are relegated to the lumen of the intestine by the intestinal barrier composed of the mucus (green), which contains IgA bound antibodies (blue) and epithelial cells. The epithelial-cell layer contains intraepithelial lymphocytes (yellow) and mucin-secreting goblet cells (pink). At the base of the intestinal crypts lie Paneth cells (light blue), which secrete alpha-defensins. Directly below the epithelial layer lies the lamina propria. Dendritic cells sample the luminal bacterial contents and migrate to Peyer’s patches (gray) within the small intestine, where they interact with T cells (orange). M cells allow the passage of antigens into Peyer’s patches for uptake by resident antigen presenting cells.

Forster 2008; Groschwitz and Hogan 2009).

In addition to the transmembrane proteins that constitute the paracellular barrier, tight junctions also contain a complex system of adaptor molecules and scaffold proteins that mediate crosslinks between the transmembrane proteins and the actin cytoskeletons within epithelial cells. Besides forming tight junctions, intestinal epithelial cells themselves constitute a dynamic community of cells. The crypt-villus axis (see Glossary) allows constant regeneration of cells by differentiation and migration of cryptic stem cells to maintain barrier integrity. This balance

of apoptosis and proliferation enables normal intestinal barrier function (Peterson and Artis 2014).

Intestinal Physical Barrier Following Alcohol Exposure and Trauma

Disruptions in either the intestinal mucus or epithelial barrier can result in pathogenic bacterial translocation. This can lead to systemic infections, sepsis, and multiple organ failure, which underscores the importance of maintaining barrier integrity (Choudhry

et al. 2000, 2004; Napolitano et al. 1995). Alcohol exposure can cause disruptions in all components of the intestinal barrier (Farhadi et al. 2003; Keshavarzian and Fields 2003). Such alterations may subsequently lead to an increase in bacterial translocation and infection among hospitalized trauma patients who have detectable blood alcohol levels at the time of their admittance (Bird and Kovacs 2008; Maier 2001; McGill et al. 1995; Molina et al. 2013; Silver et al. 2008; Valatas and Kolios 2009). Researchers have started to identify alcohol's specific effects on different parts of the physical barrier.

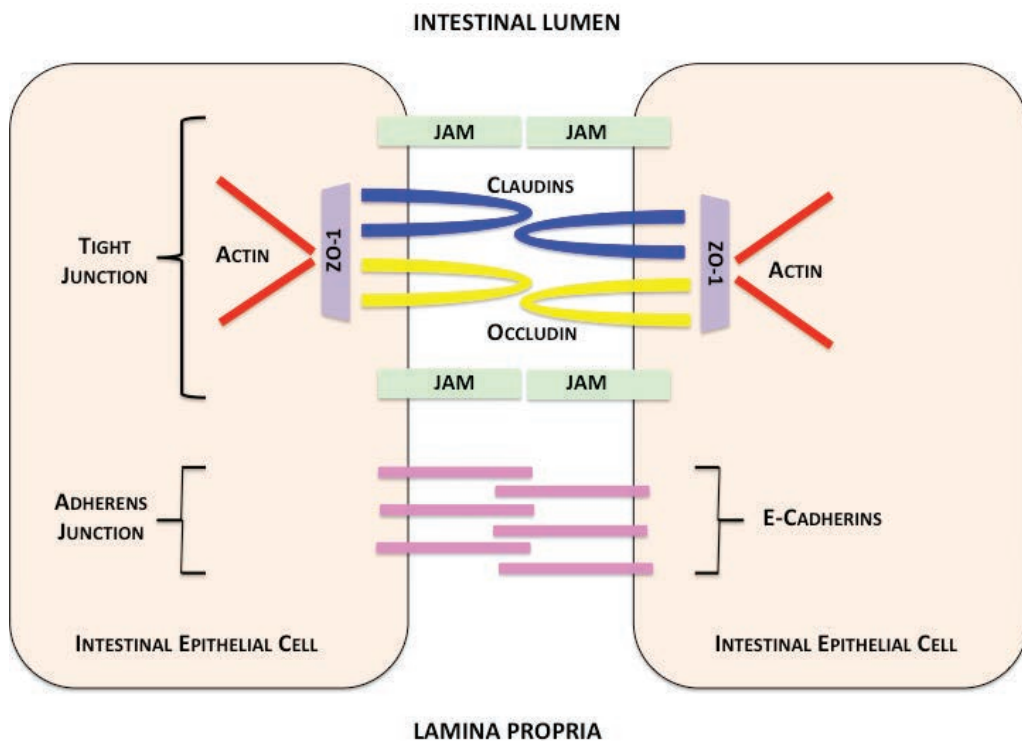


Figure 2 Intestinal epithelial-cell junctions. Contents within the intestinal lumen are prevented from passing between epithelial cells by apical tight-junction complexes. Tight junctions are composed of claudin proteins (blue) and regulated by occludin proteins (yellow). Claudin and occludin proteins are transmembrane proteins attached to an adaptor molecule, zonula occludens protein 1 (ZO-1) (purple), which anchors tight-junction proteins to intracellular actin (red). Alcohol causes disruption of occludin and ZO proteins by an unknown mechanism. Junctional adhesion molecules (JAMs) (green) also support tight-junction interactions. Intestinal epithelial cells are further supported by adherens molecules, including E-cadherins (pink), which also contribute to cell-cell contact. These junctions allow selective separation of the intestinal lumen (top) and lamina propria (bottom).

As the first line of defense against pathogenic organisms within the intestinal lumen, the mucus layer and its alteration by alcohol exposure are of particular research interest. Grewal and Mahmood (2009) investigated the role of chronic alcohol exposure on mucin production in a rat model. They demonstrated that prolonged alcohol exposure (25 to 56 days) resulted in increased mucin production. This study also discovered that several components of the mucin biochemical composition were altered following prolonged alcohol exposure. Modulation of glycosylation and enzymatic activity within the mucus layer could potentially affect the barrier's integrity, as these sites could begin to harbor adherent pathogenic bacteria (Van Klinken et al. 1995). In contrast to this finding, others have shown that chronic alcohol exposure results in decreased mucin production in the intestines of rats (Slomiany et al. 1997, 2000). Furthermore, Hartmann and colleagues (2013) demonstrated that MUC2 knockout mice are less susceptible to bacterial overgrowth and translocation following chronic alcohol exposure and are thus less prone to alcoholic liver disease. These findings suggest a relationship between alcohol exposure and mucus production. Further investigation will be required to establish the effects of alcohol on mucin production and to elucidate the mechanism by which alcohol alters the intestinal mucus layer.

Not surprisingly, alcohol and trauma also disrupt the integrity of tight junction complexes between intestinal epithelial cells (Choudhry et al. 2002; Li et al. 2008a; Tang et al. 2009). An *in vitro* study showed that Caco-2 human intestinal epithelial cells exposed to a daily regime of alcohol demonstrated a reduction in membrane localization of the adherens protein ZO-1. Furthermore, allowing the alcohol-treated cells to “recover” from alcohol exposure by culturing them for 2 weeks in alcohol-free media improved ZO-1 localization (Wood et al. 2013). Studies by Rao and colleagues have also demonstrated that acetalde-

hyde, a metabolite of alcohol, results in similar disruption of occludin and ZO-1 proteins by altering their phosphorylation status (Atkinson and Rao 2001; Dunagan et al. 2012; Rao 2008). Another study conducted by Ma and colleagues (1999) using Caco-2 cells showed identical perturbation of ZO-1 proteins. The study further demonstrated that alcohol activates an enzyme, myosin light-chain kinase (MLCK), that phosphorylates myosin regulatory light-chain (MLC), promoting its interaction with actin to cause cytoskeletal sliding (Ma et al. 1999). This interaction is important in tight junction function and may be one cause of the alcohol-related disruption of tight junctions in intestinal epithelial cells (Groschwitz and Hogan 2009). Zahs and colleagues (2012) examined the role of MLCK in gut barrier disruption following combined binge alcohol exposure and burn injury. They showed that the combination of alcohol intoxication and burn injury results in both elevated MLCK and phosphorylated MLC and decreased co-localization of both occludin and ZO-1. Such changes could alter barrier permeability.

In an *in vivo* study of acute alcohol exposure and burn injury in rats, Li and colleagues (2012) showed that the combined insult resulted in a significant reduction in phosphorylation and expression of occludin and claudin-1, which was correlated with increased epithelial cell apoptosis. Yoseph and colleagues (2013) further demonstrated that the combination of chronic alcohol and cecal ligation and puncture (CLP)-sepsis resulted in elevated intestinal epithelial apoptosis as well as decreased proliferation of cells compared to CLP-sepsis alone. Clearly, exposure to alcohol and trauma greatly affects all components of the intestinal physical barrier through changes in mucosal production and biochemical structure, disruptions of tight junction protein complexes, and increasing susceptibility to apoptosis in epithelial cells. The mechanisms by which alcohol and trauma cause these alterations are just beginning to be elucidated. Future

work will focus on how to prevent such disruptions.

The Intestinal Immune System

Beyond the physical barrier, the next line of defense against invading pathogens is the immune system within the gut, which has the most difficult task in the body. Not only does it protect the host from invading pathogens, but it also maintains homeostasis with the vastly diverse microbiome within the intestinal lumen. The immune system must distinguish between commensal and pathogenic bacteria so that it does not mount a damaging autoimmune inflammatory response. The immune cells that carry out these tasks comprise parts of both innate and acquired immune functions. They can be found in all areas of the intestines, especially in regions called gut associated lymphoid tissue (GALT). GALT includes the gut epithelium, PPs, MLNs, and LP (Choudhry et al. 2004; Mowat and Viney 1997). Intestinal T cells are found in GALT sites and exist closely with antigen presenting cells (APCs), such as DCs and macrophages, that aid in T cell differentiation and activation (figure 3). Scientists are beginning to define the roles of macrophages and DCs in gut immune functions following alcohol exposure or trauma, as well as the initial innate immune responses that occur following these insults. These immune cells activate or suppress one another using highly complex chemical signaling pathways that researchers are beginning to uncover. Alcohol could produce disruptive effects at any point along these pathways (see figure 3).

Innate Immunity

A key part of the innate immune response, neutrophils, or polymorphonuclear leukocytes (PMNs), make up a significant portion of the innate immune cells present in humans. They play integral roles in initial responses to infection including degranulation and phagocytosis (Amulic et al. 2012).

It appears that one of the main functions of gut neutrophils under homeostatic conditions is to prevent the translocation of bacteria across the epithelial barrier (Choudhry et al. 2002; Kuhl et al. 2007; Li et al. 2008*b*). In addition, IL-17 cytokine released by activated T cells known as Th17 cells supports an inflammatory immune response through recruitment of neutrophils (Hundorfean et al. 2012). It is important to note that the role of neutrophils under pathologic conditions in the intestines remains unclear. In models of inflammatory bowel disease (IBD), different studies have shown neutrophils to be beneficial (Kuhl et al. 2007; Zhang et al. 2011), harmful (Kankuri et al. 2001; Natsui et al. 1997), or indifferent (Yamada et al. 1991). Interestingly, understanding of the function of neutrophils within the intestines of mice and humans has diverged slightly as studies show that murine neutrophils secrete defensins (see Glossary), whereas human neutrophils do not (Ganz 2003; Ouellette and Selsted 1996; Risso 2000).

Neutrophil Activity Following Alcohol Exposure and Trauma

Following alcohol intoxication and trauma, neutrophil infiltration increases into different organs, including the lungs and intestines (Akhtar et al. 2009; Bird et al. 2010; Li et al. 2008*b*; Scalfani et al. 2007). Although the role of neutrophils is unclear in disease models such as IBD, neutrophils appear to have detrimental effects after alcohol exposure and trauma (Li et al. 2008*b*). Several studies have found that the inflammatory microenvironment following alcohol exposure and/or trauma may allow neutrophils to exacerbate tissue damage in numerous organs including intestine (Amin et al. 2007*a,b*; Bird and Kovacs 2008; Li et al. 2007, 2008*a*, 2011). Studies in animal models provide details surrounding neutrophil activity after alcohol intoxication and trauma. These publications show that not only are neutrophils recruited by the pro-inflammatory

cytokines IL-6 and IL-18, but they also have a prolonged presence at the injury sites (Akhtar et al. 2009; Scalfani et al. 2007; Zahs et al. 2013). Scientists do not know whether IL-6 and/or IL-18 directly recruit neutrophils, or whether these cytokines signal through other molecules such as monocyte chemoattractant-1 (MCP-1) or myeloperoxidase (MPO) (Li et al. 2011; Rana et al. 2005). They also do not know what role alcohol plays in neutrophil recruitment. However, previous work showed that alcohol intoxication leads to increased recruitment of neutrophils to the intestine following ischemic injury (Tabata and Meyer 1995). One proposal suggests that this may occur through upregulation of intestinal ICAM-1 expression following ischemic/reperfusion injury (Olanders et al. 2002). Once at the injury site, neutrophils secrete superoxide anions that kill any invading pathogens entering through the compromised intestinal barrier (Li et al. 2008*b*, 2011). Although this response is helpful at initially protecting from invading pathogens, prolonged neutrophil responses mediate tissue damage in multiple organs under inflammatory conditions (Fukushima et al. 1995; Partrick et al. 2000). Further studies will be necessary to determine how neutrophils respond following alcohol exposure, and also how they mediate the subsequent adaptive immune response.

Adaptive Immunity

T lymphocytes form a large part of the adaptive immune response in the intestine. Under homeostatic conditions, the balance between inflammatory and immunosuppressive T cells is maintained through cell-to-cell cytokine signaling. Although the intestines contain a large and diverse population of T lymphocytes, the major subsets of resident T cells within the gut include Th1, Th2, Th17, and T-regulatory (Treg) cells (Belkaid et al. 2013). The default T cell response in the intestines under normal condi-

tions is immunosuppressive. This occurs through the production of TGF- β , primarily by APCs, which drives Treg development (figure 3). In addition to TGF- β , IL-4 production drives Th2 cell development and B cell IgA antibody production. IgA also maintains gut homeostasis, in part by regulating the microbiome (Weaver et al. 2006).

The production of these immunomodulatory cytokines largely depends on resident DCs that sample the luminal contents at the epithelial barrier (Cerovic et al. 2014). DCs decipher commensal and pathogenic bacterial antigens to modulate appropriate T-cell development by a mechanism now under investigation (Cerovic et al. 2014). Naïve CD4⁺/Foxp3-T cells within GALT are driven toward specific T-cell phenotypes, depending upon the milieu of extrinsic factors present. Once activated, these T cells release cytokines to generate an immune response. Development of the Th1 phenotype depends on cytokines including IL-12, which is augmented by the presence of IL-18. IL-12 binds to its cognate receptor (IL-12R), which results in downstream signaling through the transcription factors STAT4 and T-box protein 21 (T-bet) (Amsen et al. 2009). Interestingly, recent reports show that STAT4 and T-bet may act in unison to drive Th1 differentiation. Thieu and colleagues (2008) have described a role for STAT4 in chromatin remodeling that promotes *Ifng* gene transcription by T-bet to drive Th1 differentiation. This signaling is initiated following antigen recognition on MHC-II molecules, whereupon Th1 cells secrete the cytokines IFN- γ and lymphotoxin alpha (LT- α), a member of the pro-inflammatory TNF family (Weaver et al. 2006). Some have hypothesized that Th1 cells may play a role in regulating innate mucosal responses; however, further investigation must confirm this (Belkaid et al. 2013). As mentioned above, other cytokines such as TGF- β keep development of Th1 cells in

check under homeostatic conditions. TGF- β plays an important role in preventing the differentiation of naïve T cells into inflammatory phenotypes (Sansonetti and Di Santo 2007).

Th17 cells form the other major inflammatory T cell subset found in the intestines. Intestinal Th17 development also depends heavily on the cytokine milieu. It is largely driven by the presence of IL-6. More recent studies have implicated IL-23 in Th17 differentiation, but it appears that IL-23 may only augment Th17 differentiation as opposed to being an essential component (Maynard and Weaver 2009). IL-6 and IL-23, which

are mainly produced by DCs and macrophages, signal through their cognate receptors on naïve CD4⁺ T cells, which in turn signal through the ROR- γ T transcription factor. ROR- γ T transcription drives Th17 cells to produce a host of different cytokines including IL-17A, IL-17F, IL-21, and IL-22 (Maloy and Kullberg 2008). Many contrasting studies have been published regarding the roles of Th17 cytokines. Although IL-17A and IL-17F are generally present under inflammatory conditions (Ahern et al. 2010; Leppkes et al. 2009; Wu et al. 2009; Yang et al. 2008), scientists have also observed contradictory

protective roles of IL-17A in models of IBD (Yang et al. 2008). Fewer studies have examined the actions of IL-21 and IL-22, but both cytokines seem to play a protective role in epithelia regeneration following injury (Maloy and Kullberg 2008; Sonnenberg et al. 2010). Although it is clear that Th17 cells play an essential part in modulating intestinal inflammatory immune responses, more studies will be needed to elucidate their specific functions in homeostatic and diseased conditions within the intestines.

Balancing the inflammatory T cells within the intestines, modulatory T cells are an important subset made up

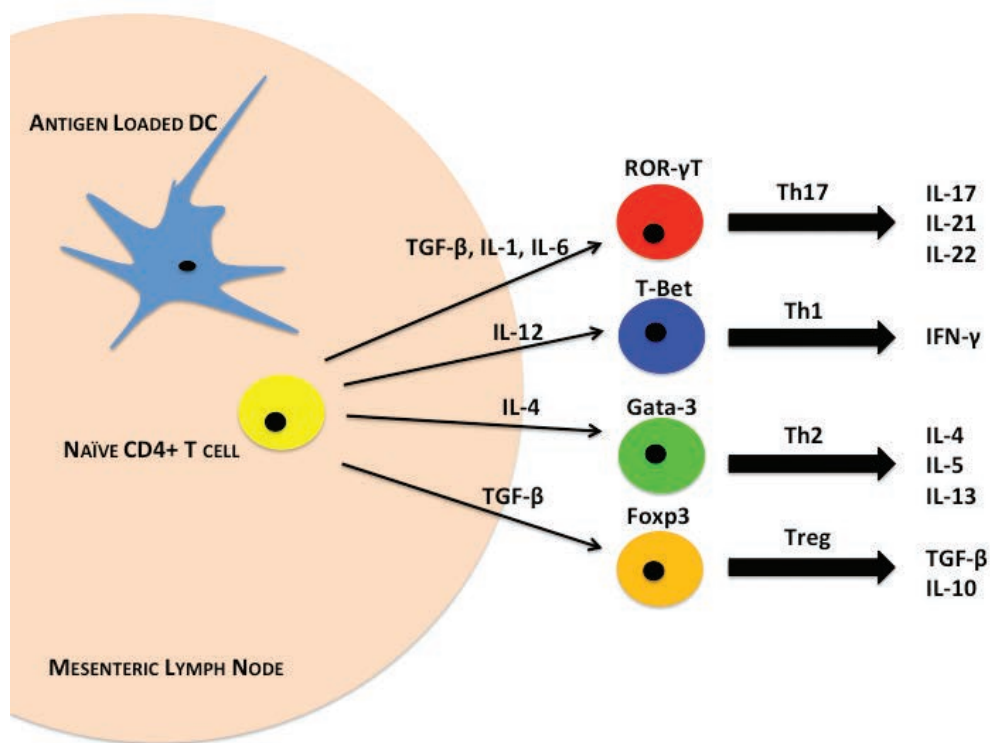


Figure 3 Intestinal CD4⁺ T-cell differentiation. Antigen-loaded dendritic cells (DCs) interact with naïve CD4⁺ T cells (yellow) in mesenteric lymph nodes through MHC-II molecules. DCs secrete different cytokines depending on this interaction. Following alcohol and burn injury, antigen-presenting cells (APCs) such as DCs may have a significantly altered cytokine expression profile. The cytokine profiles present lead to the expression of different transcription factors that promote differentiation of T cells into either Th17 (red), Th1 (blue), Th2 (green), or Treg (orange) phenotypes. These T-cell subsets secrete different cytokines that lead to inflammatory or immunosuppressive immune responses. Combined alcohol and burn injury has been shown to suppress T-cell cytokines including interferon (IFN)- γ , interleukin (IL)-17, and IL-22 from T cells.

of Th2 and Treg cells. Antigen-loaded DCs that have sampled the luminal contents release IL-4 to drive the differentiation of Th2 cells. Activation of the IL-4 receptor leads to downstream signaling through the transcription factor STAT6, which mediates the expression of another transcription factor, Gata3 (Ansel et al. 2006). Gata3 plays a major role in mediating production of key Th2 cytokines IL-4, IL-5, and IL-13. Gata3 also prevents Th1 differentiation through its inhibitory effects on IL-12 receptor and STAT4 signaling (Amsen et al. 2009; Ansel et al. 2006). One of the most important roles for Th2 cells in the maintenance of gut homeostasis is their interaction with B cells to aid in the development of IgA-producing plasma cells. IgA antibodies function to regulate homeostasis of the microbiome, as well as act as a first line of immune defense against pathogens in the GI lumen. They are by far the most highly expressed class of antibodies in the intestines of humans (Mantis et al. 2011).

Treg cells also serve a critical function in modulating the immune responses within the intestines. Populations of Tregs within the gut derive both from thymic CD4⁺CD25⁺Foxp3⁺ precursors that migrate to the gut, as well as from the gut itself, where resident naïve CD4⁺ T cells are preferentially driven towards a Treg phenotype by TGF- β , IL-10, and Foxp3 expression (Fontenot et al. 2005). Studies show that the recognition of self-antigens presented by DCs initiates Treg activation (Hsieh et al. 2006; Nishikawa et al. 2005; Watanabe et al. 2005). After sampling the luminal contents in the intestine, DCs migrate to MLNs where some present self-antigens on MHC-II molecules to naïve CD4⁺ T cells. Activation of T-cell receptors by self-antigens stimulates Foxp3 signaling to drive anti-inflammatory TGF- β and IL-10 secretion. In this regard, Tregs are able not only to inhibit inappropriate inflammatory responses to these self-antigens by Th1 and Th17 cells, but also to drive Th2

and subsequent IgA production to maintain intestinal homeostasis. More recent observations have demonstrated that T cell lineages can interconvert, specifically Treg-to-Th17 and Th17-to-Th1 (Lee et al. 2009; Zhou et al. 2009). In light of these studies, it is important to highlight that while each subset of T cells found in the intestines plays a crucial role in balancing homeostasis, these relationships are dynamic and can be altered by changes within the intestinal environment, such as those following alcohol exposure.

Intestinal T Cells Following Alcohol Exposure and Trauma

Surprisingly, few studies in the current literature have examined the effects of alcohol specifically on intestinal immunity. However, alcohol has significant, well-documented impacts on immune cells at sites outside the intestine, including in the spleen, thymus, and on circulating lymphocytes (Curtis et al. 2013; Ippolito et al. 2013; Messingham et al. 2002). Intestinal studies suggest that alcohol may have inflammatory effects, and subsequently compromise the intestine's ability to prevent bacteria from passing into the body.

Of course, an important consideration in studying the effects of alcohol on immune function is the nature of the alcohol exposure (acute vs. chronic). The authors examined the effects of alcohol exposure in an acute model, which is followed by a second traumatic burn injury. In this model, mice are given a single dose of alcohol to produce a blood alcohol level of 90–100 mg/dL 4 hours after alcohol administration, at which time they are given a full thickness ~12.5% total body surface area dorsal scald burn. Findings demonstrate that alcohol intoxication or burn injury alone does not cause significant changes to immune profiles within the gut in the first 24 hours. However, combined alcohol and burn injury lead to great perturbations resulting in high levels of inflammation accompanied by

neutrophil infiltration, T-cell suppression, and bacterial translocation (Brubaker et al. 2013; Li et al. 2008^{a,b}, 2011, 2012, 2013; Rendon et al. 2012, 2013, 2014; Zabs et al. 2013). These results clearly demonstrate that alcohol intoxication leads to greater susceptibility to secondary insults by sensitizing the immune system through an unknown mechanism.

Studies from the authors' laboratory also show a decrease in Th1 cells, particularly in MLNs, paired with decreases in IL-12 following alcohol intoxication and burn injury (Choudhry et al. 2002; Li et al. 2006). Intriguingly, restoration of IL-12 following alcohol and burn treatment restores Th1 profiles of the cytokines IFN- γ and IL-2 via an ERK-dependent pathway (Li et al. 2009). IL-12 is largely produced by resident APCs, and thus alcohol intoxication and burn injury may have both direct (i.e., on T cells) and indirect (on APCs) effects on Th1 function. Diminished Th1 effector cells present following alcohol intoxication and burn injury may allow bacteria and other pathogens to progress across the intestinal barrier. However, future studies will further address the signaling pathway(s) involved.

The authors also examined the effect of alcohol and traumatic burn injury on intestinal Th17 cells. They previously discovered a decrease in IL-23 and the Th17 effector cytokines IL-17 and IL-22 in PPs following alcohol and burn (Rendon et al. 2014). Due to the decreased presence of IL-23, they examined the effects of adding IL-23 following alcohol and burn injury (Rendon et al. 2014). Interestingly, IL-23 restored IL-22 production in an aryl hydrocarbon receptor (AhR)-dependent fashion, but IL-23 had no effect on IL-17 levels. These data give new insight into the role of IL-23 in mediating Th17 IL-22 responses, but not IL-17 responses. Like Th1 cells, the suppression of Th17 cells in the context of the alcohol/burn model may mean enhanced susceptibility to bacterial translocation and infection. Future studies will further examine

the role of both Th1 and Th17 cells and their functions following alcohol intoxication and trauma. Th2 and Treg activity following alcohol and burn injury also has not been well studied.

Another research group published recent studies examining the effects of alcohol on intestinal immunity in the context of chronic alcohol exposure followed by sepsis (Yoseph et al. 2013). Studies performed in this model showed disruptions in intestinal permeability similar to those in the studies discussed above. In addition, a significant increase in CD4⁺ production of IFN- γ and TNF- α was observed in alcohol-treated mice compared with controls (Yoseph et al. 2013). Interestingly, studies of non-alcoholic

human sepsis patients have shown lower levels of IFN- γ and TNF- α production in the spleen, which highlights the fact that local and systemic immune responses may differ greatly regardless of the presence of alcohol (Boomer et al. 2011).

Only a few studies in the literature have examined the effects of alcohol alone on intestinal immunity (Sibley and Jerrells 2000). An early study by Lopez and colleagues (1997) examined the effects of both acute and chronic alcohol exposure on PPs. They observed a significant decrease in the total number of cells within PPs of mice given a brief alcohol exposure of 5 weeks. In a more chronic exposure model, mice receiving alcohol for 19

weeks showed both a significant decrease in total PP cells, as well as a significant reduction of T and B cells present in PPs (Lopez et al. 1997). This study was important in demonstrating that alcohol administration affects the mucosal immune system, particularly PPs, suggesting that alcohol may thus affect T-cell differentiation within the intestines.

A more recent study demonstrated that alcohol exposure causes disruption of the epithelial barrier in the stomach and upper intestines (Bode and Bode 2003). It has been reported that even a single dose of alcohol at binge consumption levels can result in epithelial barrier disruptions within the gut (Bode and Bode 2005).

Glossary

AhR: Aryl Hydrocarbon Receptor: Transcription factor that drives Th17 cell differentiation.

β -Catenin: Transcription factor involved heavily in cell adhesion regulation.

CD(4/8): Cluster of differentiation: proteins expressed on the surface of cells used to identify specific cell phenotypes.

Crypt-Villus Axis: The plane that exists from the base of intestinal crypts to the tops of the villi. Epithelial cells divide from stem cells at the base of crypts and migrate to the tops of villi as they mature.

C-Type Lectins: Carbohydrate binding proteins with a diverse range of functions, including mounting immune responses against pathogens.

Defensins: Small proteins secreted by paneth cells that mediate defense against harmful microbes.

Dysbiosis: Any perturbation in the normal intestinal microbiota.

Extracellular Signal-Related Kinase (ERK): Signaling molecules that transmit a variety of intracellular signaling following activation.

Foxp3: Transcription factor that drives regulatory T cell differentiation.

Gata3: Trans-acting T-cell-specific transcription factor involved in the development of Th2 cells.

Glycosylation: A post-translational modification that involves the attachment of a carbohydrate to the specific region of a protein to enhance its function.

Intracellular Adhesion Molecule-1 (ICAM-1): Expressed mainly on endothelial cells and immune cells to mediate migration from circulation into tissues.

Microbiome: The entire makeup of bacteria that inhabit the intestines.

Nuclear Factor Kappa-Light-Chain Enhancer of Activated B Cells (NF- κ B): Transcription factor considered to be the master regulator of inflammation.

Retinoic Acid-Related Orphan Receptor Gamma T (ROR- γ T): Transcription factor that mediates Th17 development.

Sepsis: Life-threatening whole-body inflammatory response in order to fight systemic infection.

Signal Transducer and Activator of Transcription (STAT): Following receptor activation, STAT family proteins mediate transcription events to drive specific gene expression.

T-Box Transcription Factor (T-bet): Transcription factor that mediates development of Th1 T cells.

Zonula Occludins Protein 1 (ZO-1): Adherens trans-membrane junction proteins linking to the actin cytoskeleton to occludin and claudin proteins support tight junctions.

Interestingly, in an acute model of alcohol exposure, mice displayed higher numbers of Treg cells in the LP in response to barrier disruption (Boirivant et al. 2008). These results contrast with studies of chronic alcohol exposure that show increased levels of inflammatory neutrophil, Th1, and Th17 activation and production of IL-17A, IFN- γ , IL-1, and TNF- α (Bode and Bode 2005; Koivisto et al. 2008). Thus, acute alcohol exposure may result in suppression of inflammation, allowing pathogens past the intestinal barrier, while chronic exposure may produce an inflammatory state. In addition, one report with human subjects showed increases in IgA antibody production coupled with increases in TNF- α and IL-8 production in chronic alcoholics (Koivisto et al. 2008). Chronic alcohol consumption studies have reported significant effects on the liver and connected the inflammatory conditions observed in the intestines with alcoholic liver disease (Bode and Bode 2005; Koivisto et al. 2008).

Microbiota and Intestine Immune Homeostasis Following Alcohol and Burn Injury

The adaptive T-cell response provides a critical component of pathogen protection, and innate responses conducted mainly by neutrophils also play a large role in maintaining intestinal homeostasis. Importantly, however, both of these immune responses are shaped by their interactions with the intestinal microbiome. The intestinal immune system encounters more antigens than any other part of the body. Therefore, the recognition of “self” and “non-self” antigens is critical to discriminate the harmless commensal microbiota and food antigens from harmful pathogenic microbes. In part, this equilibrium is established by the balance of effector T cells discussed earlier. Antigens from the intestinal microbiota presented in GALT by APCs shapes this balance

of Treg/Th17 cells, which drives pro- or anti-inflammatory signaling.

In addition to affecting the T-cell balance, the composition of the intestinal microbiota facilitates development of lymphoid organs and directs immune cell responses and production of effector cytokines. Studies using germ free mice—that is, mice devoid of any microbes—reveal that these mice are more susceptible to colonization by pathogenic microbes; have small and undeveloped lymphoid organs; and show reductions in CD4⁺ and CD8⁺ T-cells, IgA secretion, and production of antimicrobial peptides (AMPs) including β -defensins and C-type lectins such as Reg3 γ (Bouskra et al. 2008; Cash et al. 2006; Zachar and Savage 1979). Further, following combined alcohol and burn injury, Reg3 β and Reg3 γ are significantly decreased in the small intestines of wild-type mice (Rendon et al. 2013). Together, these findings suggest that following alcohol intoxication and injury, bacterial overgrowth and translocation may be partially mediated through the inhibition of AMPs.

Several recent studies demonstrate that certain bacterial species have specific effects on immune system balance. The commensal microbes, it turns out, are essential for regulating immune physiology and the innate and adaptive immune systems. One commensal, *Bacteroides fragilis*, produces an immunomodulatory molecule called polysaccharide A (PSA), which regulates the Th1 and Th2 balance and directs Treg development to protect against intestinal inflammation (Mazmanian et al. 2005; Round and Mazmanian 2010; Round et al. 2011; Xu et al. 2003). Mazmanian and colleagues (2005) showed that therapeutic treatment with PSA led to the production of anti-inflammatory IL-10 and alleviated intestinal inflammation in various models of IBD. Segmented filamentous bacteria (SFB), a group of Gram-positive bacteria, attach to small intestine epithelial cells and lead to the production of serum amyloid A (SAA). SAA then stimu-

lates dendritic cells in the LP to secrete IL-6 and IL-23, which promotes Th17 cell differentiation and maturation (Ivanov et al. 2009). Littman’s laboratory and coauthor Ivanov and their team showed that germ-free mice have reductions of Th17 cells in the small intestine, but that levels could be restored by colonizing mice with feces taken from germ-free, SFB mono-colonized mice (Ivanov et al. 2009). Furthermore, they determined the specific membrane bound antigenic proteins of SFB that direct Th17 production (Yang et al. 2014). This bacterial group is also necessary for the secretion of IgA (Wu et al. 2011). Nevertheless, overgrowth of this bacterium may upset the Th17/Treg balance in favor of overactive Th17 cells. This shift can potentially lead to autoimmune diseases: inflammatory bowel disease, arthritis, and multiple sclerosis (Lee et al. 2011; Wu et al. 2010).

The Intestinal Microbiota Following Alcohol Exposure and Trauma

Unexpectedly, few studies in the current literature have examined the effects of alcohol exposure on the microbiome within the intestines. A recent study examining the effects of chronic daily alcohol consumption found dysbiosis—a microbial imbalance—in the colons of rats after 10 weeks (Mutlu et al. 2009). Others have correlated microbial dysbiosis to alcoholic liver disease and demonstrated that administration of probiotics reduces hepatic inflammation associated with it (Mutlu et al. 2009; Wang et al. 2013). The work done by the authors showed that combined alcohol intoxication followed by traumatic burn injury results in a significant increase in bacterial translocation across the intestinal barrier (Choudhry et al. 2002; Kavanaugh et al. 2005; Li et al. 2012; Rendon et al. 2013), and this work is supported by a previous study (Napolitano et al. 1995).

However, the long-term impact of alcohol on different microbiota and the host's health and immune function remains to be shown. Classification of the healthy intestinal microbiome is clinically necessary for determining how alcohol may alter the microbiota composition and lead to disease development and progression. Thus, whether bacterial translocation after alcohol and trauma is related to changes in the microbiome remains largely unknown. Furthermore, studies are needed to establish whether changes in the biome have any role in epithelial barrier disruption following alcohol and burn injury.

Future Directions and Perspectives

Taken together, the range of effects alcohol has on the intestines is extremely broad and alters all levels of intestinal homeostatic regulation. In parallel, alcohol exposure predisposes its users to more complications following major injury and trauma; however, the underlying mechanisms remain largely unexplored. Although studies have demonstrated that alcohol modulates the various components of the intestinal barrier, making any causal connections between these effects and complication from trauma requires more study. The balance of inflammatory and immunosuppressive T cells can be skewed following alcohol exposure. Current research suggests inflammatory conditions are mediated through both neutrophil infiltration and Th17 recruitment leading to tissue damage within the intestines. Whether alcohol influences this also needs to be explored. Many studies now show roles for the intestinal microbiome in developing the immune profiles within the intestines. Although few studies have explored whether alcohol exposure alters the composition of the microbiome, it is not far-fetched to hypothesize that this is likely the case. Together, the authors believe that the largest gap in the field remains the lack of mecha-

nistic support for the changes observed following alcohol exposure with and without burn trauma. More studies are needed to understand the molecular signaling pathways mediating changes in the barrier, immune system, and biome to give a clearer understanding of the relationship between these components and how they overlap after alcohol and burn injury.

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Alcohol's Effects on Lung Health and Immunity

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It has long been known that people with alcohol use disorder (AUD) not only may develop physical dependence but also may experience devastating long-term health problems. The most common and identifiable alcohol-associated health problems include liver cirrhosis, pancreatitis, cardiomyopathies, neuropathies, and dementia. However, the lung also is adversely affected by alcohol abuse, a fact often overlooked by clinicians and the public. Individuals with AUD are more likely to develop pneumonia, tuberculosis (TB), respiratory syncytial virus (RSV) infection, and acute respiratory distress syndrome (ARDS). Increased susceptibility to these and other pulmonary infections is caused by impaired immune responses in people with AUD. The key immune cells involved in combating pulmonary conditions such as pneumonia, TB, RSV infection, and ARDS are neutrophils, lymphocytes, alveolar macrophages, and the cells responsible for innate immune responses. Researchers are only now beginning to understand how alcohol affects these cells and how these effects contribute to the pathophysiology of pulmonary diseases in people with AUD.

Key words: Alcohol use, abuse, and dependence; alcohol use disorder; immunity; impaired immune response; innate immune response; lung disorders; pneumonia; tuberculosis; respiratory syncytial virus infection; acute respiratory distress syndrome; pulmonary infection; neutrophils, lymphocytes, alveolar macrophages; pathophysiology

People have been drinking alcoholic beverages for millennia, and alcohol consumption has played an important role throughout human history, being linked to ancient and modern religions, early medicine, and social occasions and celebrations. Although alcohol consumption is socially accepted across many cultures, heavy and prolonged alcohol intake can lead not only to physical dependence but also to devastating long-term health problems. An estimated 18 million Americans have alcohol use disorder (AUD), including alcoholism and harmful drinking (National Institute on Alcohol Abuse and Alcoholism [NIAAA] 2014). NIAAA (2014) has established guidelines for low-risk drinking that are age and gender specific. Thus, for men ages 21–64, low-risk drinking is defined as consumption of no more than 4

drinks per day or 14 drinks per week. For women, as well as for men ages 65 and older, drinking levels for low-risk drinking are defined as no more than 3 drinks per occasion or 7 drinks per week. Exceeding these daily or weekly drinking limits significantly increases the risk of developing AUD and problematic health outcomes (NIAAA 2014).

The most common health problems associated with AUD are liver cirrhosis, pancreatitis, damage to the heart muscles (i.e., cardiomyopathies), nerve damage (i.e., neuropathies), and dementia (Lieber 1995). However, the lung also is adversely affected by alcohol abuse, a fact that often is overlooked by clinicians and the public. For example, it is clear that heavy drinkers are more likely to have pneumonia (Jellinek 1943; Samokhvalov et al. 2010), tuberculosis (TB) (Borgdorff et al. 1998; Buskin et

al. 1994; Kline et al. 1995; Narasimhan et al. 2013), respiratory syncytial virus (RSV) infection (Jerrells et al. 2007), and acute respiratory distress syndrome (ARDS) (Moore et al. 2003; Moss et al. 1996). In recent years, researchers have come to better understand the pathophysiology of lung injury in individuals with AUD and the role that alcohol's effects on lung immune responses play in this process. This review focuses on these four common pulmonary conditions associated with AUD and their pathophysiologic lung immune responses.

Bacterial Pneumonia

One of the most common and deadliest conditions afflicting individuals with AUD is bacterial pneumonia.

Dr. Benjamin Rush, the first Surgeon General of the United States, described some of the earliest links of alcohol abuse to pneumonia over two centuries ago, reporting that pneumonia was more common in drinkers than nondrinkers (Jellinek 1943; Rush 1810). Two centuries later, the correlation between alcohol abuse and lung infections still remains strong. According to the Centers for Disease Control and Prevention (CDC), people who abuse alcohol are 10 times more likely to develop pneumococcal pneumonia and 4 times more likely to die from pneumonia than nondrinkers (Lujan et al. 2010).

Pneumococcal pneumonia, caused by the bacterium *Streptococcus pneumoniae*, is the most common type of pneumonia in both healthy individuals and heavy alcohol users (Ruiz et al. 1999). In addition, the incidence of infections with *Klebsiella pneumoniae* also is increased in people with AUD and seems to cause disproportionate rates of lung infection and high mortality in this population (Feldman et al. 1990; Limson et al. 1956). Regardless of the bacterial pathogen causing the infection, dysfunction of the host's immune responses to bacterial pneumonia, particularly those involving macrophages in the lungs (i.e., alveolar macrophages) and neutrophils, is an important contributor to the pathogenesis of the disease in people with AUD. The alveolar macrophages eliminate pathogens by ingesting them—a process known as phagocytosis—whereas neutrophils are involved in inflammatory responses.

Alveolar macrophages are the first line of defense in lung cellular immunity. These phagocytic cells ingest and clear inhaled microbes and foreign particles from the lungs. The release of cytokines and chemokines by these cells, in turn, mediates the influx of neutrophils into the lungs that occurs in response to infection. Chronic alcohol exposure significantly interferes with alveolar macrophage function. Prolonged alcohol consumption impairs the cells' phagocytic capacity (Joshi et al.

2005, 2009), release of cytokines and chemokines (D'Souza et al. 1996), and release of neutrophil chemoattractants (Craig et al. 2009). Although alveolar macrophages are the primary residential innate immune cells and play a pivotal role in the clearance of bacterial and viral pathogens, understanding of and research on their specific function in the context of heavy alcohol consumption and AUD still is lacking. It is clear, however, that prolonged alcohol consumption alters the pathophysiology and key factors involved in neutrophil-driven lung immunity in response to *S. pneumoniae* infection. Thus, studies have shown that exposure to alcohol impairs neutrophil recruitment (Gluckman and MacGregor 1978), weakens phagocytosis of pathogens by neutrophils (Boe et al. 2001; Jareo et al. 1995), and reduces neutrophil production and release of neutrophils into circulating blood (Melvan et al. 2011; Siggins et al. 2011). The following paragraphs outline the data supporting these deleterious effects of heavy alcohol consumption on neutrophil function in the context of *S. pneumoniae* lung infections.

Neutrophils are the earliest immune effector cells recruited to the site of inflammation during a bacteria-triggered inflammatory response. In the case of pneumonia, neutrophil recruitment to the lung is a critical early step in the host's immune response. In the early stages of infection, circulating neutrophils are recruited to sites of inflammation by a gradient of inflammatory mediators, including proinflammatory cytokines and chemokines. Neutrophils traverse the cells lining the blood vessels (i.e., vasculature endothelial cells) into the space between the lung cells (i.e., the interstitial space of the lung). From there, they migrate into the airspace within the alveoli to the sites of microbial invasion. Once in the alveolar space, neutrophils ingest, degrade, and remove invading pathogens (Nathan 2006). This neutrophil-recruitment process is impaired by alcohol; even brief alcohol exposure decreases neutrophil recruitment to

infected sites (Astry et al. 1983). For example, alcohol studies in rodents infected with aerosolized *Staphylococcus aureus* or *Proteus mirabilis* have demonstrated that alcohol intoxication decreases bacterial clearance in conjunction with decreased pulmonary neutrophil recruitment (Astry et al. 1983). Similarly, Boe and colleagues (2001) found that alcohol-exposed rats had decreased pulmonary neutrophil recruitment for up to 18 hours following *S. pneumoniae* challenge; after that, however, neutrophil recruitment remained elevated even 40 hours post-challenge compared with nondrinking rats. This observation suggests that in individuals with heavy alcohol exposure, the host neutrophils arrive late at the infected lung but stay longer (Sisson et al. 2005). Impaired neutrophil recruitment also has been reported in human volunteers with blood alcohol concentrations (BACs) of 0.10 percent and 0.24 percent (Gluckman and MacGregor 1978)—that is, even at BACs that only slightly exceed the threshold for legal intoxication in the United States (i.e., 0.08 percent). These findings highlight that alcohol intoxication impairs neutrophil recruitment into infected tissues and the lung and also hinders neutrophil clearance from the lung.

The alcohol-induced dysregulation of lung neutrophil recruitment and clearance is only part of the problem in people with AUD, because alcohol also has harmful effects on other aspects of neutrophil functioning. However, alcohol's effects on neutrophil phagocytosis and pathogen killing are less clear than the effects on neutrophil recruitment, and the findings to date are inconclusive. Thus, some studies indicate that alcohol has no effect on neutrophil phagocytosis or pathogen killing (Nilsson et al. 1996; Spagnuolo and MacGregor 1975), whereas other studies demonstrate that acute alcohol exposure impairs functional activities of neutrophils. For example, Davis and colleagues (1991) found that alcohol-fed rats failed to clear bacteria from the lungs and had increased

mortality. Some of this discrepancy likely is related to differences in the bacterial pathogens studied. Thus, Jareo and colleagues (1995) noted impaired neutrophil killing of selected strains of *S. pneumoniae* in vitro and a complete absence of killing of other bacterial strains in alcohol-exposed animals. In human studies, BACs as low as 0.2 percent (i.e., approximately 2.5 times the legal intoxication level) impaired neutrophil degranulation and bactericidal activity (Tamura et al. 1998).

In addition to neutrophil recruitment to infected areas and reduced neutrophil-killing potential, production of these cells also is affected. In healthy individuals, the bone marrow produces approximately 120 billion neutrophils per day (Cartwright et al. 1964; von Vietinghoff and Ley 2008). Moreover, bone-marrow neutrophil production is significantly increased 24 to 48 hours after a systemic bacterial infection (Melvan et al. 2011). Several studies observed decreased numbers of neutrophils in people with AUD. Alcohol exposure suppresses neutrophil production by the bone marrow and other blood cell-producing (i.e., hematopoietic) tissues (Melvan et al. 2011; Raasch et al. 2010; Siggins et al. 2011). This decreased neutrophil proliferation may account for the decreased number of neutrophils found in the lungs during the host response to pneumonia following alcohol consumption. Alcohol primarily suppresses neutrophil production by interfering with the actions of granulocyte colony-stimulating factor (G-CSF), which is the principal driver of neutrophil production, maturation, and function in the bone marrow and inflamed tissues (Bagby et al. 1998). G-CSF levels normally increase in situations where more neutrophils are needed. Thus, G-CSF levels rise significantly within 3 hours of pulmonary bacterial infections, peaking at 12 hours, and plateauing around 18 hours post-infection within the lung and systemic circulation. Additional studies have demonstrated that alcohol-consuming animals are more likely to

succumb to *S. pneumoniae* within 2 to 4 days following infection compared with their nondrinking counterparts (Boe et al. 2001). Alcohol-induced suppression of G-CSF-driven neutrophil production combined with impaired bacterial clearance likely account for the high severity and mortality of bacterial infections among the alcohol-fed mice observed in these studies.

Because of the key role of G-CSF in neutrophil regulation, investigators have hypothesized that alcohol-induced neutrophil dysfunction can be prevented by pretreatment with G-CSF (Nelson et al. 1991). Indeed, pre-treatment of alcohol-consuming mice with G-CSF for 2 days before *K. pneumoniae* infection increased neutrophil recruitment compared with that of control animals not receiving G-CSF. In addition to increased neutrophil recruitment, the pre-treated animals also exhibited improved bacterial killing and decreased mortality (Nelson et al. 1991). The findings indicate that G-CSF can prevent alcohol-induced deficits in neutrophil-dependent pulmonary defenses by increasing neutrophil production and bacterial killing function.

In summary, in the context of lung bacterial infections, alcohol impairs neutrophil recruitment (Gluckman and MacGregor 1978), reduces pathogen killing through phagocytosis (Boe et al. 2001; Jareo et al. 1995), and decreases neutrophil production and release of neutrophils into circulating blood (Melvan et al. 2011; Siggins et al. 2011). Pretreatment with G-CSF ameliorates alcohol-induced neutrophil dysfunction, including impairments in neutrophil recruitment and bacterial killing.

Tuberculosis

Bacterial pneumonia is not the only infectious disease with an increased risk among people with AUD. Lung infections with *Mycobacterium tuberculosis*, the underlying pathogen of TB, also occur at higher rates in this population (Jellinek 1943; World

Health Organization [WHO] 2014). TB is the second-leading cause of death worldwide, accounting for 1.3 million deaths in 2012. The disease is spread from person to person through the air, when infected people cough, sneeze, speak, or sing, thereby releasing *M. tuberculosis* into the air (WHO 2014). Interestingly, not everyone infected with *M. tuberculosis* becomes sick. The infection can remain latent for years while the host's immune system is able to combat it. The infected individual will have no symptoms and is not infectious to others. However, latent TB may become active when the immune system is weakened. Alcohol abuse is therefore a risk factor for active TB (Borgdorff et al. 1998; Buskin et al. 1994; Kline et al. 1995; Narasimhan et al. 2013).

Although TB is treatable with antibiotics, the prevalence of multidrug-resistant tuberculosis (MDRTB) is on the rise and has been reported worldwide (WHO 2014). One of the main factors increasing the prevalence of MDRTB is noncompliance by patients who do not complete their normal 6-month treatment regimen, leading to the emergence of drug-resistant *M. tuberculosis*. A recent study of MDRTB in South Africa reports that of 225 patients diagnosed with MDRTB, only 50 percent were cured or completed treatment. Treatment default rates were highest among alcohol users (Kendall et al. 2013). Other countries also report similar TB treatment defaults in individuals with AUD, resulting in poorer treatment outcomes and increased mortality rates (Bumburidi et al. 2006; Jakubowiak et al. 2007). Along with noncompliance, people with AUD have compromised lymphocytes, which are among the main immune components combating TB infections. The three main types of lymphocytes are natural killer (NK) cells, T cells, and B cells. Chronic alcohol intake modulates the functions of all three of these lymphocyte populations (Cook 1998; Lundy et al. 1975; Meadows et al. 1992; Spinuzzi et al. 1992; Szabo 1999).

NK cells do not need previous exposure to their target cells to recognize, bind to, and destroy these targets (e.g., cancer and virus-infected cells) (Vivier et al. 2008). In a mouse model, NK cells also become activated during the early response to *M. tuberculosis* infection and produce interferon γ (INF- γ), an important cytokine that stimulates cell-mediated immunity (Junqueira-Kipnis et al. 2003). Alcohol consumption in mice reduces the *in vitro* killing capacity of NK cells compared with control animals not exposed to alcohol (Meadows et al. 1992).

Chronic alcohol intake impairs not only the killing capacity of NK cells but also diminishes normal functioning of various types of T cells, which primarily mediate the immune response to TB (Gambon-Deza et al. 1995). (For more information on the types of T cells, see the textbox.) Alcohol exposure affects T-cell function through a variety of pathways:

- People with AUD often have reduced numbers of lymphocytes (i.e., lymphopenia), alterations in the T-cell compartments (Cook 1998; Szabo 1999; Tonnesen et al. 1990), decreased response to substances that stimulate cell division (i.e., mitogen-stimulation response) (Spinozzi et al. 1991), and impaired delayed-type hypersensitive responses (Lundy et al. 1975).¹
- Chronic alcohol consumption interferes with the proper presentation of pathogen-derived molecules (i.e., antigens), which is required for T- and B-cell activation (Ness et al. 2008).
- Alcohol-exposed T cells have a reduced capacity to produce INF- γ compared with control cells (Chadha et al. 1991).

- Alcohol-fed mice infected with TB exhibit decreased numbers of the two main subtypes of T cells (i.e., CD4⁺ and CD8⁺ T cells) as well as decreased proliferation of these cells compared with control mice (Mason et al. 2004).

INF- γ -producing (i.e., type 1) T cells mediate immune reactions that are responsible for fighting not only *M. tuberculosis* infections but also infections by other bacterial pathogens, such as *K. pneumoniae* (Greenberger et al. 1996; Moore et al. 2002). Infection with *K. pneumoniae* induces time-dependent release of IL-12 from T cells, which in turn drives T cell INF- γ production. This chain of reactions is disrupted by alcohol, because the levels of both IL-12 and INF- γ were decreased in alcohol-exposed mice infected with *K. pneumoniae* (Zisman et al. 1998). These deficits could account for decreased clearance of these bacteria from the lungs. In addition to this flawed type-1 (Th1) response, the lungs of alcohol-fed rodents exhibit increased amounts of the inflammatory cytokine IL-10,

which also may contribute to impaired lung clearance because normalizing IL-10 levels within the pulmonary system improves bacterial lung clearance (Greenberger et al. 1995).

B cells are responsible for the second arm of the immune response (i.e., the humoral immunity) that is mediated not by specific cells but by immune molecules (i.e., antibodies) produced and secreted by B cells in response to exposure to a pathogen. These antibodies consist of molecules called immunoglobulins (Igs). There are different types of Igs (e.g., IgA, IgM, and IgG) that all have specific functions during the immune response. Alcohol exposure in the context of TB also affects this arm of the immune response. Thus, although the total number of circulating B cells does not differ significantly between people with and without AUD, people with AUD have elevated levels of circulating IgA, IgM, and IgG (Spinozzi et al. 1992). In the lungs of people with AUD, however, Ig levels are reduced as determined by bronchoalveolar lavage (BAL) (Spinozzi et al. 1992). Replacement IgG therapy only

Types of T Cells

T cells are an important part of the immune system and fulfill a variety of functions in defending the organism against various pathogens. To do this, T cells are divided into different subgroups that all have specific functions. The two main subgroups are T helper cells and cytotoxic T cells. T helper cells, as the name implies, assist other immune cells in various ways. These T cells are characterized by the presence of a molecule called CD4 on their surface and therefore also are called CD4⁺ cells. When they become activated, CD4⁺ cells secrete various cytokines to facilitate different types of immune responses. Depending on the exact cytokines they produce, they can be further classified. For example, type 1 CD4⁺ cells are characterized by the secretion of interferon γ (INF- γ); they act primarily against pathogens that are found within cells. Conversely, type 2 CD4⁺ cells do not produce INF- γ but various types of interleukins. These cells act primarily against pathogens that are found outside the cells.

The other main subgroup of T cells, the cytotoxic T cells, has CD8 molecules on their surfaces. They are therefore also known as CD8⁺ cells. These T cells directly destroy virus-infected and tumor cells.

¹ Delayed-type hypersensitivity responses are excessive immune reactions that occur only a few days after the body has been exposed to the pathogen. These responses are not mediated by immune molecules produced by B cells (i.e., antibodies) but by T cells.

partially restored Ig levels in these people, although it decreased the rates of pulmonary infections (Spinozzi et al. 1992).

RSV Infection

Although much of the attention concerning lung infections in people with AUD has been focused on bacterial infections, these individuals also have an increased susceptibility to viral airway infections. RSV is one of the most common lower respiratory tract viral pathogens and is a major cause of respiratory infections in children. Although RSV infections once were thought to be limited to children, it is now clear that RSV also is a serious problem in older people, patients with chronic obstructive pulmonary disease (COPD), and people with AUD. Prolonged alcohol exposure alters the first line of the innate cellular defense, the mucociliary apparatus, against invading pathogens such as RSV. This defense system propels inhaled particles, microbes, toxins, and debris out of the lungs and airways with the help of the fine hairs (i.e., cilia) on the cells that line the respiratory tract.

Alcohol has unique effects on the ciliated airways because it is rapidly and transiently absorbed from the bronchial circulation directly across the ciliated epithelium of the conducting airways. It then is vaporized into the airways and excreted during exhalation. However, when the exhaled air cools as it reaches the trachea, the alcohol vapor condenses and is dissolved back into the fluid in periciliary airway lining (George et al. 1996). This recycling of alcohol vapor continually subjects the conducting airways to high concentrations of alcohol (George et al. 1996), which modify airway-epithelium host defenses by altering cytokine release, barrier function (Simet et al. 2012), and cilia function (Sisson 1995; Sisson et al. 2009; Wyatt and Sisson 2001).

As is the case with other organs, alcohol's specific effects on the conducting airways depend on the route, dose, and length of the exposure (Sisson 2007). Early studies found that direct exposure of the ciliated airways to very high and nonbiologically relevant alcohol concentrations (i.e., 4 to 10 percent or 0.8–3.2 M) interfere with the movement of the cilia (i.e., cause ciliostasis) in a concentration-dependent manner (Nungester and Klepser 1938; Purkinje and Valentine 1835). More recent studies have established that biologically relevant alcohol concentrations have very focused and specific effects on the lung airways. Over the past two decades, studies demonstrated that brief exposure to modest alcohol concentrations triggers generation of nitric oxide (NO) in the airway epithelial cells. This NO production stimulates a signaling pathway that involves the enzyme guanylyl cyclase, which produces a compound called cyclic guanosine monophosphate (cGMP). cGMP, in turn, activates cGMP-dependent protein kinase (PKG), followed by activation of the cyclic adenosine monophosphate (cAMP)-dependent protein kinase A (PKA). Activation of this dual kinase signaling pathway results in faster cilia beat frequency (CBF) in cilia briefly exposed to a moderate alcohol dose compared with controls (Sisson 1995; Sisson et al. 2009; Stout et al. 2007; Wyatt et al. 2003). More recent studies demonstrated that this rapid and transient alcohol-induced increase in NO levels was triggered by the alcohol-induced phosphorylation of heat shock protein 90 (HSP90) (Simet et al. 2013*b*). Upon phosphorylation, HSP90 increases its association with endothelial nitric oxide synthase (eNOS) in cilia, which then activates the cyclase-kinase cascade, resulting in increased CBF (Simet et al. 2013*b*). These findings are counterintuitive to the conventional wisdom that alcohol interferes with lung host defenses because stimulation of CBF should protect the lung; however, the clinical observation is that heavy alcohol expo-

sure impairs lung host defenses. Indeed, that is just the first part of the story.

In contrast to brief alcohol exposure, prolonged alcohol exposure completely desensitizes lung airway cilia such that they can no longer beat faster when exposed to inhaled pathogens. This cilia-desensitization effect is known as alcohol-induced cilia dysfunction (AICD). In AICD, prolonged alcohol exposure results in failure to stimulate CBF, thereby desensitizing cilia to activating agents such as beta agonists (Wyatt and Sisson 2001). AICD likely results from decreased HSP90/eNOS association, which in turn attenuates the NO-stimulated cGMP/cAMP-dependent kinase activation pathway (Simet et al. 2013*a*; Wyatt and Sisson 2001). Alternatively, AICD may be related to oxidant-driven eNOS uncoupling, because AICD can be prevented in alcohol-drinking mice by concurrently feeding the animals dietary antioxidants, such as Procyetine™ or N-acetylcysteine (Simet et al. 2013*a*).

Regardless of the exact underlying mechanism, the consequence of alcohol-induced impairment in airway ciliary function is increased susceptibility to airway bacterial and viral infections, such as RSV. For example, Jerrells and colleagues (2007) demonstrated that alcohol-fed mice are inefficient in clearing RSV from the lungs. In addition, the alcohol-consuming mice exhibited enhanced and prolonged RSV infection compared with nondrinking RSV-infected animals. RSV infection itself causes a significant loss of ciliated cells from the airway epithelium and the remaining cilia beat more slowly compared with control cells from uninfected epithelia (Slager et al. 2006). This ciliary slowing is regulated by the activation of another signaling protein called protein kinase Cε (PKCε); moreover, once PKCε becomes inactivated again, the ciliated cells detach from the epithelium (Slager et al. 2006). It is unknown how concurrent alcohol exposure impacts these consequences of RSV infection. In summary, these

studies demonstrate that alcohol exposure compromises innate defenses against viral pathogens such as RSV in part by disrupting airway ciliary function.

ARDS

People with AUD who experience any type of lung injury—be it caused by infections with bacteria, TB-causing *M. tuberculosis*, or viruses or by noninfectious events such as trauma, pancreatitis, or burns—are at high risk for developing ARDS. The syndrome is characterized by endothelial and alveolar epithelial barrier dysfunction, severe inflammation, and surfactant dysfunction.² During ARDS, robust lung inflammation results in increased accumulation of fluid and inflammatory cells in the alveolar spaces. This causes impaired gas exchange in the lung, resulting in decreased oxygenation of the blood and multiple organ failure caused by the insufficient oxygen levels. ARDS is a life-threatening complication that develops in response to several events, including lung infection, non-lung sepsis, aspiration of stomach contents, trauma, and/or inhaled toxins. Among the most common causes of ARDS are bacterial pneumonia and an associated severe inflammatory response (i.e., alveolar sepsis). Alcohol abuse also has been identified as an independent risk factor that increases the odds of at-risk individuals to develop ARDS (Moss et al. 1996). Indeed, ARDS is two to four times more common in individuals with AUD than in non-AUD individuals (Moss and Burnham 2003).

One of the central features of ARDS is an impaired barrier function of the alveolar epithelial and endothelial cells.³ Studies on the effect of alcohol alone on alveolar barrier function have

revealed that chronic alcohol intake alters physical barrier properties within alveoli (Guidot et al. 2000). Interestingly, alveolar cells from ethanol-fed rats had increased expression of sodium channels in the membrane facing the interior of the alveoli (i.e., the apical membrane). This up-regulation of sodium channels may counteract the increased paracellular leak from the blood space into the alveolar airspace observed in the lungs of alcoholic subjects, and may explain why prolonged alcohol intake, in the absence of inflammation, does not result in fluid accumulation in the lungs (i.e., pulmonary edema) (Guidot and Hart 2005). However, these alcohol-fed rats had diminished airway clearance when challenged with saline, even in the absence of an inflammatory challenge (Guidot et al. 2000). These data suggest that the alveolar epithelium actually is dysfunctional after alcohol exposure, even though it seems normal and is able to regulate the normal air-liquid interface by enhancing sodium channels at the apical surface. In the presence of an inflammatory reaction, the compensatory mechanism likely becomes overwhelmed, resulting in greater susceptibility to barrier disruption and flooding of the alveolar space with protein-containing fluid.

One of the molecules involved in disrupting epithelial integrity is the cytokine transforming growth factor β_1 (TGF- β_1). Studies in rats that had been fed alcohol for a prolonged period of time found that expression of inactive TGF- β_1 protein doubled in lung tissue compared with nondrinking animals; however, there was no evidence of TGF- β_1 release or activation in the absence of an infection (Bechara et al. 2004). Nevertheless, alcohol-fed rats released five times more activated TGF- β_1 into the alveolar airspaces than did nondrinking rats in the presence of bacterial toxins in their blood (i.e., during endotoxemia). Additional studies using alveolar epithelial cell layers derived from these alcohol-fed rats found that this perme-

ability defect was inhibited by neutralizing antibodies to TGF- β_1 (Bechara et al. 2004). Together, these data suggest that prolonged alcohol intake increases TGF- β_1 levels, which during inflammatory responses can be released and activated in the alveolar space, where it can directly impair epithelial barrier properties (Guidot and Hart 2005).

Another fundamental component contributing to alcohol's effects on the lungs is oxidative stress and the resulting alterations in alveolar macrophage function. As mentioned previously, alveolar macrophages are key components of both innate and acquired immunity against invading pathogens in the lung. After mucociliary clearance, these cells are the next line of cellular defense against invading pathogens through their phagocytic, microbicidal, and secretory functions (Rubins 2003). Chronic alcohol ingestion decreases alveolar macrophage function by inhibiting the release of cytokines and chemokines as well as other factors essential for microbial killing and immune response (Frankel-Ullmann et al. 1996; Omidvari et al. 1998). Alcohol-induced alveolar macrophage dysfunction likely occurs primarily as a result of alcohol-induced increases in oxidative stress, which is reflected by depletion of the antioxidant glutathione (GSH) in BAL fluid (Brown et al. 2007; Yeh et al. 2007). Impaired secretion of granulocyte monocyte colony-stimulating factor (GM-CSF) by type II alveolar cells likely also contributes to alcohol-induced oxidative stress (Joshi et al. 2005).

The alcohol-associated oxidative stress in the lungs is related at least in part to alcohol-driven changes in NADPH oxidase (Nox) enzyme function and GSH depletion. Nox enzymes generally promote oxidative stress, whereas antioxidants such as GSH help protect the cells against oxidative stress. Increased levels of Nox enzymes (e.g., Nox₄) and decreased GSH pools are emerging as significant components of the processes through which alcohol induces oxidative stress

² Surfactant is a lipoprotein complex produced by alveolar cells that covers alveoli and helps ensure proper lung function.

³ The epithelial cells line the alveolar surface that faces the inside (or airspace) of alveoli, whereas the endothelial cells line the surface that faces the outside of the alveoli and the surrounding blood vessels.

that then causes alveolar macrophage dysfunction. As mentioned previously, chronic alcohol intake increases the levels of activated TGF- β_1 , which then upregulates and activates Nox₄ (Brown and Griendling 2009). Nox₄ activation in turn leads to activation of Nox₁ and Nox₂, both of which cause production of reactive oxygen species (ROS) in the alveolar macrophages (Yeligar et al. 2012). At the same time, chronic alcohol consumption depletes levels of GSH in the lungs. Both of these processes promote chronic oxidative stress, which then impairs alveolar macrophage functions (Brown et al. 2004, 2007; Holguin et al. 1998; Yeh et al. 2007). Thus, both cellular-based microbial lung clearance and alveolar macrophage cell viability are decreased after chronic alcohol exposure and the resulting increase in oxidative stress (Velasquez et al. 2002). This role of alcohol-induced oxidative stress in macrophage dysfunction has been demonstrated in animal models in which chronic alcohol-drinking mice had decreased levels of GSH and increased levels of Nox enzymes and Nox-associated proteins in alveolar macrophages (Yeligar et al. 2012, 2014).

The identification of alcohol-driven oxidative stress as a contributor to alveolar macrophage dysfunction has led to promising antioxidant treatment approaches aiming to prevent alcohol-induced lung conditions in rodent models of prolonged alcohol consumption. For example, oral GSH treatment in alcohol-drinking mice was able to restore GSH pools, reverse alcohol-induced Nox increases, and restore alveolar macrophage function (Yeligar et al. 2012, 2014). Other studies have demonstrated that treatment with GSH precursors such as Procysteine™, N-acetylcysteine, or s-adenosylmethionine was able to improve alveolar macrophage phagocytosis (Brown et al. 2007) and promote differentiation of interstitial macrophages into mature alveolar macrophages (Brown et al. 2009) during chronic alcohol ingestion. These results suggest

that GSH is a vital component in restoring alcohol-induced alveolar macrophage function by decreasing Nox proteins and restoring GSH pools.

Studies also have analyzed the role of GM-CSF in alcohol-induced oxidative stress and impaired lung immunity. GM-CSF is secreted by type II alveolar cells and is required for terminal differentiation of circulating monocytes into mature, functional alveolar macrophages (Joshi et al. 2006). The levels of GM-CSF are reduced in chronic alcohol-drinking mice (Joshi et al. 2005). Studies have shown that mice that have been genetically modified to no longer produce GM-CSF (i.e., GM-CSF knockout mice) exhibit a variety of changes contributing to impaired lung immune responses, including impaired surfactant expression, clearance, and phagocytosis; decreased expression of GM-CSF receptor; and impaired alveolar macrophage development (Dranoff et al. 1994; Joshi et al. 2005; Trapnell and Whitsett 2002). Conversely, overexpression of GM-CSF in genetically modified (i.e., transgenic) mice causes increased lung size, excessive growth (i.e., hyperplasia) of alveolar epithelial cells, and improved surfactant protein removal from the alveolar space (Ikegami et al. 1997). Other studies using a rat model of chronic alcohol consumption found that although the levels of GM-CSF in the alveolar space were not affected by alcohol exposure, the expression of GM-CSF receptors was significantly decreased in the membranes of alveolar macrophages (Joshi et al. 2005). Chronic alcohol intake also decreased alveolar binding of PU.1, a transcription factor responsible for GM-CSF activation. When the animals were treated with recombinant GM-CSF, alveolar macrophage bacterial phagocytic capacity, GM-CSF receptor expression, and PU.1 nuclear binding were restored (Joshi et al. 2005). These studies offer the groundwork for understanding the importance of GM-CSF within the lung for the maturation and host immune function of the alveolar macrophage as well

as the deleterious impact of chronic alcohol use on these processes.

As these experimental studies have demonstrated, chronic alcohol intake exerts a detrimental effect on the function of alveolar macrophages, an important cell type involved in limiting ARDS risk and severity. Restoration of GM-CSF following alcohol exposure, replenishing of GSH pools, and normalization of Nox enzymes restore alveolar macrophage functions. The use of recombinant GM-CSF and antioxidants potentially could improve alveolar macrophage function in people with AUD. Preventing the pathophysiological consequences of lung injury, including excessive inflammation, and the resulting pulmonary edema and insufficient oxygen supply (i.e., hypoxia) in the tissues associated with ARDS remains the goal of research on alcohol-enhanced ARDS.

Summary

For centuries, it has been known that people with AUD are more likely to have pulmonary infections such as pneumonia and TB. Over the past two decades, it has become clear that other conditions such as RSV and ARDS also are linked to high-risk alcohol consumption. Even with the development of antibiotics, vaccinations, health education, and preventative medicine, a strong correlation still exists among heavy alcohol consumption, pulmonary infections, and ARDS. Over the past 30 years, however, research has vastly enhanced our understanding of the pathophysiology of the immunocompromised “alcoholic lung.” This includes new insight into the mechanisms that cause the harmful effects of heavy alcohol intake on neutrophils, lymphocytes, airway ciliary function, and alveolar macrophages, all of which contribute to the prolonged and often more severe pulmonary diseases observed in people who abuse alcohol. Armed with a better understanding of the lung pathophysiology unique to the

heavy drinker, clinicians now are better prepared to combat these diseases through various treatment regimens. Preclinical models suggest that antioxidant nutritional supplements may prevent alcohol-induced lung oxidative stress, allowing mucociliary clearance and alveolar macrophage functions to be preserved. Promising animal studies also show that restoration of normal G-CSF, IgG, and GM-CSF levels could permit normal lung recovery following infection and injury in individuals with AUD. These disease- and cell-associated studies offer hope for novel preventative and therapeutic options for restoration of a normal lung immune response in people with AUD.

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Measuring the Burden— Current and Future Research Trends

Results From the NIAAA Expert Panel on Alcohol and Chronic Disease Epidemiology

Rosalind A. Breslow, Ph.D., M.P.H., R.D.,
and Kenneth J. Mukamal, M.D.

Alcohol has a significant impact on health and well-being, from the beneficial aspects of moderate drinking to the detrimental effects of alcoholism. The broad implications of alcohol use on public health have been addressed through a wide range of epidemiological and clinical studies, many of which are described in this issue of *Alcohol Research: Current Reviews*. Where chronic disease is involved, alcohol use can be a risk factor that not only affects the onset of various chronic diseases but also exacerbates the ongoing extent and severity of those diseases. Lifestyle choices and genetic influences also contribute to, or help to alleviate, that risk. **KEY WORDS:** NIAAA Expert Panel on Alcohol and Chronic Disease Epidemiology; alcohol consumption; alcohol burden; chronic disease; risk factors; epidemiology; research; diabetes; cardiovascular disease; cancer; stroke; liver disease; genetic factors; eating behaviors; clinical trials

Research is continuing to investigate how alcohol impacts chronic disease. The National Institute on Alcohol Abuse and Alcoholism (NIAAA) hosted a 2-day Expert Panel on Alcohol and Chronic Disease Epidemiology in August 2011 to review the state of the field on alcohol and chronic disease. The panel was chaired by Kenneth J. Mukamal, M.D., and Rosalind A. Breslow, Ph.D., M.P.H., R.D., and was convened by NIAAA's Division of Epidemiology and Prevention Research.

Panel members (see textbox) represented a wide range of backgrounds and expertise, ranging from alcohol-related chronic diseases and risk factors to methods and technology. Among the chronic diseases addressed were diabetes, cardiovascular disease, cancer, stroke, and liver disease. The broader aspects

of the design and implementation of clinical trials and the implication of technological advances for research also were considered. Other topics included the links between genetics and other lifestyle factors, such as eating behavior, and the relationship between drinking and various chronic diseases. Taken together, these summaries provide unique insight into the current state of research on alcohol's role in chronic disease and the direction these investigations may take in the future. (For more information on the epidemiological challenges of elucidating the effects of alcohol consumption and drinking as they relate to the initiation/ exacerbation and treatment of chronic diseases, see the article by Shield and colleagues [pp. 155–173]). Panel members also were asked what research they would most strongly support if funds were unlimited and how they might scale back that research if funding were limited (see Future Ideas textbox). Highlights from this panel are presented below and specific recommendations are listed in the accompanying sidebar.

Clinical Trials

Clinical studies include clinical nutrition studies, controlled feeding studies, and metabolic studies. This type of research has numerous strengths for studying alcohol and chronic disease, including the ability to control alcohol dose and diet, collect abundant biologic samples from a variety of tissues, assess cause and effect, and examine mechanisms—all with a relatively small number of participants enrolled for a short period of time.

Clinical study end points typically are surrogate markers for chronic diseases because the disease itself may take years or even decades to develop. For example, lipoproteins and markers of inflammation have been used as surrogates for cardiovascular disease, insulin sensitivity for diabetes, and DNA damage for cancer.

According to Dr. David J. Baer, considerable need for controlled clinical studies on alcohol and chronic disease still exists. There have been few clinical studies, even on cardiovascular disease (Brien et al. 2011), which is the focus of most alcohol-related chronic disease research. He also noted the relatively few controlled clinical studies of alcohol and obesity (Sayon-Orea et al. 2011) that were advocated by the

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Report of the Dietary Guidelines Advisory Committee on the Dietary Guidelines for Americans (U.S. Department of Agriculture 2010).

Dr. Baer suggested the following future opportunities for alcohol and chronic disease research:

- Drinking patterns;
- Effects on metabolism and disease risk;
- Non-ethanol components of alcoholic beverages;
- Possible effects on cardiovascular disease, diabetes (insulin sensitivity), cancer, and bone metabolism;
- Gender and age differences (pre- and postmenopausal women, men);
- Genetic basis for response of chronic disease surrogate markers to alcohol;
- Energy metabolism, body weight regulation, and insulin sensitivity;
- Interaction of alcohol with lower-fat or higher-protein diets; and
- Bone metabolism.

Cardiovascular Disease

Studies on alcohol and cardiovascular disease have yielded important findings with regard to public health. For example, we now know that the association of alcohol use within recommended limits with lower risk of heart disease depends more on the frequency with which alcohol is consumed and not on the type (Cleophas 1999). Wine, beer, and spirits all have been associated with reduced risk of myocardial infarction. Modest differences in the effects of those different types of alcohol are thought to be more a result of lifestyle differences among drinkers rather than a direct link to a specific type of alcohol. How often people drink alcohol has a larger impact on cardiovascular disease. Among men, drinking more frequently seems to have a greater impact than the actual amount consumed (Mukamal et al. 2003); effects are less clear among women. The beneficial effects of alcohol also have been shown to be similar for people with existing cardiovascular disease or diabetes (Costanzo et al. 2010; Koppes et al. 2006) and those in the general population. In addition to its beneficial effects on coronary heart disease, moderate drinking has been found to reduce the risk of ischemic stroke but at a lesser magnitude and with lower levels of consumption (Klatsky et al. 2001).

Although the exact mechanisms involved in these cardio-protective effects still are under investigation, the putative benefits on cardiovascular disease likely are the result of alcohol's effects on lipids and insulin sensitivity (Dijousse et al. 2009).

In his presentation, Dr. Kenneth J. Mukamal noted that standard epidemiologic studies of alcohol consumption and coronary heart disease incidence or mortality are no longer useful, as virtually all prospective studies performed since 1980 have shown that moderate drinking reduces risk (Corrao et al. 2000; Mukamal et al. 2010; Ronksley et al. 2011). Recent analytic strategies have resulted in more precise statistical estimates, but the conclusion is unchanged. In essence, he stated, "We've been doing the same epidemiology since 1992."

Dr. Mukamal suggested the following future opportunities for alcohol and cardiovascular disease research:

- Effects of heavy and binge drinking;
- Effects of changes in alcohol consumption over time;
- Differences in effect of gender-specific drinking patterns;
- Genetic interactions;
- Studies of new mechanisms directly related to alcohol's effects (for example, cholesterol efflux capacity) (Khera et al. 2011);
- Pooling projects for questions that require large samples; and
- Use of case crossover designs to account for both triggering events and chronic use (Mostofsky 2011).

Cancer

Alcohol consumption increases the risk for several cancers, including breast, colon, liver, and upper aero-digestive cancers (oral, pharynx, larynx, and esophagus) (Schutze et al. 2011; World Cancer Research Fund 2007). The potential mechanisms underlying alcohol's effects include the carcinogenicity of acetaldehyde (for colorectal cancer and upper aero-digestive tract cancers), which is an intermediate product of alcohol metabolism; impairment of the one-carbon nutrient metabolism (for colorectal cancer); alteration of hormone levels (for breast cancer); and oxidative stress resulting from alcohol metabolism.

Dr. Edward Giovannucci noted the paucity of research on drinking patterns and cancer. He acknowledged too that studies can yield disparate findings, describing a study that initially showed no relationship between average alcohol consumption and prostate cancer but which in a posteriori analyses hinted at a possible relationship with high-quantity/low-frequency drinking (Platz et al. 2004).

In identifying areas for future research, Dr. Giovannucci discussed the importance of studying cancer–nutrient interactions, particularly for colon cancer. For example, the epidemiologic literature has consistently shown an interaction between alcohol and folate, a nutrient that seems to be protective at higher levels of drinking (Ferrari et al. 2007; Jiang et al. 2003). This suggests that the excess risk of cancer resulting from alcohol use potentially could be modified by a nutrient or combination of nutrients.

Further study also is needed to better understand the role of genetics and family history in cancer risk. The genes involved in alcohol metabolism (Yokoyama et al. 2001) and nutrient metabolism (for example, the gene *methylenetetrahydrofolate reductase* [*MTHFR*] for folate as well as other

genes involved in the one-carbon metabolism pathway) are other areas that warrant additional study. Determining the molecular characteristics of tumors, such as tumor subtypes classified by level of methylation, which might reflect defects in one-carbon metabolism (Schernhammer et al. 2010), is another area that requires further investigation. In addition, little research has been conducted with cancer survivors, a group that may be especially willing to modify their drinking habits.

Finally, as noted by Dr. Giovannucci, alcohol increases the risk for many cancers, but not all. Recent studies have found that alcohol is associated with a lower risk of kidney cancer (Lee et al. 2007) and non-Hodgkins lymphoma

Future Research Ideas, Large and Small, for Consideration

In addition to the full panel discussions, panelists were asked to consider directions for future studies—both large and small. Specifically, the panelists described what studies they would suggest for future research and how they would refine those visions when funds are limited. Selected noteworthy examples are described below.

- A randomized trial to evaluate alcohol consumption and risk of multiple clinical outcomes with sufficient power to evaluate prespecified genetic environmental interactions would be ideal. However, with limited resources, it might be more realistic to use a hybrid design, with a prospective cohort study and a smaller nested trial. For example, a trial might evaluate if recommending moderate alcohol consumption, versus no recommendation, had an effect on cardiovascular and stroke outcomes among patients with a high risk for vascular problems.
- Clinical trials to establish the effects of alcohol consumption on clinical cardiovascular and cancer outcomes. A large-scale trial using high-risk populations with standardized exposure to alcohol would be ideal. A more practical approach would be to conduct shorter trials with subclinical measures of both cardiovascular disease and, to a lesser degree, cancer, using such techniques as serial computed tomography angiography and colonography.
- Studies to identify factors that influence the risk for liver disease among moderate drinkers. A large, prospective study would be ideal and would include serial measures of genomic, dietary, anthropometric, and behavioral risk factors obtained as objectively as possible, coupled with serial noninvasive measures of liver disease using magnetic resonance imaging for fat and fibroscan for fibrosis. Such a cohort could additionally fold in cardiovascular disease risk factors and clinical and subclinical cardiovascular disease. Among other things, this study would help to address the simultaneous associations of alcohol consumption with lower risk of cardiovascular disease but higher risk of fatty liver, which is associated with a higher risk for cardiovascular disease. Although of more limited utility, a cross-sectional study with the same measures would also be of clear import.
- Studies to verify estimates of drinking patterns. This is particularly important as self-reported estimates form the basis for epidemiological studies but have yet to be validated, particularly in the context of eating patterns, portion sizes, and health beliefs.
- Studies of how alcohol ingestion impacts energy balance in both moderate and binge drinkers.
- Studies to better understand the risk factors underlying alcohol-related chronic disease. These factors range from fixed characteristics, such as genetics and ethnic background, to broader modifiable behaviors, such as diet, exercise, or smoking. An ideal study would be multifaceted and include both disease-specific and composite global endpoints, such as healthy aging or survival free of chronic disease. A more limited study could simply compile data from the dozens of cohort studies worldwide where much of this data already have been collected. A more comprehensive effort would use ongoing studies prospectively to incorporate novel measures of drinking patterns, biomarkers of health status, or greater assessment of quality of life and mental health.

(Kroll et al. 2012). Understanding how these two cancers differ from others is another area requiring additional research.

Dr. Giovannucci suggested the following future opportunities for alcohol and cancer research:

- Effects of drinking patterns on cancer risk;
- Nutrient interactions;
- Genetic susceptibility (genes related to alcohol metabolism, genes related to one-carbon metabolism);
- Tumor subtypes;
- Cancer survivors; and
- Pathways that might explain the limited protective aspects of alcohol consumption.

Diabetes

Evidence that alcohol can impact diabetes has been consistent over several studies. Results from the Nurses' Health Study (Stampfer et al. 1988), the Health Professionals Follow-up Study (Conigrave et al. 2001), a systematic review (Howard et al. 2004), and two meta-analyses (Baliunas et al. 2009; Koppes et al. 2005) all show that moderate drinking is associated with a lower risk of diabetes. Heavy drinking, on the other hand, seems to lead to an increased risk of diabetes, although sample sizes generally have been too small to draw firm conclusions.

Dr. Eric Rimm described specific areas of research that warrant further study. For example, only about 30 to 50 percent of alcohol's beneficial effects on diabetes can be linked to biomarkers studied to date. In addition to its overall effect on insulin sensitivity (Davies et al. 2002), moderate alcohol consumption improves adiponectin, a fat-tissue hormone associated with insulin sensitivity; inflammatory status (Joosten et al. 2008); and HDL cholesterol. With regard to metabolic studies, he noted the value of using short-term feeding studies because they provide an opportunity to control and simultaneously examine drinking (for example, with meals or without) and diet (for example, high versus low glycemic load) (Mekary et al. 2011). He also discussed the importance of studying genetic predisposition (Beulens et al. 2007).

In addition to these areas, Dr. Rimm suggested several future opportunities for alcohol and type 2 diabetes research:

- Pool large cohort studies to maximize power to look at subpopulations where alcohol may be most detrimental or most beneficial.

- Pool data from large cohort studies with genetic information on alcohol metabolizing and diabetes-related genes to examine the interactions between alcohol, genetic predisposition, and diabetes risk.
- Conduct metabolic studies specifically within subgroups to examine how alcohol modifies risk based on lifestyle characteristics, such as body mass index, diet, and physical activity.

Stroke and Cognition

Several important findings on the effects of alcohol consumption on the incidence of stroke have emerged from the Northern Manhattan Study, a prospective, multiethnic cohort study (Elkind et al. 2006; Sacco et al. 1999). In that study, subjects with the lowest risk for ischemic stroke consumed, on average, two drinks per day. Those effects were similar among drinkers of wine, beer, and liquor. In contrast, no protective effect was found for hemorrhagic stroke.

The study's principal investigator, Dr. Ralph Sacco, presented the results of two meta-analyses. One found the greatest protection against all strokes combined was most evident at a lower level of drinking, less than or equal to one drink per day (Ronksley et al. 2011). Other analyses compared results from ischemic with hemorrhagic strokes (Reynolds et al. 2003). For ischemic stroke, moderate drinking was protective, whereas heavy drinking was associated with an increased risk; for hemorrhagic stroke, heavy drinking increased risk (although sample size was insufficient to study the effects of moderate drinking on hemorrhagic stroke).

The heterogeneity of strokes underscores the importance of studying stroke subtypes. Both ischemic strokes (the majority of all strokes) and hemorrhagic strokes (about 17 percent of all strokes) have subtypes with differing etiologies that may respond differently to alcohol consumption. Little research has been conducted on these subtypes, partly because of the small numbers of each that occur within most studies and the need for relatively large samples to obtain sufficiently precise estimates of risk. Numerous subclinical markers of stroke, such as endothelial function, currently are being pursued by researchers (Suzuki et al. 2009).

Cognition

The prevalence of cognitive impairment is growing rapidly as the population ages, and, like stroke, cognitive impairment is not a single disease or condition. Studies of alcohol use and cognition have examined a variety of outcomes, including Alzheimer's disease, cognitive function, dementia, and mild cognitive impairment (Lee et al. 2010). Studies and meta-analyses generally show that moderate drinking is associated with a decreased risk of dementia (Mukamal et al. 2003b; Peters et al. 2008), Alzheimer's disease (Peters et al. 2008), vascular dementia (Peters et al. 2008), and cognitive

Recommendations for Strengthening Studies

In addition to offering ideas for future studies, the Expert Panel also made recommendations for strengthening research in the field. Specific suggestions include:

1. Standardize alcohol consumption measurement in prospective and retrospective studies of alcohol and chronic disease to the greatest degree possible. Standardized measures:
 - a. Should include consumption quantity, frequency, and binge drinking (i.e., basic drinking patterns).
 - b. Should consider drinking over the lifespan (for example, during youth, middle age, menopause, and during time of heaviest drinking) as the critical time periods for effects of alcohol on chronic disease development are uncertain.
 - c. Are available from NIAAA and from the NIH/National Human Genome Research Institute Phenx Toolkit: <http://www.niaaa.nih.gov/Resources/ResearchResources/Pages/TaskForce.aspx>; <https://www.phenx.org/Default.aspx?tabid=36>
2. Strongly encourage collection of biological material and broad consent for genetic studies in all clinical trials and in as many population studies as possible.
3. Objectively validate standardized alcohol measures using novel technologies as they become available. Examples may include implantable biosensors and point-of-care devices with wireless transmission capability.
4. Develop new biomarkers for moderate alcohol consumption to complement those used for heavy drinking.
5. Identify surrogate markers for chronic disease (including measures of subclinical disease) that will have utility in small-scale studies and for elucidating mechanisms and pathways linking alcohol to chronic disease.
6. Pool data from existing cohort studies to facilitate examination by population subgroups, including but not limited to age, lifespan phase, race/ethnicity, menopausal status, body mass index/anthropometrics, dietary intake/nutritional status, smoking status, physical activity/fitness, cancer survivorship, and age of drinking onset. Pooled data also may facilitate studies of rare or understudied outcomes such as liver disease.
 - a. Standardized alcohol questions should be used where possible.
 - b. Confounding and interaction should be considered to ensure robust estimates and define susceptible subgroups.
 - c. Targeted sub-studies within large cohorts should be considered as a cost-efficient way to better understand and explain results in the full cohort. For example, when data on alcohol consumption are not gathered in enough detail in the original study, targeted follow-up studies may be used among stratified subsets of subjects to collect biological samples and to obtain more detailed data on consumption for extrapolating to the parent study.
7. Include associations between alcohol dependence/abuse and chronic disease outcomes. Studies using pooled data or sub-studies within large cohorts may have the power to address these drinking problems. Data on period of maximum drinking could be important, particularly given the marked variation in alcohol intake during the lifespan.
8. Perform studies in understudied areas, including but not limited to the effects of alcohol on diabetes, obesity, cognition, healthy aging, and food intake.
9. Focus on relationships between drinking patterns and chronic disease. Drinking patterns include but are not limited to basic patterns such as usual quantity, frequency, and binge drinking as well as when, where, and with whom alcohol was consumed and whether it was consumed with a meal.
10. Encourage clinical trials across the spectrum of chronic disease from studies that examine key physiological parameters and intermediate studies such as feeding studies that examine surrogates or subclinical

phenotypes to practical trials that examine chronic disease outcomes.

- a. Physiologic studies are preferred when epidemiologic evidence is relatively limited.
 - b. Practical trials are preferred when there is extensive evidence from physiological and epidemiological studies.
11. Encourage studies examining the interactions between the genetics that predispose individuals to drink and the genetics that modify how alcohol affects chronic disease.
 12. Encourage studies of carefully defined homogeneous phenotypes. For example, studies are needed to clarify the effects of alcohol on thrombotic versus embolic ischemic stroke, Alzheimer's disease versus other dementias, specific eye diseases, etc.

13. Encourage studies on moderate drinking patterns and metabolism ranging from total energy and macronutrient metabolism to specific metabolic pathways for small molecules such as vitamins, amino acids, sugars, and steroids and their products and precursors.
14. Examine the effectiveness of communication messages about drinking. Studies may include, but are not limited to, how to disseminate cost-benefit messages, individualized messages based on patient demographic and clinical history, and guidance for health care professionals on how to advise patients.
15. Encourage the use of natural experiments to examine whether policy interventions or alcohol intervention studies might change the relationship between alcohol and chronic disease.

decline (Peters et al. 2008). According to Dr. Sacco, there currently is great interest in vascular risk factors for dementia, yet little alcohol research has been done in that area.

Other future opportunities for research into alcohol and chronic neurological disease noted by Dr. Sacco include the following:

- Cohort studies with careful end point adjudication to separate ischemic stroke subtypes and different etiologies of dementia and cognitive impairment;
- Examination of interactions with race and ethnicity and other neurological risk factors;
- Comparison of associations across beverage types for neurological outcomes; and
- Understanding protective alcohol mechanisms including inflammatory relationships, subclinical measures and biomarkers, and gene–environment interactions.

Chronic Liver Disease

Chronic liver disease has long been associated with alcohol consumption and includes alcoholic liver disease, hepatitis C, and nonalcoholic steatohepatitis. Despite this clear associ-

ation, however, there is a lack of strong clinical measures to describe and predict the progression of chronic liver disease. Dr. James Everhart noted that the course of alcoholic liver disease is several decades in duration and begins as simple steatosis (fatty liver) before progressing to more advanced stages including steatohepatitis, alcoholic cirrhosis, and, eventually, liver failure.

Dr. Everhart noted that alcoholic liver disease may be overrepresented in terms of mortality because of the current classification system. Histologically, alcoholic fatty liver and nonalcoholic fatty liver look similar (Scaglioni et al. 2011), and patients with otherwise similar multiple risk factors and histology may be classified as having alcoholic liver disease rather than nonalcoholic steatohepatitis simply because they do or do not drink. According to Dr. Everhart, the current strict separation of alcoholic and nonalcoholic fatty liver disease limits epidemiology, public health, and clinical understanding.

In examining the effects of drinking amounts on liver disease, little association has been found between moderate drinking and alcoholic liver disease, and only a minority of very heavy drinkers develops alcoholic liver disease, although the reason is not clear. It is possible that drinking patterns and diet each play a role in risk. More information also is needed to determine if drinking at times other than during meals could increase risk.

Other factors that put people at higher risk for liver disease include being obese, using cannabis, having diabetes,

and being female (Hart et al. 2010). Conversely, coffee consumption seems to lower risk and smoking seems to have no effect on the development of chronic liver disease. Genetic susceptibility is another important risk factor for liver disease. For example, a variant in one gene, *PNPLA3*, originally associated with fatty liver, has been strongly associated with alcoholic liver disease. Again, additional research is needed to determine how these factors influence alcohol's effects.

Dr. Everhart suggested several future opportunities for alcohol and chronic liver disease research:

- Improve the current chronic liver disease classification scheme;
- Develop reliable and accurate measures of progressive liver disease that can be applied serially;
- Implement better measures of alcohol consumption and its patterns to study drinking patterns and interactions between drinking and diet;
- Evaluate how genetics may influence the link between alcohol consumption and the risk of liver disease; and
- Identify determinants of chronic liver disease among heavy drinkers.

Genetics

Chronic diseases tend to run in families yet do not follow a simple genetic pattern; that is, they are complex and polygenic. Identifying the genes that affect chronic disease risk can be hampered by multiple factors, including phenotypic complexity, multiple genes with small effects, environmental variability, gene–gene interactions, and gene–environment interactions. Alcohol's role in chronic disease likely reflects a gene–environment interaction in which risk is influenced by genes, by lifestyle choices, and by a combination of both. In addition, as noted by Dr. Howard J. Edenberg, most of the variations in genes related to alcohol and chronic disease likely have only small effects, making those genetic influences especially difficult to identify.

One way of overcoming these difficulties, as proposed by Dr. Edenberg, is to obtain large sample sizes by combining data from multiple epidemiologic studies. This enables investigators to examine gene–environment associations using secondary data analyses. The drawback is that studies typically ask different questions about alcohol use and often include different time frames, often collect no data on drinking problems, and may not obtain appropriate consent for genetic testing. Dr. Edenberg suggested a number of strategies to manage these obstacles. For example, investigators could be encouraged to incorporate standardized alcohol consumption questions, particularly for patterns of consumption, and to obtain DNA samples using proper consent for genetic

studies, where appropriate. Existing studies also could be enhanced through targeted ancillary studies in which key subsets of subjects are re-contacted to provide more detailed or standardized information. The payoffs from such steps could lead to the discovery of key genes and pathways that reveal mechanisms and potential targets for therapy. Even if the effect of a variant is small, the pathway it leads to could be of major importance.

Dr. Edenberg suggested several future opportunities for the genetics of alcohol and chronic disease research:

- Design and incorporate more detailed alcohol exposure measures that include patterns of consumption and drinking problems;
- Search out ongoing and planned studies to;
 - Partner to incorporate exposure measures as early as possible;
 - Target follow-up and additional studies to gather more detailed exposure information and genetic samples; and
 - Encourage collection of samples with consent for genetic studies.

Eating Behaviors

The link between alcohol intake and eating behaviors is not well known. Studies generally show that alcohol calories, when added to the diet, increase total energy intake (Yeomans 2010). Yet despite the fact that alcohol is an energy source, is largely uncompensated (i.e., supplements rather than replaces other calories), may weaken feeding controls, and spares fat for storage, little evidence exists that moderate drinking is associated with increased body mass index or weight gain (Liangpunsakul 2010; Liu et al. 1994; Wang et al. 2010) (although a French study did show such an effect [Lukasiewicz et al. 2005]). On the other hand, certain drinking patterns, particularly binge drinking, have been associated with higher body mass index (Arif and Rohrer 2005; Breslow and Smothers 2005), although impulsivity related to both eating and drinking could be an alternative explanation. According to Dr. Richard Mattes, determining alcohol's effects on eating behaviors is further confounded by beverage consumption itself and the fact that energy compensation for fluids is less than for semisolid or solid foods (Mattes 1996; Mourao et al. 2007).

He also suggested that what people think they are eating may be more important in terms of appetitive sensations than its true energy value, noting current research showing that manipulating food form (liquid or solid) can alter a person's expectation of how filling that food will be.

Dr. Mattes suggested several research opportunities for future studies on ingestive behavior and alcohol-related

chronic disease research, particularly in controlled experimental designs:

- Clarify the role of moderate alcohol consumption on energy balance;
- Assess which properties of alcohol contribute to hunger and satiety;
- Ascertain the true biological energy value of alcohol;
- Test the role of drinking patterns on energy balance; and
- Determine the effects of different levels of alcohol consumption on body composition and energy balance.

Technology

A number of promising technologies and medical devices currently are under development by the National Institute of Biomedical Imaging and Bioengineering and others that may enhance alcohol-related chronic disease research in the future. Dr. John Haller reviewed the research on three areas: sensors, point-of-care (POC) diagnostic devices, and imaging technologies and bioinformatics tools.

Sensors are used to detect and quantitate clinically relevant analytes. Examples include BioMEMs, microfluidics (Chin et al. 2011), and nanoscale technologies, including micro-total analysis systems, arrays, and biochips. These multifunctional devices can measure multiple analytes across a variety of diseases using a platform the size of a credit card.

Such technologies then can be combined into POC tests, which are defined as diagnostic testing at or near the site of patient care (rather than at centralized laboratories). Benefits include earlier diagnosis of disease and the ability to monitor patients at home. For example, POC tests for alcohol include a breath test and saliva-testing devices (http://www.aacc.org/events/online_progs/documents/AlcoholTesting1.2.pdf); SpectRx, a wristwatch-type device; and Giner, a WrisTas transdermal sensor for measuring alcohol consumption (Marques and McKnight 2009). Dr. Haller also reviewed implantable monitors and a tattoo using nanosensors that reside under the skin. By shining a light on the tattoo the subject enables tracking of sodium and glucose levels by portable digital devices, including smartphones. In the future, such a technology could be used to track alcohol consumption.

Biomedical imaging of the brain is another area where advances could be applied to the study of alcohol and chronic disease. Most radiology images (e.g., magnetic resonance imaging [MRI], computerized tomography) show anatomy/morphology. These images generally capture the late stages of chronic disease. An alternative approach would be to examine the physiological function (e.g., neuroreceptors) using nuclear imaging (e.g., positron emission tomog-

raphy and single-photon emission computed tomography). Magnetic resonance spectroscopy can image relative chemical composition. MRI diffusion tensor imaging can image white matter tracts (connectivity), and functional MRI can image relative blood flow, a marker of neural activity. These structural and functional neuroimaging methods currently are being used in alcohol research (Buhler and Mann 2011). Dr. Haller noted that informatics (data modeling, simulation, and analysis) also will have a significant role in making sense of the large amounts of high-dimension data now available.

Dr. Haller had the following suggestions regarding alcohol-related chronic disease research:

- Among the variety of technologies and medical devices that exist for the study of individuals and populations, those of particular interest might include sensors, POC diagnostic devices, imaging technologies, and bioinformatics tools;
- A better alternative to the “hammer-in-search-of-a-nail” approach in imaging is to define the clinical problem of interest first, then find the appropriate tools to address the problem or chronic disease under study;
- Alcohol and chronic disease epidemiology could be improved through the use of new sensors (including POC diagnostics, sensors embedded in the home or implanted in the body) to enhance alcohol measurement and by techniques that can image physiological function early in the course of chronic disease; and
- Technological advances will inevitably produce vast amounts of data about individuals and populations, but they require new informatics tools that enable meaningful use of the data in wide varieties of research settings.

Summary

This NIAAA workshop provided an excellent forum for summarizing the current state of the field and for identifying future research opportunities. Although by no means exhaustive, the ideas provided here highlight areas in need of additional study and offer a roadmap for moving forward across a variety of methodological approaches and content areas. NIAAA would like to thank all of the presenters for their insight and for taking the time to participate in this unique workshop. Our hope is that the ideas presented here will stimulate additional research and further advance our understanding of the role of alcohol in chronic disease. ■

Additional Resources

The agenda, roster of speakers, and speaker’s abstracts can be obtained from the author. A copy of the meeting transcript also is available from the author, upon request.

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Focus On: Ethnicity and the Social and Health Harms From Drinking

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Alcohol consumption is differentially associated with social and health harms across U.S. ethnic groups. Native Americans, Hispanics, and Blacks are disadvantaged by alcohol-attributed harms compared with Whites and Asians. Ethnicities with higher rates of risky drinking experience higher rates of drinking harms. Other factors that could contribute to the different effects of alcohol by ethnicity are social disadvantage, acculturation, drink preferences, and alcohol metabolism. This article examines the relationship of ethnicity and drinking to (1) unintentional injuries, (2) intentional injuries, (3) fetal alcohol syndrome (FAS), (4) gastrointestinal diseases, (5) cardiovascular diseases, (6) cancers, (7) diabetes, and (8) infectious diseases. Reviewed evidence shows that Native Americans have a disproportionate risk for alcohol-related motor vehicle fatalities, suicides and violence, FAS, and liver disease mortality. Hispanics are at increased risk for alcohol-related motor vehicle fatalities, suicide, liver disease, and cirrhosis mortality; and Blacks have increased risk for alcohol-related relationship violence, FAS, heart disease, and some cancers. However, the scientific evidence is incomplete for each of these harms. More research is needed on the relationship of alcohol consumption to cancers, diabetes, and HIV/AIDS across ethnic groups. Studies also are needed to delineate the mechanisms that give rise to and sustain these disparities in order to inform prevention strategies. **Key words:** Alcohol consumption; alcohol-attributable fractions; alcohol burden; harmful drinking; alcohol and other drug-induced risk; risk factors; ethnicity; ethnic groups; racial groups; cultural patterns of drinking; Native Americans; Hispanics; Blacks; African Americans; Asian Americans; Whites; Caucasians; injury; intentional injury; unintentional injury; fetal alcohol syndrome; gastrointestinal diseases; cardiovascular diseases; cancers; diabetes; infectious diseases

Research has shown differential social and health effects from alcohol use across U.S. ethnic groups, including Whites, Blacks, Hispanics, Asians, and Native Americans. The relationship of ethnicity to alcohol-related social and health harms partially is attributed to the different rates and patterns of drinking across ethnicities. Some ethnic groups have higher rates of alcohol consumption, putting them at greater risk of drinking harms. However, other ethnic minorities experience health harms from drinking that are

disproportionate to their consumption. Differences in social and socioeconomic factors and biological differences related to alcohol metabolism also could contribute to alcohol's varying effects across populations. This article reviews current research examining the harms of drinking for U.S. ethnic groups. It examines such social harms as driving under the influence and alcohol-attributed violence but primarily focuses on health harms like fetal alcohol syndrome (FAS), liver diseases, and cancers.

The research reviewed focuses on Whites, Blacks, Hispanics, Asians, and Native Americans (i.e., American Indians and Alaska Natives) in the United States as general ethnic groups, although significant subgroup differences within populations also are evident. There are limitations to using these general categories because ethnicity encompasses a combination of characteristics such as tribe, ancestry, national group, birthplace, and language, which could have distinct relationships to patterns of drinking and alcohol-related harms (Caetano 1986; Cheung 1993; Heath 1990–1991). People with multiethnic backgrounds also are not well represented by these general groups. Nevertheless, studies that examine ethnicity and alcohol-attributed harms provide important information about public health and serve to identify high-risk groups in the population. This article shows that Native Americans, Hispanics, and Blacks are disproportionately affected by the adverse social and health harms from alcohol consumption.

Drinking Patterns and Other Determinants of Risk for Alcohol-Related Harms

Heavy drinking and binge drinking contribute to a variety of alcohol-attributed social and health harms (Naimi et al. 2003; Rehm et al. 2010). Heavy alcohol use, as defined by the National Institute on Alcohol Abuse and Alcoholism's (NIAAA's) *Helping Patients Who Drink Too Much: A Clinician's Guide* (NIAAA 2005), is defined as consuming more than 4 standard drinks per day (or more than 14 per week) for men and more than 3 per day (or more than 7 per week) for women. One standard drink is equivalent to 12 ounces of

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beer, 5 ounces of wine, or 1.5 ounces of 80-proof spirits. Binge drinking is defined as consuming five or more drinks in approximately 2 hours for men and four or more drinks for women (NIAAA 2004).

Other than these patterns of consumption, the volume of alcohol intake, defined as the total alcohol consumed over a time period, is linked to social and health harms. Most diseases (e.g., injury, some cancers, and liver cirrhosis) have a detrimental dose-response relationship with alcohol as risk increases with higher-volume alcohol consumption, whereas coronary heart disease and diabetes display a J- or U-shaped relationship (Howard et al. 2004; Rehm et al. 2010; Roerecke and Rehm 2012). The J and U shapes are characterized by both detrimental and beneficial (e.g., increased high-density lipoprotein “good cholesterol”) (Goldberg and Soleas 2001) effects of alcohol use, with higher risks for abstainers and heavy drinkers compared with light or moderate drinkers. However, this relationship is complex and varies by age, gender, and ethnicity (Roerecke and Rehm 2012). Drinking levels that may be protective of cardiovascular health among men also may increase the risk for other harms such as injury, violence, gastrointestinal disease, and some cancers.

Epidemiological studies show that these high-risk patterns of drinking and drinking volume vary by U.S. ethnic group. Ethnicities with greater drinking volume and higher rates of daily and weekly heavy drinking could be at greater risk for experiencing alcohol-attributed harms. Among adult drinkers in the United States, based on the 2001–2002 National Epidemiologic Survey on Alcohol and Related Conditions (NESARC) (Chen et al. 2006), Native Americans and Hispanics have greater alcohol consumption than other ethnic minority groups. Rates of daily heavy drinking were higher among Hispanics (33.9 percent), Native Americans (28.4 percent), and Whites (27.3 percent) compared with Blacks (22.5 percent) and Asians (19.2 percent). Weekly heavy drinking was highest among Native Americans (21.9 percent), followed by Blacks (16.4 percent), Whites (16.3 percent), Hispanics (11.8 percent), and Asians (9.8 percent). Based on the 2001–2002 NESARC data, Caetano and colleagues (2010) reported that White men consumed a higher volume of alcohol (22.3 drinks per month) than Black men (18.9 drinks per month) and Hispanic men (17.8 drinks per month) and that White women consumed more (6.2 drinks per month) compared with Black women (4.9 drinks per month) and Hispanic women (3.9 drinks per month). The sample for these estimates of drinking volume was the U.S. population of Whites, Blacks, and Hispanics and included abstainers. However, a study by Mulia and colleagues (2009) of current drinkers in the United States showed that Whites consumed less alcohol than Hispanics and more than Blacks. The differences between these two studies could reflect a higher rate of abstinence from alcohol among Hispanics (25.7 percent) compared with Whites (13.4 percent) in the U.S. population (Chen et al. 2006). The study that included abstainers (Caetano et al. 2010), who by definition consume zero drinks, showed higher drinking volume for Whites, whereas the study excluding abstainers (Mulia et al. 2009)

reported higher volume for Hispanics. Other ethnic minority groups with higher abstinence rates include Blacks (24.7 percent) and Asians (39.1 percent). Native Americans (17.14 percent) have lower rates of abstinence than other minority groups.

Alternatively, the negative effects from drinking could be explained by factors other than alcohol consumption. Mulia and colleagues (2009) showed that Black and Hispanic adult drinkers were more likely than White drinkers to report alcohol dependence symptoms and social problems from drinking at the no/low level of heavy drinking. Blacks also experience negative health effects from alcohol use despite showing a later onset of use and levels of use often comparable with, if not lower than, Whites (Chartier et al. 2011; Chen et al. 2006; Russo et al. 2004). Other factors associated with ethnic disparities in alcohol-related harms include social disadvantage, characterized by lower socioeconomic status, neighborhood poverty, greater neighborhood alcohol availability, reduced alcohol treatment utilization, and unfair treatment or discrimination (Chae et al. 2008; Chartier and Caetano 2011; Cunradi et al. 2000; Mulia et al. 2008; Nielsen et al. 2005; Zeng et al. 2011). Some ethnic subgroups are more likely to consume high-alcohol-content beverages (e.g., malt liquor), which could result in greater social and health harms (Vilamovska et al. 2009). Preference for such beverages seems to be more common in lower-income ethnic minority communities (Bluthenthal et al. 2005). Some ethnic minority groups also face stressors related to the acculturation process. Higher acculturation, U.S.-born nativity, and longer residence in the United States are risk factors associated with alcohol use disorders and alcohol-related social problems among Hispanics, particularly women (Alegria et al. 2007, 2008; Caetano et al. 2009, 2012; Zeng 2007). Another potential contributor is ethnic differences in the alcohol content of poured drinks. Kerr and colleagues (2009) showed that Black men had drink sizes with larger average alcohol content compared with other groups, which partially could explain the higher risks for alcohol-related harms. Genes responsible for alcohol metabolism also vary across ethnic groups and could be associated with susceptibility for alcohol-related diseases. Among Whites, Blacks, and Asians, alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) genotypes have been linked in combination with drinking to alcohol-related cancers, birth defects, and pancreatitis (Yin and Agarwal 2001).

Ethnicity and Alcohol-Attributed Harms

Alcohol-attributed harms can be both acute and chronic conditions that are wholly caused (e.g., alcoholic liver cirrhosis) or associated with alcohol use via intoxication, alcohol dependence, and the toxic effects of alcohol (Rehm et al. 2010). The major injury and disease categories linked to alcohol consumption include (1) unintentional injuries, (2) intentional injuries, (3) FAS, (4) gastrointestinal diseases, (5) cardiovascular diseases, (6) cancers, (7) diabetes, and (8) infectious diseases (World Health Organization [WHO] 2011). Evidence

is incomplete on the relationship between ethnicity, drinking, and each of these categories. Below, those alcohol-related harms are described that have available findings by ethnic group in addition to important gaps in this scientific literature. Alcohol use disorders are causally linked to drinking and vary by ethnicity (i.e., more likely in Native Americans and Whites) (Hasin et al. 2007), but this disease category is not described here.

Unintentional Injuries

Unintentional injuries associated with alcohol use include falls, drowning, and poisoning (WHO 2011). However, most available research on ethnicity, alcohol use, and injuries is focused on motor vehicle crashes. Alcohol-impaired driving and crash fatalities vary by ethnicity, with Native Americans and Hispanics being at higher risk than other ethnic minority groups. Past-year driving under the influence (DUI) estimates based on the 2007 National Survey on Drug Use and Health were highest for Whites (15.6 percent) and Native Americans (13.3 percent) relative to Blacks (10.0 percent), Hispanics (9.3 percent), and Asians (7.0 percent) (Substance Abuse and Mental Health Services Administration [SAMHSA] 2008). National surveys generally show lower DUI rates for Hispanics than Whites, but studies based on arrest data identify Hispanics as another high-risk group for DUI involvement (Caetano and McGrath 2005; SAMHSA 2005). The DUI arrest rate for Native Americans in 2001, according to the U.S. Department of Justice (Perry 2004), was 479 arrestees per 100,000 residents compared with 332 for all other U.S. ethnic groups.

Based on a 1999–2004 report from the National Highway Traffic Safety Administration (Hilton 2006), rates of intoxication (i.e., blood alcohol concentration [BAC] more than or equal to 0.08 percent) for drivers who were fatally injured in a motor vehicle crash were highest for Native Americans (57 percent) and Hispanics (47 percent) and lowest for Asians (approximately 20 percent), with Whites and Blacks falling in between. Across ethnic groups, most drinking drivers killed were male, although the proportion of female drivers who were intoxicated among fatally injured drivers was highest (i.e., more than 40 percent) for Native Americans. Centers for Disease Control and Prevention (CDC) (2009*b*) statistics on alcohol-related motor vehicle crash deaths also point to an important subgroup difference for Asians. In 2006, the overall death rate among Asians (1.8 per 100,000 people) obscured the death rate among Native Hawaiians and other Pacific Islanders (5.9), which was less than the rate for Native Americans but similar to that for Hispanics (14.5 and 5.2, respectively).

Intentional Injuries

Suicide

Native Americans are overrepresented in national estimates of alcohol-involved suicides. A CDC report (2009*a*) based

on 2005–2006 data from the National Violent Death Reporting System presented findings on alcohol and suicide across ethnic groups. Recent alcohol use was reported among suicides in 46 percent of Native Americans, 30 percent of Hispanics, 26 percent of Whites, 16 percent of Blacks, and 15 percent of Asians. Among those tested for alcohol, the rates of intoxication (BAC higher than or equal to 0.08) were highest for Native Americans (37 percent), followed by Hispanics (29 percent), Whites (24 percent), Blacks (14 percent), and Asians (12 percent). Age-groups identified as being at high risk for alcohol-involved suicide included Native Americans ages 30 to 39 (54 percent of suicide victims had BACs higher than or equal to 0.08), Native Americans and Hispanics ages 20 to 29 (50 percent and 37 percent, respectively), and Asians ages 10 to 19 (29 percent). Males were at higher risk than female drinkers in all ethnic groups except Native Americans; the percentages of alcohol intoxication among Native American suicides were equal for males and females (37 percent).

Violence

Ethnic groups are differentially affected by alcohol-attributed violence, including intimate-partner violence (IPV). Alcohol plays an important role in IPV and other types of relationship conflicts (Field and Caetano 2004; Leonard and Eiden 2007). Based on data from the National Study of Couples, general rates of male-to-female partner violence (MFPV) and female-to-male partner violence (FMPV), are highest among Black couples (23 percent and 30 percent, respectively), followed by Hispanic (17 percent and 21 percent) and White (12 percent and 16 percent) couples (Caetano et al. 2000). The National Study of Couples provides general population data on IPV, which includes mostly moderate violence and may differ from other studies of severe violence. In this study, regardless of ethnicity, men were more likely than women to report drinking during partner violence. Drinking during a violent episode by the male or the female partner, respectively, was more frequent among Blacks (MFPV: 41.4 percent and 23.6 percent; FMPV: 33.7 percent and 22.4 percent) than among Whites (MFPV: 29.4 percent and 11.4 percent; FMPV: 27.1 percent and 14.7 percent) and Hispanics (MFPV: 29.1 percent and 5.4 percent; FMPV: 28.4 percent and 3.8 percent). Longitudinal findings, using 5-year National Study of Couples data, identified female-partner alcohol problems (i.e., alcohol dependence symptoms and social problems) in Black couples and male- and female-partner alcohol consumption in White couples as risk factors for IPV (Field and Caetano 2003). Some evidence also suggests that interethnic couples, involving White, Black, and Hispanic partners of different ethnic backgrounds, are a high-risk group for relationship violence. Relative to intraethnic couples, these interethnic couples had higher prevalence rates of IPV, which was associated with binge drinking and alcohol problems among male partners (Chartier and Caetano 2012).

Alcohol also contributes to violence victimization among Native Americans (Yuan et al. 2006). Several studies

indicate that Native Americans are at greater risk for alcohol-related trauma (e.g., IPV, rape, and assault) compared with other U.S. ethnic groups (Oetzel and Duran 2004; Wahab and Olson 2004). Based on 1992–2001 National Crime Victimization Survey data, the U.S. Department of Justice (Perry 2004) reported that 42 percent of all violent crimes (i.e., rape, sexual assault, robbery, aggravated assault, and simple assault) were committed by an offender who was under the influence of alcohol. In particular, Native American violent crime victims were more likely (62 percent) than other violent crime victims to report alcohol use by their offender, including Whites (43 percent), Blacks (35 percent), and Asians (33 percent).

respectively) compared with other ethnic groups (0 percent to 10.7 percent and 21.0 percent to 37.3 percent). White women in this study were at greater risk for an alcohol-exposed pregnancy. However, other studies found that Black, Hispanic, and Asian women were less likely to reduce or quit heavy drinking after becoming pregnant (Morris et al. 2008; Tenkku et al. 2009). Blacks and Native Americans are at greater risk than Whites for FAS and fetal alcohol spectrum disorders (Russo et al. 2004). From 1995 to 1997, FAS rates averaged 0.4 per 1,000 live births across data-collection sites for the Fetal Alcohol Syndrome Surveillance Network and were highest for Black (1.1 percent) and Native American (3.2 percent) populations (CDC 2002).

Fetal Alcohol Syndrome

Using data from the 2001–2002 NESARC, Caetano and colleagues (2006) examined alcohol consumption, binge drinking, and alcohol abuse and dependence among women who were pregnant during the past year. Most women (88 percent) who reported being pregnant and also a drinker at any point in the past 12 months indicated that they did not drink during pregnancy. Rates of past-year alcohol abuse (0.8 percent to 2.3 percent) and dependence (1.2 percent to 2.8 percent) were similar and low in White, Black, Hispanic, and Asian pregnant women. Binge drinking and alcohol consumption without binge drinking among pregnant women were highest in Whites (21.1 percent and 45.0 percent,

Gastrointestinal Diseases

Liver disease is an often-cited example of the disproportionate effect of alcohol on health across ethnic groups. Native Americans have higher mortality rates for alcoholic liver disease than other U.S. ethnic groups (see figure). According to the National Vital Statistical Reports (Miniño et al. 2011) on 2008 U.S. deaths, age-adjusted death rates attributed to alcoholic liver disease for Native American men and women were 20.4 and 15.3 per 100,000 people, respectively, compared with 6.9 and 2.4 per 100,000 for men and women in the general population.

Blacks and Hispanics have greater risk for developing liver disease compared with Whites (Flores et al. 2008), and

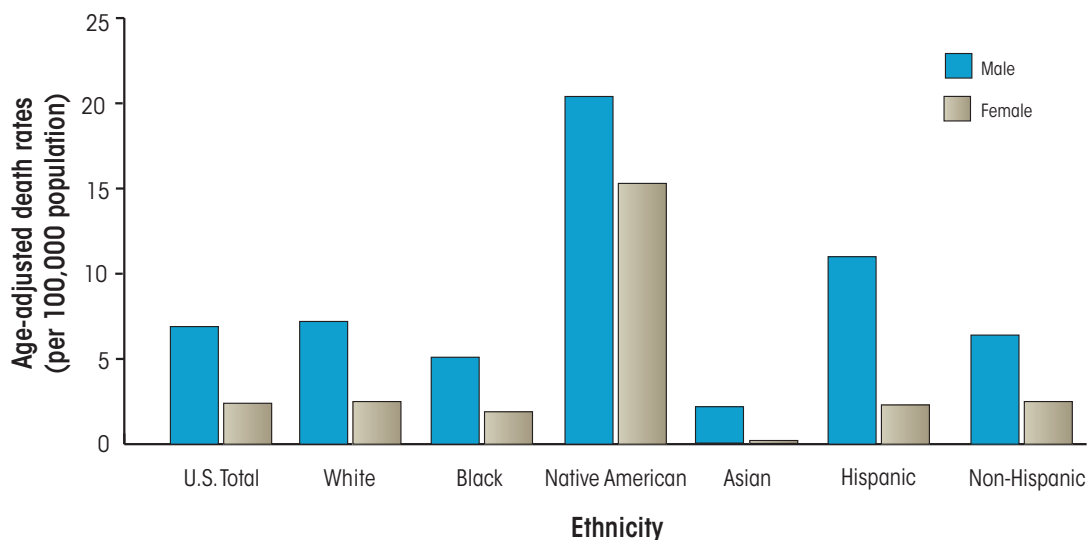


Figure In 2008, age-adjusted death rates attributed to alcoholic liver disease for Native American men and women were 20.4 and 15.3 per 100,000 people, respectively, compared with 6.9 and 2.4 for men and women in the general population.

SOURCE: Miniño, A.M. et al., Deaths: Final data for 2008. *National Vital Statistics Reports* 59(10):1–52, 2011.

death rates attributed to alcohol-related cirrhosis across populations of Whites, Blacks, and Hispanics are highest for White Hispanic men (Yoon and Yi 2008). Blacks show a greater susceptibility than Whites to alcohol-related liver damage, with risk differences amplified at higher levels of consumption (Stranges et al. 2004). Based on data from the National Center for Health Statistics, 1991–1997, mortality rates for cirrhosis with mention of alcohol were higher in White Hispanics and Black non-Hispanics compared with White non-Hispanics (Stinson et al. 2001). Male mortality rates for alcohol-related cirrhosis in White Hispanics and non-Hispanic Blacks were 114 percent and 24 percent higher, respectively, than the overall male rate (5.9 deaths per 100,000 people); female rates in White Hispanics and non-Hispanic Blacks were 16 percent and 47 percent higher than the overall female rate (1.9 deaths per 100,000 people). In contrast, death rates for White non-Hispanic and Black Hispanic males and females were lower than overall rates for each gender. In addition, there is considerable variation in deaths from liver cirrhosis across Hispanic subgroups, with mortality rates highest in Puerto Ricans and Mexicans and lowest in Cubans (Yoon and Yi 2008).

Cardiovascular Diseases

Although moderate alcohol consumption has been associated with a reduced risk for coronary heart disease (CHD) (Goldberg and Soleas 2001), there is some evidence that ethnic groups differ in terms of this protective effect, particularly for Blacks compared with Whites. Sempos and colleagues (2003) found no protective health effect for moderate drinking in Blacks for all-cause mortality, as previously reported in Whites. Kerr and colleagues (2011) reported the absence of this protective effect for all-cause mortality in Blacks and Hispanics. Similar findings have been described for hypertension and CHD risks in Black men compared with White men and women (Fuchs et al. 2001, 2004) and for mortality among Black women without hypertension (Freiberg et al. 2009). Mukamal and colleagues (2010) also showed that the protective effects of light and moderate drinking in cardiovascular mortality were stronger among Whites than non-Whites. Pletcher and colleagues (2005) found evidence that the dose-response relationship between alcohol consumption and increased coronary calcification, a marker for CHD, was strongest among Black men.

Cancers

In 1988, the WHO International Agency for Research on Cancer (IARC) reviewed the epidemiologic evidence on the association between alcohol consumption and cancer and found a consistent association between alcohol consumption and increased risk for cancers of the oral cavity, pharynx, larynx, esophagus, and liver (IARC 1988). Regardless of ethnicity, the risk of developing these cancers is significantly

higher among men than women (National Cancer Institute 2011*c, d, e*). The incidence and mortality rates for these cancers also vary across ethnic groups. Regarding cancers of the oral cavity and pharynx, incidence rates among White and Black men are comparable (16.1 and 15.6 per 100,000, respectively); however, mortality rates are higher among Black men (6.0 versus 3.7 per 100,000 for White men) (National Cancer Institute 2011*e*). For cancer of the larynx, both incidence and mortality rates are higher among Black men than among White men (incidence, 9.8 and 6.0; mortality, 4.4 and 2.0) (National Cancer Institute 2011*c*). Although these differences may be explained by differential use of alcohol and tobacco in relation to gender and ethnicity, there is some evidence that even after controlling for alcohol and tobacco use, Blacks continue to be at increased risk for squamous cell esophageal cancer and cancers of the oral cavity and pharynx (Brown et al. 1994; Day et al. 1993).

The majority (approximately 90 percent) of all primary liver cancers are hepatocellular carcinomas (HCC) (Altekruse et al. 2009). Alcohol-related and non-alcohol-related liver cirrhosis usually precede HCC and are the two most common risk factors (Altekruse et al. 2009; El-Serag 2011; Pelucchi et al. 2006). The relative risk for developing this cancer increases with increased levels of alcohol consumption (Pelucchi et al. 2006). By ethnic group, 2003–2005 age-adjusted incidence rates for HCC per 100,000 persons were highest among Asians (11.7), followed by Hispanics (8.0), Blacks (7.0), Native Americans (6.6), and Whites (3.9) (Altekruse et al. 2009). Death rates for HCC per 100,000 people also are higher among minority groups (i.e., 8.9, 6.7, 5.8, 4.9, and 3.5 for Asians, Hispanics, Blacks, Native Americans, and Whites, respectively).

In 2007, the IARC reconvened and added breast and colorectal cancers to the list of cancers related to alcohol use (Baan et al. 2007). Research has demonstrated consistent, albeit weak, dose-response relationships between alcohol consumption and these cancers (Cho et al. 2004; Collaborative Group on Hormonal Factors in Breast Cancer 2002; Moskal et al. 2007; Singletary and Gapstur 2001). Alcohol consumption also contributes to the stage at which breast cancer is diagnosed (Hebert et al. 1998; Trentham-Dietz et al. 2000; Vaeth and Satariano 1998; Weiss et al. 1996). This could be because of the timing of disease detection, since heavy drinking has been associated with a lack of mammography utilization (Cryer et al. 1999). Alcohol consumption also may contribute to more rapid tumor proliferation (Singletary and Gapstur 2001; Weiss et al. 1996). Data from the Surveillance, Epidemiology, and End Results (SEER) Program indicate that White women, relative to women from ethnic minority groups, have higher incidence rates of breast cancer (i.e., Whites, 127.3; Blacks, 119.9; Asians, 93.7; Native Americans, 92.1; and Hispanics, 77.9 per 100,000 people) (National Cancer Institute 2011*a*). Black women, however, are more likely to be diagnosed with advanced disease (Chlebowski et al. 2005) and have significantly higher mortality rates than White women (i.e., 32.0

per 100,000 versus 22.8 per 100,000 people) (Chlebowski et al. 2005; National Cancer Institute 2011*a*). Regarding colorectal cancer, Blacks have higher incidence (67.7) and mortality (51.2) rates than all ethnic groups combined (55.0 and 41.0, respectively) (National Cancer Institute 2011*b*). Unfortunately, little is known about how drinking differentially affects ethnic differences in breast and colorectal cancers.

Diabetes

In 2010, the prevalence of diabetes was 7.1 percent, 12.6 percent, 11.8 percent, and 8.4 percent among Whites, Blacks, Hispanics, and Asians, respectively (National Institute of Diabetes and Digestive and Kidney Diseases 2011). Age-adjusted mortality rates in 2007 were 20.5, 42.8, 28.9, and 16.2 per 100,000 people among Whites, Blacks, Hispanics, and Asians (National Center for Health Statistics 2011). Data on mortality rates for diabetes among Hispanics may be underreported as a result of inconsistencies in the reporting of Hispanic origin on death certificates (Heron et al. 2009). Despite higher risks for the development of and death from diabetes in Hispanics and Blacks compared with Whites, little evidence is available to delineate the relationship of alcohol to diabetes across ethnic groups. Studies among both diabetics and nondiabetics demonstrate a J- or U-shaped curve between alcohol consumption and insulin sensitivity (Bell et al. 2000; Davies et al. 2002; Greenfield et al. 2003; Kroenke et al. 2003). Likewise, two large epidemiologic studies among diabetic subjects show that moderate alcohol consumption is associated with better glycemic control (Ahmed et al. 2008; Mackenzie et al. 2006). An important limitation of these studies, however, is that few included ethnic minority groups or failed to emphasize possible differences in relation to ethnicity in their analyses.

Infectious Diseases

Among the infectious diseases attributable to alcohol (e.g., pneumonia, tuberculosis) (WHO 2011), human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS) are most relevant to U.S. ethnic health disparities. In 2009, Blacks represented 44 percent of new HIV infections and Hispanics represented 20 percent. Infection rates by gender for Blacks were 15 times (for men) and 6.5 times (for women) those of Whites, and rates for Hispanics were 4.5 times for men and 2.5 times for women, compared with rates for Whites (CDC 2011). In addition, alcohol consumption has been associated with increased HIV infection risk (Bryant et al. 2010). Caetano and Hines (1995) showed that heavy drinking predicted high-risk sexual behaviors in White, Black, and Hispanic men and women, with more Blacks than Whites and Hispanics reporting risky sexual behaviors. Among HIV-infected patients, there also is evidence that increased alcohol consumption negatively affects adherence to antiretroviral medication regimens (Chander et al. 2006;

Cook et al. 2001; Samet et al. 2004) and HIV disease progression (Conigliaro et al. 2003; Samet et al. 2003). Despite these strong individual associations between ethnicity and HIV/AIDS and alcohol and HIV/AIDS, there is limited research across ethnicities on alcohol use and HIV infection or disease progression.

Conclusions

This article identifies U.S. ethnic-group differences in alcohol-attributed social and health-related harms. Three minority ethnicities are particularly disadvantaged by alcohol-related harms. Native Americans, relative to other ethnic groups, have higher rates of alcohol-related motor vehicle fatalities, suicide, violence, FAS, and liver disease mortality. Unlike other ethnic groups, in which men are primarily at risk for alcohol-related harms, both Native American men and women are high-risk groups. Hispanics have higher rates of alcohol-related motor vehicle fatalities, suicide, and cirrhosis mortality. Blacks have higher rates of FAS, intimate partner violence, and some head and neck cancers, and there is limited empirical support in Blacks for a protective health effect from moderate drinking. These patterns of findings provide recognition of the health disparities in alcohol-attributed harms across U.S. ethnicities. However, further research is needed to identify the mechanisms that give rise to and sustain these disparities in order to develop prevention strategies. The contributing factors include the higher rates of consumption found in Native Americans and Hispanics, but more broadly range from biological factors to the social environment. More research on the relationship of alcohol to some cancers, diabetes, and HIV/AIDS across ethnic groups is also needed. There is limited evidence for how drinking differentially affects ethnic differences in breast and colorectal cancers and in diabetes and HIV/AIDS onset and care, and few findings for how alcohol-attributed harms vary across ethnic subgroups. ■

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Alcohol and Mortality

Global Alcohol-Attributable Deaths From Cancer, Liver Cirrhosis, and Injury in 2010

Jürgen Rehm, Ph.D., and Kevin D. Shield, MH.Sc.

Alcohol consumption has long been recognized as a risk factor for mortality. By combining data on alcohol per capita consumption, alcohol-drinking status and alcohol-drinking patterns, risk relationships, and mortality, the Comparative Risk Assessment Study estimated alcohol-attributable mortality for 1990 and 2010. Alcohol-attributable cancer, liver cirrhosis, and injury were responsible for the majority of the burden of alcohol-attributable mortality in 1990 and 2010. In 2010, alcohol-attributable cancer, liver cirrhosis, and injury caused 1,500,000 deaths (319,500 deaths among women and 1,180,500 deaths among men) and 51,898,400 potential years of life lost (PYLL) (9,214,300 PYLL among women and 42,684,100 PYLL among men). This represents 2.8 percent (1.3 percent for women and 4.1 percent for men) of all deaths and 3.0 percent (1.3 percent for women and 4.3 percent for men) of all PYLL in 2010. The absolute mortality burden of alcohol-attributable cancer, liver cirrhosis, and injury increased from 1990 to 2010 for both genders. In addition, the rates of deaths and PYLL per 100,000 people from alcohol-attributable cancer, liver cirrhosis, and injury increased from 1990 to 2010 (with the exception of liver cirrhosis rates for women). Results of this paper indicate that alcohol is a significant and increasing risk factor for the global burden of mortality. **KEY WORDS:** Alcohol consumption; alcohol burden; alcohol-attributable mortality; alcohol-attributable fractions; global alcohol-attributable mortality; risk factor; cancer; liver cirrhosis; injury; burden of disease; Global Burden of Disease and Injury study

Alcohol and Mortality

Alcohol is causally linked to more than 200 different diseases, conditions, and injuries (as specified in the *International Classification of Diseases, Revision 10* [ICD-10] three-digit codes [see Rehm 2011; Rehm et al. 2009; Shield et al., 2013c [pp. 155–173 of this issue)]. All of these disease, condition,

and injury categories cause mortality and disability, and, thus, alcohol consumption causes a net burden of mortality and disability (Rothman et al. 2008). However, certain patterns of alcohol consumption are protective for ischemic diseases (Roerecke and Rehm 2012a) and diabetes (Baliunas et al. 2009), and, thus, alcohol can prevent death and disability from these causes. The total mortality and disability caused by and prevented by the consumption of alcohol is calculated by comparing the expected mortality under current conditions to a counterfactual scenario where no one has consumed alcohol (Ezzati et al. 2006; Walter 1976). Although the counterfactual scenario seems unrealistic as almost one-half of the global population consumes alcohol (for the most up-to-date statistics on alcohol consumption, see Shield et al. 2013b; World Health Organization 2011a), recent natural experiments in countries where there has been a considerable reduction in alcohol consumption showed a clear reduction in mortality (e.g., Russia) (Leon et al. 1997; Neufeld and Rehm 2013). Accordingly, the calculations of the deaths and disability caused by alcohol consumption seem to correspond to real phenomena and, thus, could predict an approximate level of reduction in mortality if alcohol consumption were to be reduced.

This article outlines the alcohol-attributable mortality burden from three major causes: cancer, liver cirrhosis, and injury. All three categories have long been identified as causally linked to alcohol consumption. With respect to cancer, in 1988 the International Agency for Research on Cancer established alcohol as a carcinogen (International Agency for Research on Cancer 1988), and in its latest monograph has found alcohol consumption to be causally associated with oral cavity, pharynx, larynx, esophagus, liver, colon, rectum, and female breast cancers (International Agency for Research on Cancer 2010, 2012). Studies have shown that stomach cancer may be associated with alcohol consumption, but evidence on the causal relationship between stomach cancer

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and alcohol consumption is not yet conclusive (International Agency for Research on Cancer 2012; Rehm and Shield, in press). Biologically, it has been established that ethanol, and not other ingredients of alcoholic beverages, is the ingredient that mainly causes cancer (Lachenmeier et al. 2012), with acetaldehyde (the first metabolite of ethanol) likely being the most important biological carcinogen (International Agency for Research on Cancer 2010, 2012; Rehm and Shield, in press). In addition, observational studies have found a clear dose-response relationship between alcohol consumption and the risk of cancer, with no observed threshold for the effect of alcohol, as an elevated risk of cancer has been observed even for people who consume relatively low amounts of alcohol (Bagnardi et al. 2013; Rehm et al. 2010a).

Liver cirrhosis has been associated with alcohol consumption, especially heavy consumption, since the seminal work of Benjamin Rush (Rush 1785). The causal link between alcohol consumption and liver cirrhosis is so strong and important that the World Health Organization has created a specific category for alcoholic liver cirrhosis (World Health Organization 2007). As with cancer, there is a dose-response relationship between alcohol consumption and the risk of liver cirrhosis, with no lower threshold being observed (Rehm et al. 2010c); however, the majority of the effect can be seen for heavy drinking (Rehm et al. 2010c).

The risk of injury also has been causally linked to alcohol consumption, with this relationship fulfilling all of the classic Bradford Hill criteria (e.g., consistency of the effect, temporality, a dose-response relationship with the risk of an injury [biological gradient]) (Rehm et al. 2003a). The effect of alcohol on injury is acute; the level of risk for both intentional and unintentional injuries is clearly linked to blood alcohol level (Taylor and Rehm 2012; Taylor et al. 2010), with a very low threshold (Eckardt et al. 1998). There also is an association between average consumption of alcohol and injury (Corrao et al. 2004).

Alcohol-attributable cancer, liver cirrhosis, and injury constitute the majority of the burden of alcohol-attributable mortality. Collectively, they were responsible for 89 percent of the net burden of alcohol-attributable mortality (i.e., the mortality rate after including the beneficial effects of alcohol on ischemic diseases and diabetes) and for 79 percent of the gross burden of alcohol-attributable mortality (Shield et al. 2013a) in the United States in 2005, for people 15 to 64 years of age. Additionally, they were responsible for 91 percent of the net alcohol-attributable mortality and 79 percent of the gross alcohol-attributable mortality in the European Union (Rehm et al. 2012) and 80 percent of the net alcohol-attributable mortality and 72 percent of the gross alcohol-attributable mortality globally (Rehm et al. 2009) in 2004. This article does not review the other causes of alcohol-attributable deaths included in the latest Comparative Risk Assessment (CRA) Study (Lim et al. 2012). The CRA study estimates as published in December contained significant errors in the calculation of alcohol-attributable cardiovascular deaths, estimating that 33 percent of all ischemic heart disease

deaths were attributed to alcohol, which is an impossibility as the relationship between alcohol consumption and this disease category is mainly protective (for details on relationship between alcohol and heart disease, see Roerecke and Rehm 2010, 2012b). A comparison with other alcohol-attributable disease and protective effects will thus only be possible once the corrected CRA results are published.

Methodology Underlying the Estimation of the Mortality Burden of Alcohol-Attributable Diseases and Injuries

The number of alcohol-attributable cancer, liver cirrhosis, and injury deaths in 1990 and 2010 were estimated using alcohol-attributable fractions (AAFs) (Benichou 2001; Walter 1976, 1980). AAFs are calculated by comparing the population risk of a disease under current conditions to a counterfactual scenario where no one has consumed alcohol. This is achieved by using information on the distribution of levels of alcohol consumption and the associated relative risks (RRs) (i.e., risks of disease for different levels of alcohol consumption versus abstainers). These calculated AAFs then were applied to mortality data obtained from the 2010 Global Burden of Disease (GBD) Study for 1990 and 2010 (Lim et al. 2012). Mortality data for 1990 and 2010 were modelled using data on mortality from 1980 to 2010. Data on mortality were imputed for those countries with little or no data by using data from other countries and were smoothed over time (in addition to other data corrections procedures that corrected for cause of death recording errors) (Lozano et al. 2012).

Calculating the Alcohol-Attributable Mortality Burden of Cancer and Liver Cirrhosis

Alcohol consumption is causally related to mouth and oropharynx cancers (ICD-10 codes: C00 to C14), esophageal cancer (C15), liver cancer (C22), laryngeal cancer (C32), breast cancer (C50), colon cancer (C18), and rectal cancer (C20). Alcohol RR functions for cancer were obtained from Corrao and colleagues (2004) (For information about the causal relationship between alcohol and cancer, see Baan et al. 2007; International Agency for Research on Cancer 2010.) The alcohol RR for liver cirrhosis (ICD-10 codes: K70 and K74) was obtained from Rehm and colleagues (Rehm et al. 2010c). The above-noted RRs were modelled based on drinking status and average daily alcohol consumption among drinkers. The same RRs were used to estimate the AAFs by country, sex, and age for 1990 and for 2010.

Alcohol-drinking statuses and adult (people 15 years of age and older) per capita consumption data for 1990 were obtained from various population surveys (Shield et al. 2013b), and the Global Information System on Alcohol and Health (available at: <http://apps.who.int/ghodata/?theme=GISAH>), respectively. Data on drinking status and adult per

capita consumption for 2010 were estimated by projections (performed using regression analyses) using data from years prior to 2010 (Shield et al. 2013b). Average daily alcohol consumption was modelled using a gamma distribution (Rehm et al. 2010b) and data on per capita consumption for 1990, which was projected to 2010 (Shield et al. 2013b). (For more information on the methodology of how alcohol consumption was modelled, see Kehoe et al. 2012; Rehm et al. 2010b). This paper presents alcohol consumption data from 2005, the latest year with actual data available.

Calculating the Alcohol-Attributable Mortality Burden of Injuries

The burden of injury mortality attributable to alcohol consumption was modelled according to methodology outlined by Shield and colleagues (2012), using risk information obtained from a meta-analysis (Taylor et al. 2010) and alcohol consumption data from 1990 and 2010. The risk of an injury caused to the drinker over a year was calculated based on alcohol consumed during normal drinking occasions and alcohol consumed during binge-drinking occasions. Alcohol-

attributable injuries caused to nondrinkers also were estimated (Shield et al. 2012).

Global Consumption of Alcohol

In 2005 adult per capita consumption of alcohol was 6.1 litres of pure alcohol. Figure 1 shows the adult per capita consumption of alcohol by country. Alcohol consumption per drinker in 2005 was 17.1 litres (9.5 litres per female drinker and 20.5 litres per male drinker). Of all adults, 45.8 percent were lifetime abstainers (55.6 percent of female adults and 36.0 percent of male adults), 13.6 percent were former drinkers (13.1 percent of female adults and 14.1 percent of male adults), and 40.6 percent were current drinkers (31.3 percent of female adults and 49.9 percent of male adults). The global pattern of drinking score (a score from 1 to 5 that measures the harmfulness of alcohol drinking patterns, with 1 being the least harmful and 5 being the most harmful [Rehm et al. 2003b]) was 2.6 in 2005 and ranged from 4.9 for Eastern Europe to 1.5 for Western Europe. Thus, alcohol consumption in Eastern Europe can be characterized by fre-

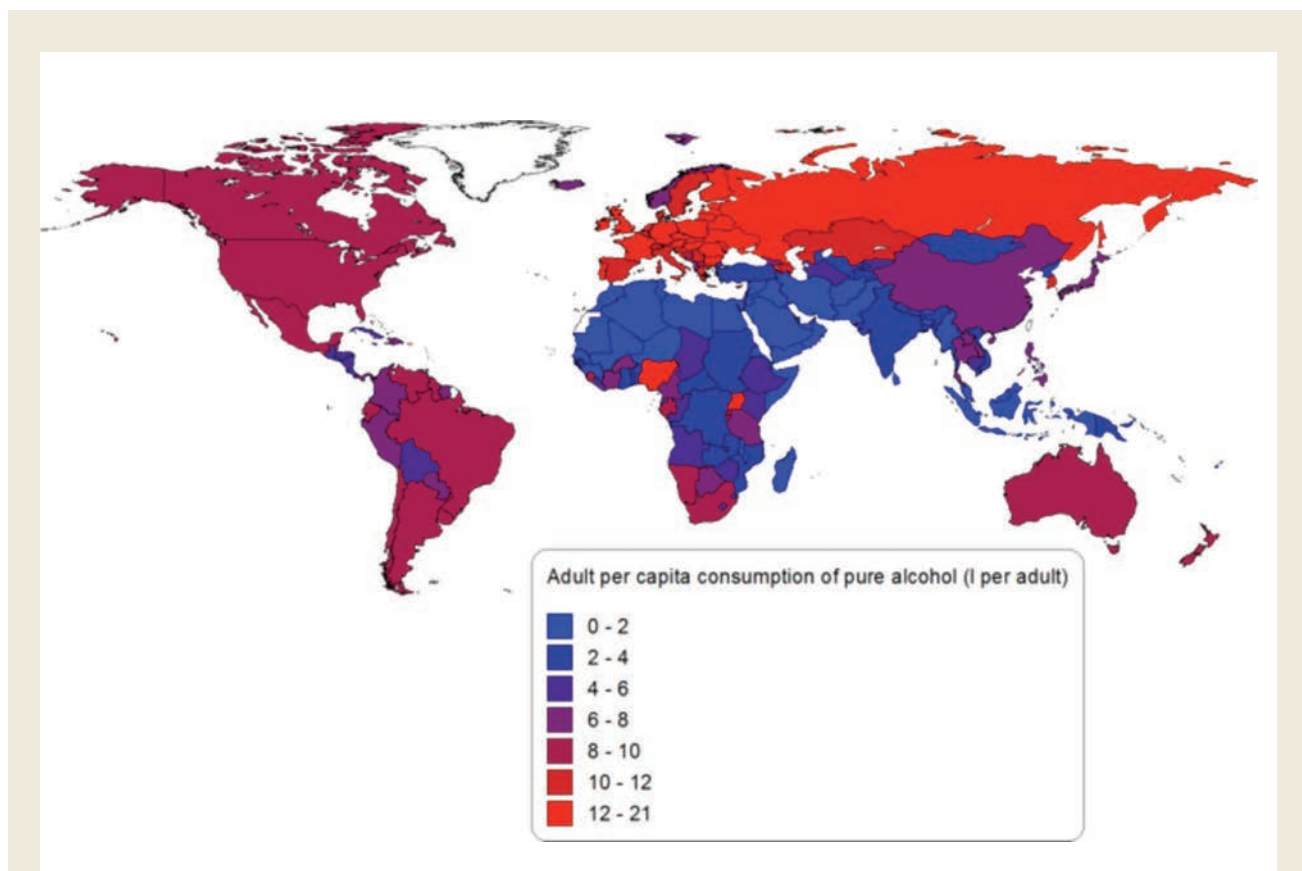


Figure 1 Adult per capita consumption of pure alcohol by country in 2005.

NOTE: More detailed information can be obtained from the author.

quent heavy alcohol consumption outside of meals and drinking to intoxication.

Global Alcohol-Attributable Mortality From Cancer

In 2010, alcohol-attributable cancer caused 337,400 deaths (91,500 deaths among women and 245,900 deaths among men) and 8,460,000 PYLL (2,143,000 PYLL among women and 6,317,000 PYLL among men). This burden is equal to 4.9 deaths per 100,000 people (2.7 deaths per 100,000 women and 7.1 deaths per 100,000 men) and 122.8 PYLL per 100,000 people (62.8 PYLL per 100,000 women and 181.9 PYLL per 100,000 men). Stated another way, alcohol-attributable cancer was responsible for 4.2 percent of all cancer deaths in 2010 and 4.6 percent of all PYLL caused by cancer. Figure 2 shows the number of alcohol-attributable cancer deaths per 100,000 people by region in 2010. Eastern Europe had the highest burden of mortality and morbidity from alcohol-attributable cancer, with 10.3 deaths and 272.0 PYLL per 100,000 people. North Africa and the Middle East had the lowest mortality burden of

alcohol-attributable cancer, with 0.6 deaths and 17.1 PYLL per 100,000 people.

In 1990, alcohol-attributable cancer caused 243,000 deaths worldwide (70,700 deaths among women and 172,300 deaths among men) and 6,405,700 PYLL (1,762,200 PYLL among women and 4,643,500 PYLL among men). This mortality burden is equal to 4.6 deaths per 100,000 people (2.7 deaths per 100,000 women and 6.5 deaths per 100,000 men) and 120.8 PYLL per 100,000 people (67.0 PYLL per 100,000 women and 173.9 PYLL per 100,000 men) caused by alcohol-attributable cancer. From 1990 to 2010 the absolute mortality burden of alcohol-attributable cancer (measured in deaths and PYLL) and the rates of deaths and PYLL per 100,000 people have each increased.

Global Alcohol-Attributable Mortality From Liver Cirrhosis

In 2010, alcohol-attributable liver cirrhosis was responsible for 493,300 deaths worldwide (156,900 deaths among women and 336,400 deaths among men) and 14,327,800 PYLL

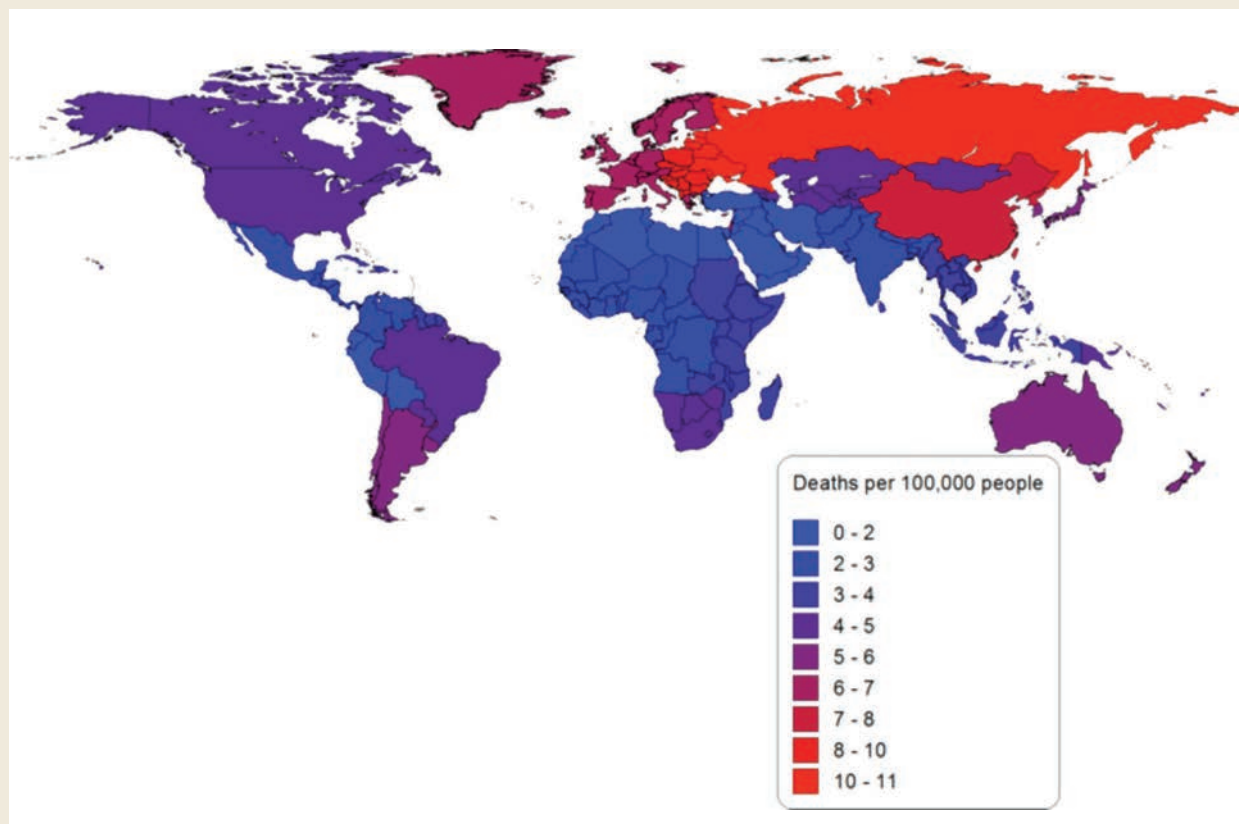


Figure 2 Alcohol-attributable cancer deaths per 100,000 people in 2010 by global-burden-of-disease region.

NOTE: More detailed information can be obtained from the author.

(4,011,100 PYLL among women and 10,316,800 PYLL among men). This mortality burden is equal to 7.2 deaths per 100,000 people (4.6 deaths per 100,000 women and 9.7 deaths per 100,000 men) and 208.0 PYLL per 100,000 people (117.5 PYLL per 100,000 women and 297.0 PYLL per 100,000 men) caused by alcohol-attributable liver cirrhosis in 2010. Overall, in 2010 alcohol-attributable liver cirrhosis was responsible for 47.9 percent of all liver cirrhosis deaths and 47.1 percent of all liver cirrhosis PYLL. Figure 3 outlines the number of alcohol-attributable liver cirrhosis deaths per 100,000 people by region in 2010, showing strong regional variability.

In 1990, alcohol-attributable liver cirrhosis was responsible for 373,200 deaths worldwide (125,300 deaths among women and 247,900 deaths among men) and 10,906,200 PYLL (3,253,300 PYLL among women and 7,652,900 PYLL among men). That is, 7.0 deaths per 100,000 people (4.8 deaths per 100,000 women and 9.3 deaths per 100,000 men) and 205.7 PYLL per 100,000 people (123.7 PYLL per 100,000 women and 286.6 PYLL per 100,000 men) were caused by liver cirrhosis attributable to alcohol consumption. From 1990 to 2010, the absolute mortality burden of alcohol-

attributable liver cirrhosis (measured in deaths and PYLL) and this mortality burden per 100,000 people have each increased (except for women, where alcohol-attributable liver cirrhosis deaths and PYLL per 100,000 decreased slightly).

Global Alcohol-Attributable Mortality From Injury

Globally in 2010, alcohol-attributable injuries were responsible for 669,300 deaths (71,100 deaths among women and 598,200 deaths among men) and 29,110,600 PYLL (3,060,200 PYLL among women and 26,050,400 PYLL among men). This mortality burden is equal to 9.7 deaths per 100,000 people (2.1 deaths per 100,000 women and 17.2 deaths per 100,000 men) and 422.6 PYLL per 100,000 people (89.6 PYLL per 100,000 women and 750.0 PYLL per 100,000 men). Overall, in 2010 alcohol-attributable injuries were responsible for 13.2 percent of all injury deaths and 12.6 percent of all injury PYLL. Figure 4 outlines the number of alcohol-attributable injury deaths per 100,000 people in 2010. Eastern Europe had the greatest mortality burden of alcohol-attributable injuries, with 76.7 deaths and

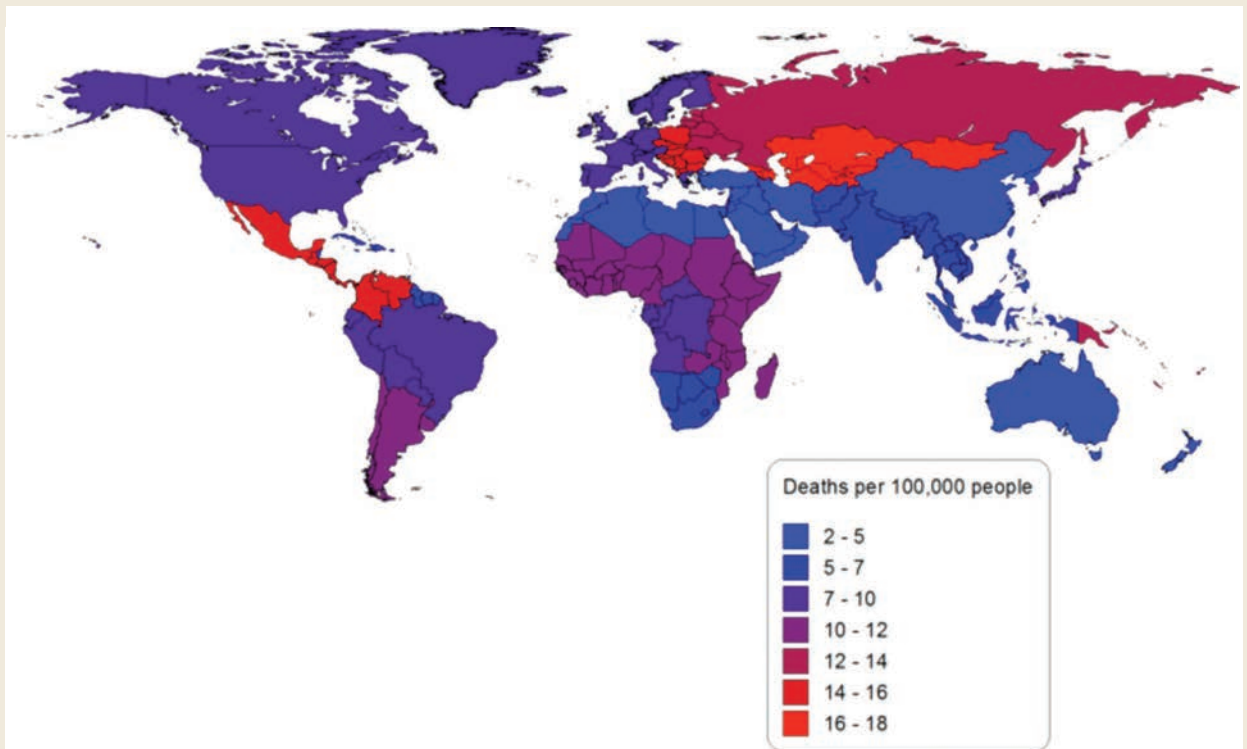


Figure 3 Alcohol-attributable liver cirrhosis deaths per 100,000 people in 2010 by global-burden-of-disease region.

NOTE: More detailed information can be obtained from the author.

3,484.7 PYLL per 100,000 people, whereas North Africa and the Middle East had the lowest mortality burden, with 2.0 deaths and 117.2 PYLL per 100,000 people.

In 1990, alcohol-attributable injuries were responsible for 485,100 deaths (54,700 deaths among women and 430,400 deaths among men) and 21,934,800 PYLL (2,409,100 PYLL among women and 19,525,700 PYLL among men), equal to 9.2 deaths (2.1 deaths per 100,000 women and 16.1 deaths per 100,000 men) and 413.8 PYLL per 100,000 people (91.6 PYLL per 100,000 women and 731.3 PYLL per 100,000 men). The absolute number of alcohol-attributable injury deaths and PYLL and the number of alcohol-attributable injury deaths and PYLL per 100,000 people each increased from 1990 to 2010.

Appendix 1 presents the number and percentage of alcohol-attributable cancer, liver cirrhosis, and injury deaths and PYLL by GBD study region for 1990 and 2010. Appendix 2 presents the number of alcohol-attributable cancer, liver cirrhosis, and injury deaths per 100,000 people. Unlike figures 1, 2, and 3, the figures in Appendix 2 use the same scale for each cause of death.

Global Alcohol-Attributable Cancer, Liver Cirrhosis, and Injury Mortality As Part of Overall Mortality

In 2010, alcohol-attributable cancer, liver cirrhosis, and injury caused 1,500,000 deaths (319,500 deaths among women and 1,180,500 deaths among men). This represents 2.8 percent of all deaths (1.3 percent of all deaths among women and 4.1 percent of all deaths among men), or 21.8 deaths per 100,000 people (9.4 deaths per 100,000 women and 34.0 deaths per 100,000 men). In 1990, alcohol-attributable cancer, liver cirrhosis, and injury caused 1,101,400 deaths (250,800 deaths among women and 850,600 deaths among men), representing 20.8 deaths per 100,000 people (9.5 deaths per 100,000 women and 31.9 deaths per 100,000 men). The table outlines the mortality burden (measured in deaths and PYLL) of alcohol-attributable cancer, liver cirrhosis, and injury for 1990 and 2010 by age and by sex. Compared with the mortality burden in 1990, the absolute number of alcohol-attributable deaths from cancer, liver cirrhosis, and injury in 2010 is higher, and the rate of deaths per 100,000 also increased for men but decreased slightly for women in 2010.

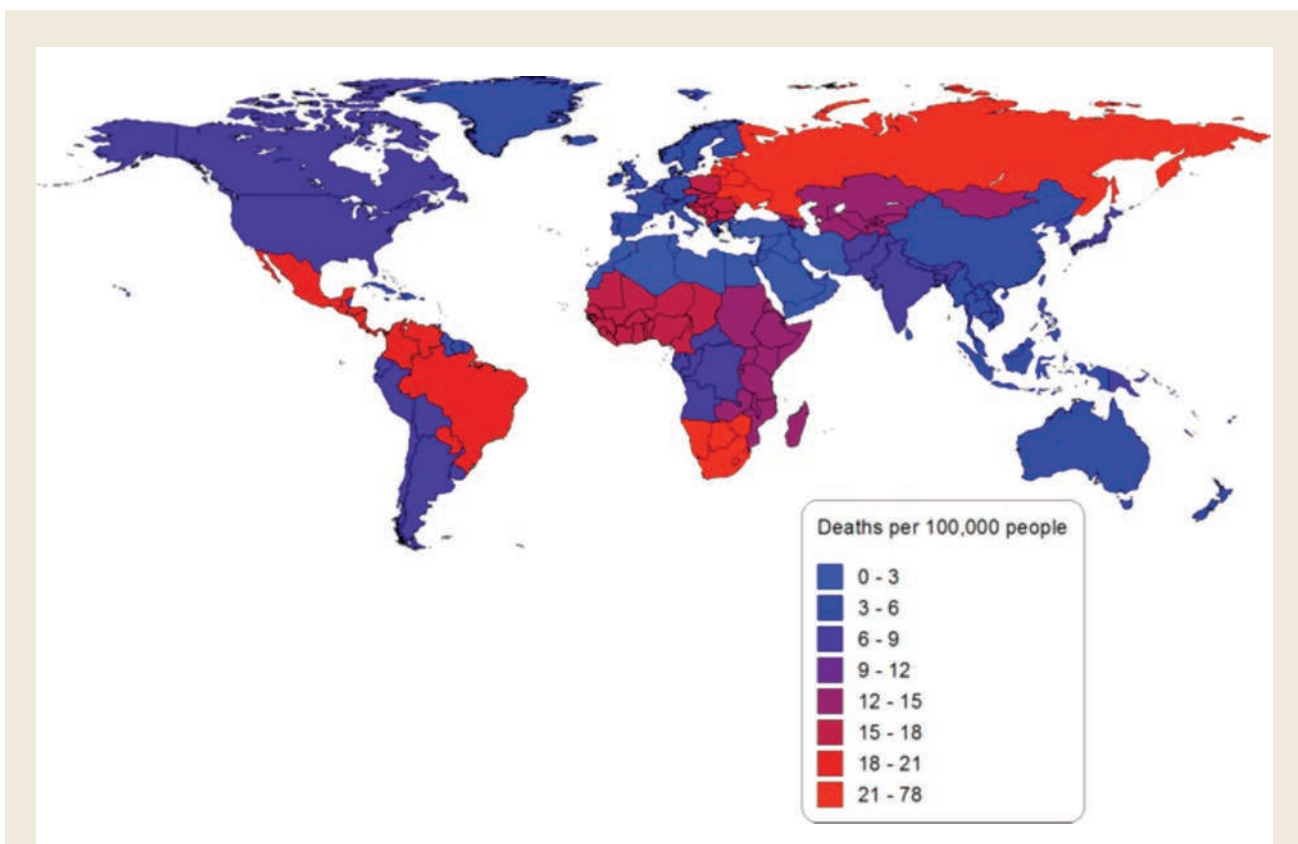


Figure 4 Alcohol-attributable injury deaths per 100,000 people in 2010 by global-burden-of-disease region.

NOTE: More detailed information can be obtained from the author.

The burden of mortality from alcohol-attributable cancer, liver cirrhosis, and injury led to 51,898,400 PYLL (9,214,300 PYLL among women and 42,684,100 PYLL among men) in 2010 and 39,246,800 PYLL (7,424,600 PYLL among women and 31,822,100 PYLL among men) in 1990. This mortality burden represents 3.0 percent (1.3 percent for women and 4.3 percent for men) of all PYLL in 2010 and 2.0 percent (0.9 percent for women and 2.9 percent for men) of all PYLL in 1990. In 2010, alcohol-attributable cancer, liver cirrhosis, and injury led to 753.4 PYLL per 100,000 people (269.8 PYLL per 100,000 women and 1,228.9 PYLL per 100,000 men) and to 740.4 PYLL per 100,000 people (282.2 PYLL per 100,000 women and 1,191.9 per 100,000 men) in 1990. Again, the overall rates of PYLL per 100,000 people increased, but this effect was attributed to increases for men, coupled with slight decreases for women.

Measurement Limitations

The methods used to estimate the number of alcohol-attributable cancer, liver cirrhosis, and injury deaths and PYLL have limitations as a result of the available data on mortality and the measurement of alcohol consumption and RRs. Most low- and middle-income countries do not have reliable mortality data and measurement of adult mortality in these countries (through verbal autopsies or surveys) is infrequent. Therefore, estimates of mortality and PYLL have a large degree of uncertainty (Wang et al. 2012). Additionally, for high-income countries, information concerning the cause of death has long been acknowledged as containing inaccuracies (James et al. 1955), and more recent studies have confirmed considerable degrees of error in this information (Nashelsky and Lawrence 2003; Shojania et al. 2003). To adjust for inaccuracies and inconsistencies in mortality data,

Table 1 Deaths and Years of Life Lost (YLL) From Cancer, Liver Cirrhosis, and Injuries Attributable to Alcohol Consumption in 1990 and 2010

Year	Gender	Age (Years)	Deaths	% Of All Deaths	YLL	% Of All YLL
1990	Women	0 to 15	4,000	0.1	324,400	0.1
		15 to 34	22,300	1.5	1,349,500	1.5
		35 to 64	128,700	2.9	4,437,000	3.0
		65+	95,800	1.0	1,313,800	1.1
		Total	250,800	1.2	7,424,600	0.9
	Men	0 to 15	6,700	0.1	540,400	0.1
		15 to 34	174,400	8.4	10,547,900	8.4
		35 to 64	502,600	7.4	18,167,100	7.8
		65+	166,800	1.8	2,566,700	2.0
		Total	850,600	3.4	31,822,100	2.9
Total	Total		1,101,400	2.4	39,246,800	2.0
2010	Women	0 to 15	3,800	0.1	313,800	0.1
		15 to 34	28,800	1.7	1,741,700	1.7
		35 to 64	162,000	3.1	5,570,800	3.1
		65+	124,800	0.9	1,587,900	1.1
		Total	319,500	1.3	9,214,300	1.3
	Men	0 to 15	6,100	0.1	492,400	0.1
		15 to 34	214,900	8.5	12,972,300	8.5
		35 to 64	709,200	7.9	25,549,800	8.2
		65+	250,300	1.9	3,669,500	2.2
		Total	1,180,500	4.1	42,684,100	4.3
Total	Total		1,500,000	2.8	51,898,400	3.0

NOTE: More detailed information can be obtained from the author.

the 2010 GBD study modelled the number of deaths mathematically (Wang et al. 2012).

Survey data measuring alcohol consumption, patterns of alcohol consumption, and the prevalence of lifetime abstainers, former drinkers, and current drinkers also are susceptible to numerous biases (Shield and Rehm 2012). To correct for the undercoverage that is observed when alcohol consumption is measured by population surveys (as compared with per capita consumption of alcohol), alcohol consumption was modelled by triangulating per capita and survey data (see above). Total alcohol consumption was set to 80 percent of per capita consumption in order to account for alcohol produced and/or sold, but not consumed, and to account for the undercoverage of the alcohol consumption typically observed in studies that calculate the alcohol RRs (Rehm et al. 2010*b*). Additionally, although alcohol was measured using adult per capita consumption and most people 14 years and younger do not consume alcohol or binge regularly, some adolescents 10 to 14 years of age report previously trying alcohol and previously being intoxicated (Windle et al. 2008).

The CRA was based on alcohol RR functions that typically were differentiated by sex and adjusted for age, smoking status, and other potentially confounding factors. The use of adjusted RR functions may introduce bias into the estimated number of deaths and PYLL that would not have occurred if no one had ever consumed alcohol (Flegal et al. 2006; Korn and Graubard 1999; Rockhill and Newman 1998). However, most of the published literature on alcohol-as-a-risk-factor-only reports adjusted RRs, and, thus, the use of unadjusted alcohol RRs for the CRA study would have led to imprecise estimates as a result of leaving out most of the studies. The bias of using adjusted RRs is likely to be small, as most analyses of the estimated RRs show no marked differences after adjustment for the potentially confounding factors and effect measure modifiers. Future CRA studies may require more complex modelling techniques for alcohol if other dimensions of alcohol consumption, such as irregular heavy-drinking occasions, impact RR estimates.

Finally, this analysis did not account for a lag time for the calculation methods used in this paper. This is especially a problem for cancer, which has a lag time of 15 to 20 years (Holmes et al. 2012; Rehm et al. 2007). In other words, the alcohol-attributable deaths and PYLL in 2010 actually are based on consumption patterns from 1990 to 1995, but in this paper were estimated based on consumption in 1990 and 2010. Although liver cirrhosis also is a chronic disease that develops over time like cancer (Rehm et al. 2013*a*), the impact of population-level consumption on liver cirrhosis deaths can be quite abrupt. For example, Gorbachev's anti-alcohol campaign was reflected in a clear reduction in Russia's liver cirrhosis mortality (Leon 1997). Likewise, the German seizure of alcohol in France in World War II led to reduced cirrhosis mortality (Zatonski et al. 2010). Most of the effect of alcohol consumption on liver cirrhosis probably is captured within 1 year (Holmes et al. 2012). For injury, with the exception of suicide, there is no noticeable lag time as

the risk of injury is associated with blood alcohol content (Taylor and Rehm 2012; see also Cherpitel 2013).

Implications of Alcohol-Attributable Mortality

In 1990 and in 2010, alcohol consumption had a huge impact on mortality. Regions such as Europe (especially Eastern Europe) and parts of Sub-Saharan Africa (especially south Sub-Saharan Africa) that have a high per capita consumption of alcohol and detrimental drinking patterns are more affected by alcohol consumption compared with other regions. It is important to note that the alcohol-attributable mortality burden is composed of two elements: AAF and the overall mortality burden of the respective disease. Accordingly, the observed overall increase from 1990 to 2010 in alcohol-attributable cancer, liver cirrhosis, and injury deaths and in PYLL can be attributed to two different sources: (1) an increase in the number of cancer, liver cirrhosis, and injury deaths (mainly attributed to increases of these deaths in low- to middle-income countries) (Lozano et al. 2012) and (2) an increase in alcohol consumption in low- to middle-income countries (Shield et al. 2013*b*).

Low- and middle-income countries have higher rates of alcohol-attributable mortality per 100,000 people, even though these countries typically have lower AAF (as their overall burden of mortality is higher). Economic wealth is correlated with overall mortality (Lozano et al. 2012), and, thus, the mortality burden per litre of alcohol consumed is highest in low-income countries, followed by middle-income countries (Rehm et al. 2009; Schmidt et al. 2010). It follows, therefore, that increases in the alcohol-attributable mortality burden in low- and middle-income countries attributed to economic growth may be able to be reduced or controlled for by implementing alcohol control policies such as taxation (Shield et al. 2011; Sorpaisarn et al. 2012*a, b*, 2013), bans on advertising, and restrictions on availability (Anderson et al. 2009; World Health Organization 2011*b*) preferably while maintaining the relatively high levels of abstinence in these countries.

The typical causes of death associated with alcohol use disorders are liver cirrhosis and injuries, (i.e., exactly the categories described in this article). Liver cirrhosis and injuries, and to a lesser degree cancer, may primarily be responsible for the high proportion of alcohol-attributable mortality explained by alcohol use disorders (Rehm et al. 2013*b*); however, additional research is required to empirically confirm this hypothesis. By increasing the rate of treatment for alcohol use disorders (Rehm et al. 2013*b*), the mortality burden of alcohol-attributable diseases also can be reduced. Recent research has shown that the mortality burden associated with alcohol use disorders, albeit high, has been underestimated (see Harris and Barraclough 1998 for the first meta-analysis; and Callaghan et al. 2012; Campos et al. 2011; Guitart et al. 2011; Hayes et al., 2011; Saieva et al. 2012; Tikkanen et al.

2009 for recent papers that observed a markedly higher mortality risk than in the first meta-analysis). ■

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Chronic Diseases and Conditions Related to Alcohol Use

Kevin D. Shield, M.H.Sc.; Charles Parry, Ph.D.; and Jürgen Rehm, Ph.D.

Alcohol consumption is a risk factor for many chronic diseases and conditions. The average volume of alcohol consumed, consumption patterns, and quality of the alcoholic beverages consumed likely have a causal impact on the mortality and morbidity related to chronic diseases and conditions. Twenty-five chronic disease and condition codes in the International Classification of Disease (ICD)-10 are entirely attributable to alcohol, and alcohol plays a component-risk role in certain cancers, other tumors, neuropsychiatric conditions, and numerous cardiovascular and digestive diseases. Furthermore, alcohol has both beneficial and detrimental impacts on diabetes, ischemic stroke, and ischemic heart disease, depending on the overall volume of alcohol consumed, and, in the case of ischemic diseases, consumption patterns. However, limitations exist to the methods used to calculate the relative risks and alcohol-attributable fractions. Furthermore, new studies and confounders may lead to additional diseases being causally linked to alcohol consumption, or may disprove the relationship between alcohol consumption and certain diseases that currently are considered to be causally linked. These limitations do not affect the conclusion that alcohol consumption significantly contributes to the burden of chronic diseases and conditions globally, and that this burden should be a target for intervention. **KEY WORDS: Alcohol consumption; alcohol use frequency; chronic diseases; disorders; mortality; morbidity; alcohol-attributable fractions (AAF); risk factors; relative risk; AOD-induced risk; cancers; neuropsychiatric disorders; cardiovascular diseases; digestive diseases; diabetes; ischemic stroke; ischemic heart disease; burden of disease**

Alcohol has been a part of human culture for all of recorded history, with almost all societies in which alcohol is consumed experiencing net health and social problems (McGovern 2009; Tramacere et al. 2012*b, c*). With the industrialization of alcohol production and the globalization of its marketing and promotion, alcohol consumption and its related harms have increased worldwide (see Alcohol Consumption Trends, in this issue). This has prompted the World Health Organization (WHO) to pass multiple resolutions to address this issue over the past few years, including the World Health Assembly's Global Strategy to

Reduce the Harmful Use of Alcohol, which was passed in May 2010. Of growing concern are noncommunicable chronic diseases and conditions that have been shown to contribute substantially to the alcohol-attributable burden of disease (Rehm et al. 2009). Specifically, in 2004 an estimated 35 million deaths and 603 million disability-adjusted life-years (DALYs) lost were caused by chronic diseases and conditions globally (WHO 2008); alcohol was responsible for 3.4 percent of the deaths and 2.4 percent of DALYs caused by these conditions (Parry et al. 2011). To address the burden of chronic diseases and conditions, the United Nation (UN) General Assembly passed Resolution 64/265 in May of 2010, calling for their prevention and control (UN 2010). This resolution is intended to garner multisectoral commitment and facilitate action on a global scale to address the fact that alcohol (together with tobacco, lack of exercise, and diet) plays a significant role in chronic diseases and conditions. It is noteworthy that cardiovascular diseases, cancers, and diabetes in particular have been highlighted for targeted action (UN 2010) because alcohol is a risk factor for many cardiovascular diseases and cancers and has both beneficial and detrimental effects on diabetes and ischemic cardiovascular diseases,¹ depending on the amount of alcohol consumed and the patterns of consumption.

Building on previous reviews concerning alcohol and disease (Rehm et al. 2003*a*, 2009), this article presents an up-to-date and in-depth overview of the relationship of alcohol consumption and high-risk drinking patterns and the initia-

¹ Ischemic cardiovascular diseases are those caused by a blockage of blood vessels, resulting in a loss of blood supply to the tissue serviced by the affected blood vessels.

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tion/exacerbation and treatment of various chronic diseases and conditions. It also assesses the methods used to calculate the impact of alcohol consumption on chronic diseases and conditions.

Alcohol Consumption As a Risk Factor for Chronic Diseases and Conditions

Figure 1 presents a conceptual model of the effects of alcohol consumption on morbidity and mortality and of the influence of both societal and demographic factors on alcohol consumption and alcohol-related harms resulting in chronic diseases and conditions (adapted from Rehm et al. 2010a). According to this model, two separate, but related, measures of alcohol consumption are responsible for most of the causal impact of alcohol on the burden of chronic diseases and conditions—overall volume of alcohol consumption and patterns of drinking. The overall volume of alcohol consumption plays a role in all alcohol-related diseases, whereas drinking patterns only affect ischemic cardiovascular diseases. In addition to the overall volume and pattern of consumption, the quality of the alcoholic beverages consumed also may influence mortality and morbidity from chronic diseases and conditions. However, this pathway is of less importance from a public health perspective (Lachenmeier and Rehm 2009; Lachenmeier et al. 2007) because it has a much smaller impact than the other two factors.

The effects of overall volume of alcohol consumed, consumption patterns, and quality of the alcoholic beverages consumed on mortality and morbidity from chronic diseases and conditions are mediated by three main mechanisms.

These include the following:

- The toxic and beneficial biochemical effects of beverage alcohol (i.e., ethanol) and other compounds found in alcoholic beverages;
- The consequences of intoxication; and
- The consequences of alcohol dependence.

These intermediate mechanisms have been reviewed in more detail by Rehm and colleagues (2003a).

Chronic Diseases and Conditions Related to Alcohol

Chronic Diseases and Conditions Entirely Attributable to Alcohol

Of the chronic diseases and conditions causally linked with alcohol consumption, many categories have names indicating that alcohol is a necessary cause—that is, that these particular diseases and conditions are 100 percent alcohol attributable. Of these, alcohol use disorders (AUDs)—that is, alcohol dependence and the harmful use of alcohol as defined by the *International Classification of Disease, Tenth Edition* (ICD-10)—certainly are the most important categories, but many other diseases and conditions also are entirely attributable to alcohol (see table 1).

Chronic Diseases and Conditions for Which Alcohol Is a Component Cause

Alcohol is a component cause for more than 200 other diseases and conditions with ICD-10 three-digit codes—that

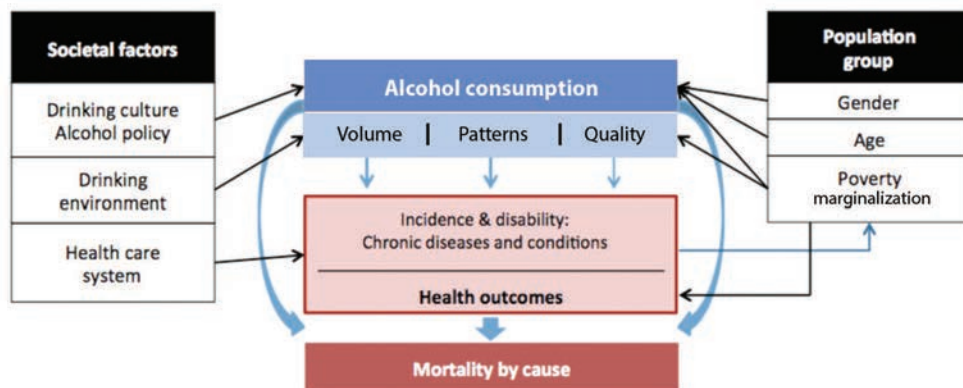


Figure 1 Causal model of alcohol consumption, intermediate mechanisms, and long-term consequences, as well as of the influence of societal and demographic factors on alcohol consumption and alcohol-related harms resulting in chronic diseases and conditions.

SOURCE: Adapted from Rehm et al. 2010a.

is, alcohol consumption is not necessary for the diseases to develop (Rehm et al. 2010a). For these conditions, alcohol shows a dose-response relationship, where the risk of onset of or death from the disease or condition depends on the total volume of alcohol consumed (Rehm et al. 2003a). Table 2 outlines these chronic diseases and conditions that are associated with alcohol consumption and lists the source of the relative risk (RR) functions if the chronic disease or condition is included as an alcohol-attributable harm in the 2005 Global Burden of Disease (GBD) Study.² Several of these chronic diseases and conditions are singled out for further discussion in the following sections to highlight alcohol's causative or protective role.

Specific Chronic Diseases and Conditions Associated With Alcohol Consumption

Malignant Neoplasms

The relationship between alcohol consumption and cancer already was suggested in the early 20th century, when Lamy (1910) observed that patients with cancer either of the esophagus or of the cardiac region were more likely to be alcoholics. The accumulation of evidence supporting the relationship between ethanol and cancers led the International Agency for Research on Cancer (IARC) to recognize the cancer-inducing potential (i.e., carcinogenicity) of ethanol in animal models and to conclude that alcoholic beverages are carcinogenic to humans (IARC 2008). Specifically, the GBD study found that alcohol increased the risk of cancers of the upper digestive track (i.e., mouth and oropharynx, esophagus, and larynx), the lower digestive track (i.e., colon, rectum, and liver), and the female breast (see figure 2). More up-to-date systematic reviews and meta-analyses on alcohol consumption and the risk of developing cancer have been published by Fedirko and colleagues (2011) for colorectal cancer, Islami and colleagues (2011) for esophageal squamous cell carcinoma, Islami and colleagues (2010) for laryngeal cancer, and Tramacere and colleagues (2010) and Turati and colleagues (2010) for oral and pharyngeal cancers.

A recent meta-analysis also has indicated that alcohol consumption is significantly linked to an increased risk of developing prostate cancer in a dose-dependent manner (Rota et al. 2012); this observation is consistent with previous meta-analyses concluding that alcohol consumption and the risk for prostate cancer are significantly correlated (Dennis 2000; Fillmore et al. 2009). Additional research, however, is required on the biological pathways to prove the role of alcohol consumption in the development of this type of cancer.

Evidence also has suggested that stomach cancer may be linked to ethanol consumption (Bagnardi et al. 2001; Tramacere et al. 2012a); however, the findings have not been unequivocal.

Thus, two recent meta-analyses found no association between alcohol drinking status (i.e., drinkers compared with non-drinkers) and risk of gastric cardia adenocarcinoma (Tramacere et al. 2012a, d). However, one meta-analysis did find an association between heavy alcohol consumption and the risk of this type of cancer (Tramacere et al. 2012a).

For several types of cancer investigators have found a nonsignificant positive association with alcohol consumption, including endometrial (Bagnardi et al. 2001; Rota et al. 2012), ovarian (Bagnardi et al. 2001), and pancreatic cancers (Bagnardi et al. 2001). However, because the relationship at least between alcohol consumption and endometrial and pancreatic cancer is modest (i.e., the point estimates of RR are low, even at high levels of average daily alcohol consumption), additional studies with large numbers of participants are needed to accurately assess the relationship (Bagnardi et al. 2001). The relationship between alcohol consumption and bladder and lung cancers is even less clear, with one meta-analysis finding that alcohol significantly increases the risk for both types of tumors (Bagnardi et al. 2001), whereas

Table 1 Chronic Diseases and Conditions That Are, by Definition, Alcohol Attributable (i.e., Require Alcohol Consumption As a Necessary Cause)

ICD-10 Code	Disease
F10	Mental and behavioral disorders attributed to the use of alcohol
F10.0	Acute intoxication
F10.1	Harmful use
F10.2	Dependence syndrome
F10.3	Withdrawal state
F10.4	Withdrawal state with delirium
F10.5	Psychotic disorder
F10.6	Amnesic syndrome
F10.7	Residual and late-onset psychotic disorder
F10.8	Other mental and behavioral disorders
F10.9	Unspecified mental and behavioral disorder
G31.2	Degeneration of nervous system attributed to alcohol
G62.1	Alcoholic polyneuropathy
G72.1	Alcoholic myopathy
I42.6	Alcoholic cardiomyopathy
K29.2	Alcoholic gastritis
K70	Alcoholic liver disease
K70.0	Alcoholic fatty liver
K70.1	Alcoholic hepatitis
K70.2	Alcoholic fibrosis and sclerosis of liver
K70.3	Alcoholic cirrhosis of liver
K70.4	Alcoholic hepatic failure
K70.9	Alcoholic liver disease, unspecified
K85.2	Alcohol-induced acute pancreatitis
K86.0	Alcohol-induced chronic pancreatitis
P04.3	Fetus and newborn affected by maternal use of alcohol
Q86.0	Fetal alcohol syndrome (dysmorphic)

² The GBD Study is a project that aims to provide a consistent and comparative description of the global burden of diseases and injuries and the risk factors that cause them.

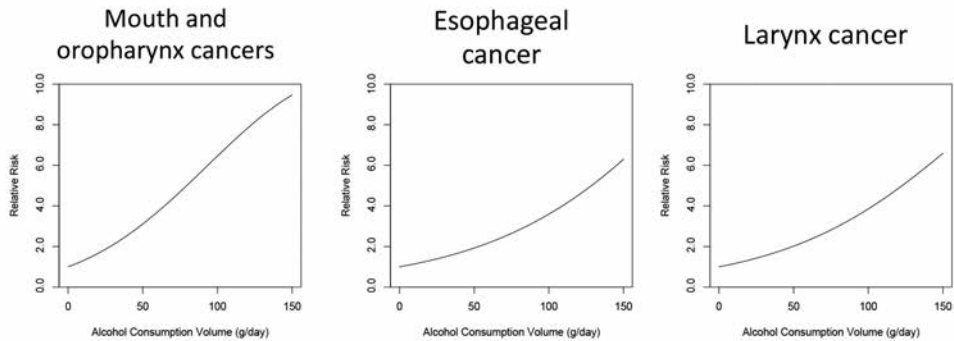
Table 2 Chronic Diseases and Conditions for Which Alcohol Consumption Is a Component Cause, Identified by Various Meta-Analyses and Reviews and Listed in the 2005 Global Burden of Disease (GBD) Study

No. of 2005 GBD Code	Disease	ICD-10	Effect	Level of Evidence Meta-Analysis	Used if Included in the GBD Study
IIA	Malignant neoplasms				
IIA1	Mouth cancer	C00–C08	Detrimental	Causally related	International Agency for Research on Cancer 2008 (based on relative risks from Corrao et al. 2004)
IIA2	Nasopharynx cancer and other pharynx cancers	C09–C13	Detrimental	Causally related	International Agency for Research on Cancer 2008 (based on relative risks from Corrao et al. 2004)
IIA3	Esophagus cancer	C15	Detrimental	Causally related	International Agency for Research on Cancer 2008 (based on relative risks from Corrao et al. 2004)
IIA4	Stomach cancer	C16	Detrimental	Insufficient causal evidence	
IIA5	Colon and rectum cancers	C18–C21	Detrimental	Causally related	International Agency for Research on Cancer 2008 (based on relative risks from Corrao et al. 2004)
IIA6	Liver cancer	C22	Detrimental	Causally related	International Agency for Research on Cancer 2008 (based on relative risks from Corrao et al. 2004)
IIA9	Larynx cancer	C32	Detrimental	Causally related	International Agency for Research on Cancer 2008 (based on relative risks from Corrao et al. 2004)
IIA10	Trachea, bronchus, and lung cancers	C33–C34	Detrimental	Insufficient causal evidence	
IIA13	Breast cancer (women only)	C50	Detrimental	Causally related	International Agency for Research on Cancer 2008 (based on relative risks from Corrao et al. 2004)
IIA16	Ovarian cancer	C56	Detrimental	Insufficient causal evidence	
IIA17	Prostate cancer	C61	Detrimental	Insufficient causal evidence	
IIA19	Kidney and other urinary organ cancers	C64–C66, C68 (except C68.9)	Beneficial (renal cell carcinoma only)	Insufficient causal evidence	
IIA23	Hodgkins lymphoma	C81	Beneficial	Insufficient causal evidence	
IIA24	Non-Hodgkins lymphoma	C82–C85, C96	Beneficial	Insufficient causal evidence	
IIB	Other neoplasms	D00–D48 (except D09.9, D37.9, D38.6, D39.9, D40.9, D41.9, 48.9)	Detrimental		

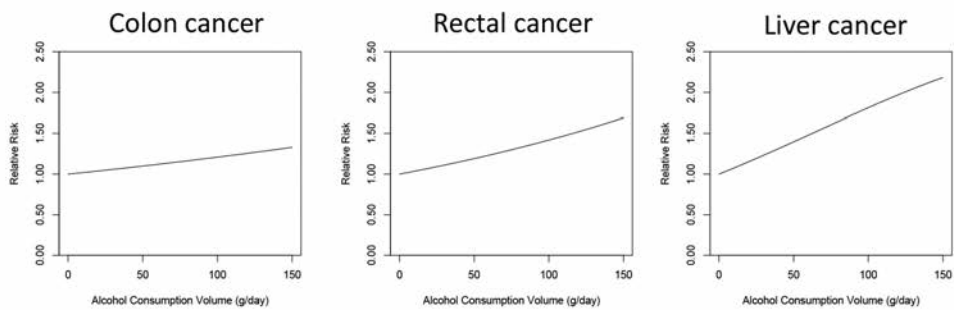
Table 2 Chronic Diseases and Conditions for Which Alcohol Consumption Is a Component Cause, Identified by Various Meta-Analyses and Reviews and Listed in the 2005 Global Burden of Disease (GBD) Study (Continued)

No. of 2005 GBD Code	Disease	ICD-10	Effect	Level of Evidence Meta-Analysis	Used if Included in the GBD Study
IIC	Diabetes	E10–E13	Beneficial (however, this depends on drinking patterns and volume of consumption)	Causally related	(Baliunas et al. 2009)
IIE	Mental and behavioral disorders				
IIE1	Unipolar depressive disorders	F32–F33, F34.1	Detrimental	Causally related	
IIF	Neurological conditions				
IIF1	Alzheimer’s disease and other dementias	F01–F03, G30–G31	Conflicting evidence (mainly beneficial)	Insufficient causal evidence	
IIF3	Epilepsy	G40–G41	Detrimental	Causally related	(Samokhvalov et al. 2010a)
IIH	Cardiovascular and circulatory diseases				
IIH2	Hypertensive heart disease	I11–I13	Detrimental (however, this depends on drinking patterns and volume of consumption)	Causally related	(Taylor et al. 2010)
IIH3	Ischemic heart disease	I20–I25	Beneficial (however, this depends on drinking patterns and volume of consumption)	Causally related	(Roerecke and Rehm 2010)
IIH4	Cerebrovascular diseases				
IIH4a	Ischemic stroke	I63–I67, I69.3	Beneficial (however, this depends on drinking patterns and volume of consumption)	Causally related	(Patra et al. 2010)

A. Neoplasms of the upper digestive tract



B. Neoplasms of the lower digestive tract



C. Other neoplasms

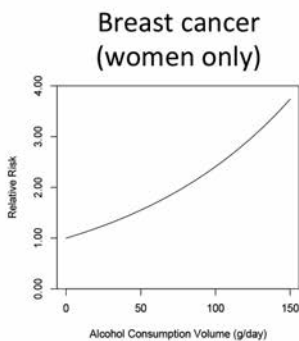


Figure 2 The relationship between increasing amounts of average daily alcohol consumption and the relative risk for cancer, with lifetime abstainers serving as the reference group.

SOURCE: Lim et al. 2012.

more recent meta-analyses have found no significant association between alcohol consumption and the risk of bladder cancer (Pelucchi et al. 2012) or the risk of lung cancer in individuals who had never smoked (Bagnardi et al. 2001). These conflicting results may stem from the studies in the more recent meta-analyses adjusting for smoking status when assessing the risk relationship between alcohol and these cancers within individual observational studies (Bagnardi et al. 2001; Pelucchi et al. 2012).

The biological pathways by which alcohol increases the risk of developing cancer depends on the targeted organ and are not yet fully understood. Factors that seem to play a role include the specific variants of alcohol-metabolizing enzymes (i.e., alcohol dehydrogenase [ADH], aldehyde dehydrogenase [ALDH], and cytochrome P450 2E1) a person carries or the concentrations of estrogen as well as changes in folate metabolism and DNA repair (Boffetta and Hashibe 2006). For example, a deficiency in ALDH2 activity in people carrying a gene variant (i.e., allele) called *ALDH2 Lys487* contributes to an elevated risk of esophageal cancer from alcohol consumption (Brooks et al. 2009). Because the *ALDH2 Lys487* allele is more prevalent in Asian populations (i.e., Japanese, Chinese, and Koreans) (Eng et al. 2007), and ALDH2 is hypothesized to impact the risk associated with alcohol for all cancers, studies should account for the presence of this allele when assessing the risk relationship between alcohol consumption and the development of any form of cancer.

However, it is important to note that alcohol not only increases the risk of cancer but also may lower the risk of certain types of cancer. For example, meta-analyses of observational studies have found that alcohol significantly decreases the risk of renal cell carcinoma (Bellocco et al. 2012; Song et al. 2012), Hodgkin's lymphoma (Tramacere et al. 2012c), and non-Hodgkin's lymphoma (Tramacere et al. 2012b). Alcohol's protective effect for renal cancer is thought to be mediated by an increase in insulin sensitivity, because light to moderate alcohol consumption has been associated with improved insulin sensitivity (Davies et al. 2002; Facchini et al. 1994; Joosten et al. 2008). Insulin resistance may play a key role in the development of renal cancer because people with diabetes, which is characterized by insulin resistance, have an increased risk of renal cancers (Joh et al. 2011; Lindblad et al. 1999). The mechanisms underlying alcohol's protective effect on the risk of developing Hodgkin's lymphoma and non-Hodgkin's lymphoma currently are unknown (Tramacere et al. 2012b, c). Thus, these observed protective effects should be interpreted with caution because the underlying biological mechanisms are not understood and confounding factors and/or misclassification of abstainers within observational studies may be responsible for these effects.

Diabetes

Research has found that moderate alcohol consumption is associated with a reduced risk of type 2 diabetes³ (Baliunas et al. 2009). Because development of insulin resistance is key in the pathogenesis of type 2 diabetes, it is thought that moder-

ate alcohol consumption protects against the disorder by increasing insulin sensitivity (Hendriks 2007). Such an alcohol-related increase in insulin sensitivity has been found in observational studies as well as in randomized controlled trials (Davies et al. 2002; Kiechl et al. 1996; Lazarus et al. 1997; Mayer et al. 1993; Sierksma et al. 2004). Alternative explanations for the protective effect of moderate alcohol consumption involve an increase in the levels of alcohol metabolites, such as acetaldehyde and acetate (Sarkola et al. 2002); an increase in high-density lipoprotein (HDL)⁴ (Rimm et al. 1999); and the anti-inflammatory effects of alcohol consumption (Imhof et al. 2001). It is important to note, however, that although there is reason to believe that alcohol consumption is causally linked to reduced risk of type 2 diabetes, it currently is unclear whether alcohol consumption itself is a protective factor or if moderate drinking is a marker for healthy lifestyle choices that may account for some of the observed protective effect.

Furthermore, the effects of alcohol consumption on risk of diabetes are dose dependent (see figure 3). Thus, in observational studies consumption of large amounts of alcohol has been related to an increased risk of type 2 diabetes because higher consumption levels may increase body weight, the concentrations of certain fats (i.e., triglycerides) in the blood, and blood pressure (Wannamethee and Shaper 2003; Wannamethee et al. 2003).

Neuropsychiatric Conditions

One of the neuropsychiatric conditions associated with alcohol consumption is epilepsy, which is defined as an enduring predisposition for epileptic seizures and requires the occurrence of at least one seizure for a diagnosis. Alcohol consumption is associated with epilepsy, whereas alcohol withdrawal can cause seizures but not epilepsy (Hillbom et al. 2003).⁵ Observational research has found that a consistent dose-response relationship exists between alcohol consumption and the risk of epilepsy (see figure 3). Multiple possible pathways may underlie this relationship. In particular, alcohol consumption may have a kindling effect, where repeated withdrawals from alcohol consumption by heavy drinkers may lower the threshold for inducing an epileptic episode (Ballenger and Post 1978). Alternatively, heavy alcohol consumption may increase the risk of epilepsy by causing shrinkage of brain tissue (i.e., cerebral atrophy) (Dam et al. 1985), cerebrovascular infarctions, lesions, head traumas, and changes in neurotransmitter systems and ionic balances

³ There are two main types of diabetes. Type 1 diabetes results from the body's failure to produce insulin, and patients therefore regularly must inject insulin. This type also is known as juvenile diabetes because of its early onset, or insulin-independent diabetes. Type 2 diabetes results from insulin resistance, which develops when the cells fail to respond properly to insulin. It develops with age and therefore also is referred to as adult-onset diabetes.

⁴ HDLs are certain types of compounds consisting of both fat (i.e., lipid) and protein components that are involved in cholesterol metabolism in the body. HDLs also are referred to as "good cholesterol."

⁵ Seizures are excluded from the 2005 GBD study definition of epilepsy.

(Barclay et al. 2008; Dam et al. 1985; Freedland and McMicken 1993; Rathlev et al. 2006).

Another neuropsychiatric disorder considered to be causally linked to alcohol consumption is unipolar depressive disorder. This association is supported by the temporal order of the two conditions, consistency of the findings, reversibility with abstinence, biological plausibility, and the identification of a dose-response relationship. One study determined the risk of depressive disorders to be increased two- to threefold in alcohol-dependent people (see Rehm and colleagues [2003a] for an examination of the causal criteria). The alcohol-attributable morbidity and mortality from unipolar depressive disorder currently cannot be calculated because the relationship may be confounded by several factors, including a genetic predisposition, environmental factors (i.e., an underlying disorder or environmental exposure that may contribute to both heavy alcohol use and depressive disorders), and potential self-medication with alcohol by individuals with unipolar depressive disorders (Grant and Pickering 1997; Rehm et al. 2004). Research findings suggest that all of these pathways may play a role. The pathways for the association between alcohol and unipolar depressive disorder in which alcohol does not play a causal role only affect the measurement of the alcohol-based RR for unipolar depressive disorder; however, they do not contradict the notion that alcohol is causally related to the development of unipolar depressive disorder via other pathways. This conclusion results from the observation that depressive symptoms increase markedly during heavy-drinking occasions and disappear or lessen during periods of abstinence (Rehm et al. 2003a).

Numerous studies also have examined the association between alcohol and Alzheimer's disease and vascular dementia.⁶ These analyses generally have determined a beneficial effect of alcohol, which has been attributed to alcohol's ability to

prevent ischemic events in the circulatory system (Peters et al. 2008; Tyas 2001). However, studies of these associations have generated highly heterogeneous results, and the design and statistical analyses of these studies make it impossible to rule out the potential effects of confounding factors (Panza et al. 2008; Peters et al. 2008).

Cardiovascular and Circulatory Diseases

Alcohol consumption affects multiple aspects of the cardiovascular system, with both harmful and protective effects. These include the following (figure 4):

- Increased risk of hypertension (at all consumption levels for men and at higher consumption levels for women);
- Increased risk of disorders that are caused by abnormalities in the generation and disruption of the electrical signals that coordinate the heart beat (i.e., conduction disorders and other dysrhythmias);
- Increased risk of cardiovascular disease, such as stroke caused by blockage of blood vessels in the brain (i.e., ischemic stroke) (at a higher volume of consumption) or rupture of blood vessels (i.e., hemorrhagic stroke); and
- Protective effects (at lower levels of consumption) against hypertension in women and against ischemic heart disease and ischemic stroke in both men and women.

⁶ Vascular dementia, the second most common form of dementia after Alzheimer's disease, is caused by problems in the supply of blood to the brain. Its most common symptoms include problems with thinking, concentration, and communication; depression and anxiety; physical weakness or paralysis; memory problems; seizures; and periods of severe confusion.

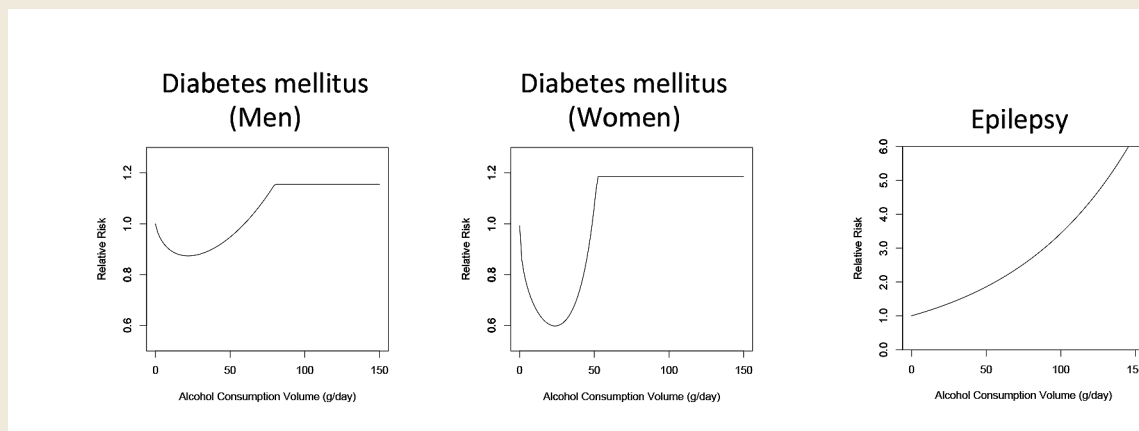
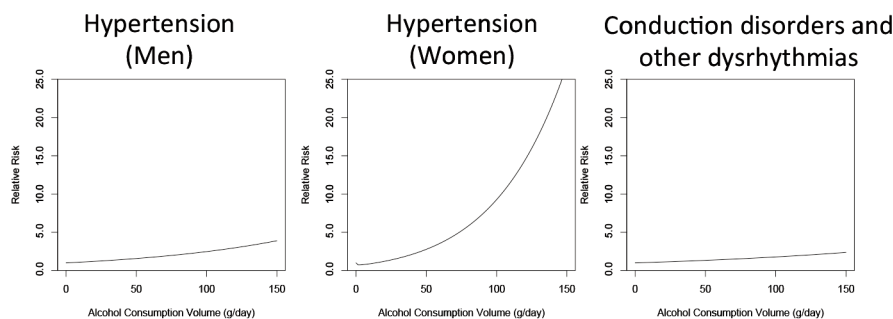


Figure 3 The relationship between increasing amounts of average daily alcohol consumption and the relative risk for diabetes and epilepsy, with lifetime abstainers serving as the reference group.

SOURCE: Lim et al. 2012.

A. Hypertension and conduction disorders



B. Cardiovascular diseases

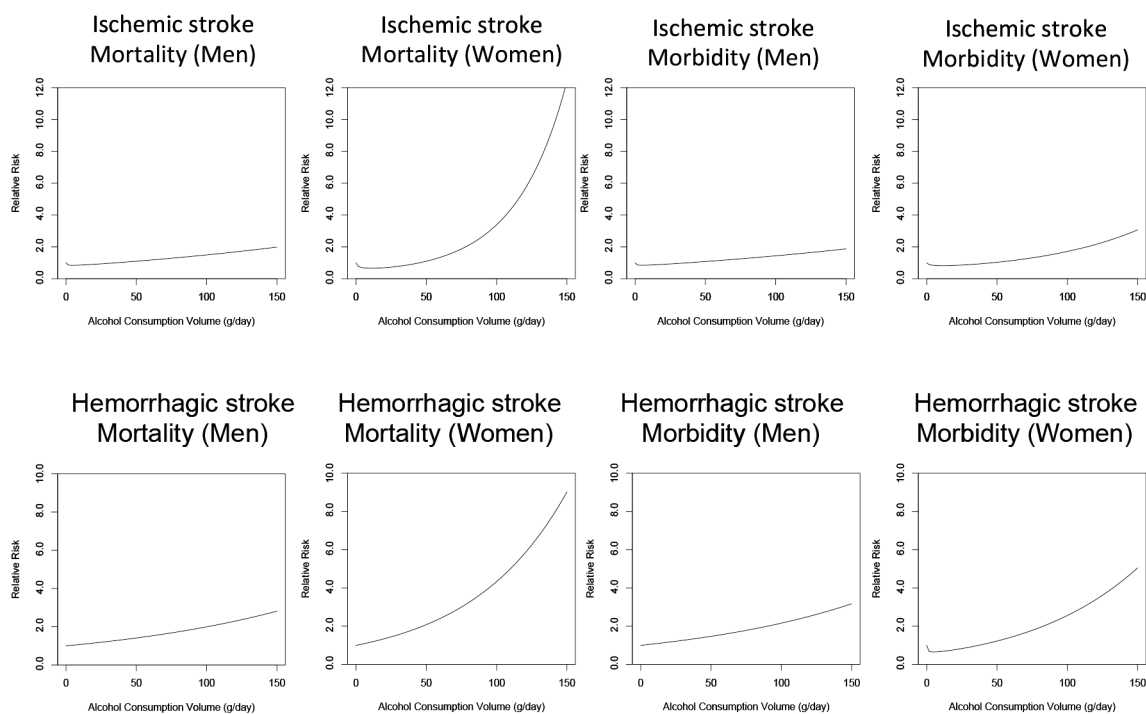


Figure 4 The relationship between increasing amounts of average daily alcohol consumption and the relative risk for cardiovascular diseases (i.e., hypertension, conduction disorders, and ischemic and hemorrhagic stroke), with lifetime abstainers serving as the reference group. For both hypertension and hemorrhagic and ischemic stroke, the relationship differs between men and women. Moreover, for both ischemic and hemorrhagic stroke, the influence of alcohol consumption on mortality is much greater than the influence on morbidity, at least in women. In men, no such difference appears to exist.

SOURCE: Lim et al. 2012.

The specific biological pathways through which alcohol consumption interacts with the cardiovascular system are not always clear, but several mechanisms have been identified that may play a role. These include increased blood concentrations of HDLs, effects on cellular signaling, decreased blood clot formation by platelets, and increased blood clot dissolution through enzyme action (Zakhari 1997). More indirect effects also may play a role. For example, alcohol may increase the risk of hypertension by enhancing the activity of the sympathetic nervous system, which results in greater constriction of the blood vessels and makes the heart contract more strongly. In addition, alcohol possibly decreases the sensitivity of the body's internal blood pressure sensors (i.e., baroreceptors), thereby diminishing its ability to regulate blood pressure.

Alcohol's protective effects against the risk of ischemic heart disease as well as against hypertension in women is hypothesized to result from its ability to increase HDL levels and/or reduce platelet aggregation on arterial walls. Differences in the effects of alcohol in men and women may stem from differing drinking patterns, with men more likely to engage in binge drinking, even at low average levels of consumption. These heavy-drinking occasions may lead to an increased risk of hypertension for men compared with women at similar alcohol consumption levels (Rehm et al. 2003*b*).

Alcohol's effect on hypertension also contributes to the risk of hemorrhagic stroke (Taylor et al. 2009), with a hypothesized dose-response effect. The mortality and morbidity from alcohol-attributable hemorrhagic stroke differ by sex (see figure 4). As with hypertension, differences in drinking pattern between men and women most likely are responsible for the differing RR functions for hemorrhagic stroke by sex. Three possible explanations have been put forth to explain the effects of drinking pattern on RR:

- Heavy drinkers also may have other comorbidities that may increase the probability of a fatal hemorrhagic stroke.
- Alcohol consumption may worsen the disease course through biological mechanisms and by decreasing compliance with medication regimens.
- Alcohol's effects on morbidity may be underestimated because of a stigmatization of heavy alcohol consumption in women, thereby potentially decreasing the probability that female heavy drinkers will be treated for stroke.

Large cohort studies and meta-analyses have shown that alcohol consumption leads to an increase in the risk for conduction disorders and dysrhythmias (Samokhvalov et al. 2010*b*). These effects are caused by changes in the electrical activity of the heart, including direct toxic effects of alcohol on the heart (i.e., cardiotoxicity), excessive activity of the sympathetic nervous system (i.e., hyperadrenergic activity) during drinking and withdrawal, impairment of the parasympathetic nervous system (i.e., of vagal tone), and increase of intra-atrial conduction time (Balbão et al. 2009).

Alcohol interacts with the ischemic system to decrease the risk of ischemic stroke and ischemic heart disease at low levels of consumption; however, this protective effect is not observed at higher levels of consumption. As mentioned above, alcohol exerts these effects mainly by increasing levels of HDL, preventing blood clots, and increasing the rate of breakdown of blood clots. However, binge drinking, even by light to moderate drinkers, leads to an increased risk of ischemic events by increasing the probability of clotting and abnormal contractions of the heart chambers (i.e., ventricular fibrillation). As with hemorrhagic stroke, alcohol has different effects on morbidity than on mortality related to ischemic events (see figure 5). Thus, meta-analyses of alcohol consumption and the risk of ischemic heart disease (Roerecke and Rehm 2012) and ischemic stroke (Taylor et al. 2009) found a larger protective effect for morbidity than for mortality related to these conditions. One possible explanation for this observation, in addition to those listed above for hemorrhagic stroke, is that patients in the morbidity studies may be younger at the time of the stroke than those in mortality studies. Despite the increased risk for ischemic heart disease at higher levels of alcohol consumption noted in observational studies (see Roerecke and Rehm 2012 for the most up-to-date meta-analysis), there was not enough evidence for a detrimental effect of alcohol consumption on ischemic heart disease for it to be modeled in the 2005 GBD study.

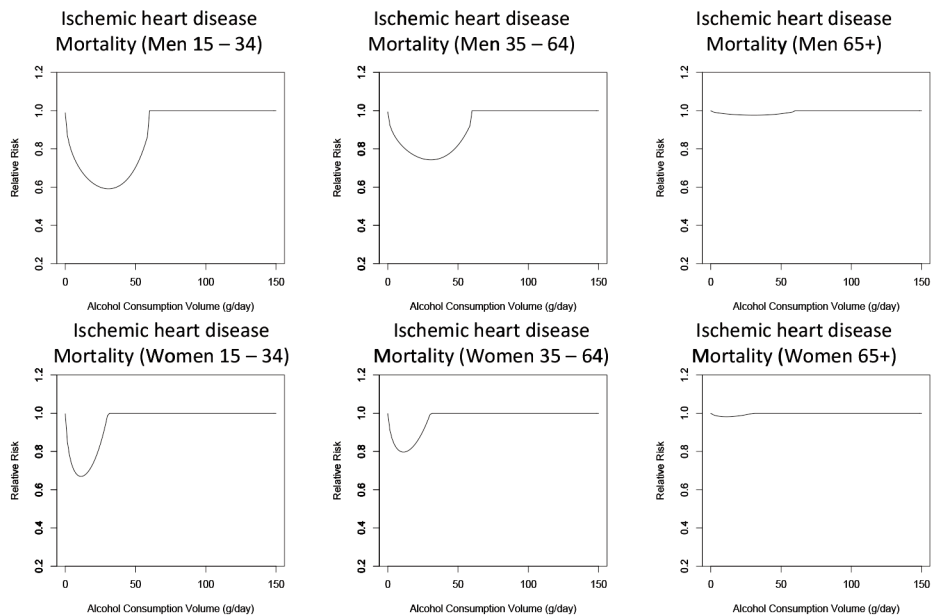
Moreover, the observational studies investigating the link between alcohol consumption and ischemic events had several methodological flaws, and the RR functions for ischemic events, especially ischemic heart disease, therefore are not well defined. A meta-analysis conducted by Roerecke and Rehm (2012) observed a substantial degree of heterogeneity among all consumption levels, pointing to a possible confounding effect of heavy drinking. In addition, previous observational studies have been limited by the inclusion of "sick quitters" in the reference groups, who have an increased risk of ischemic events compared with lifetime abstainers.

Digestive Diseases

Alcohol is associated with various liver diseases and is most strongly related to fatty liver, alcoholic hepatitis, and cirrhosis. The association between the risk of liver cirrhosis and alcohol consumption has long been recognized (see figure 6). The main biological mechanism contributing to this liver damage likely involves the breakdown of ethanol in the liver through oxidative and nonoxidative pathways that result in the production of free radicals, acetaldehyde, and fatty acid ethyl esters, which then damage liver cells (Tuma and Casey 2003). Given the same amount of alcohol consumption, alcohol increases the risk of mortality from liver cirrhosis more steeply than the risk of morbidity because it worsens the course of liver disease and has a detrimental effect on the immune system (Rehm et al. 2010*c*).

Alcohol consumption also has been linked to an increase in the risk for acute and chronic pancreatitis. Specifically, heavy alcohol consumption (i.e., more than, on average,

A. Ischemic heart disease - Mortality



B. Ischemic heart disease - Morbidity

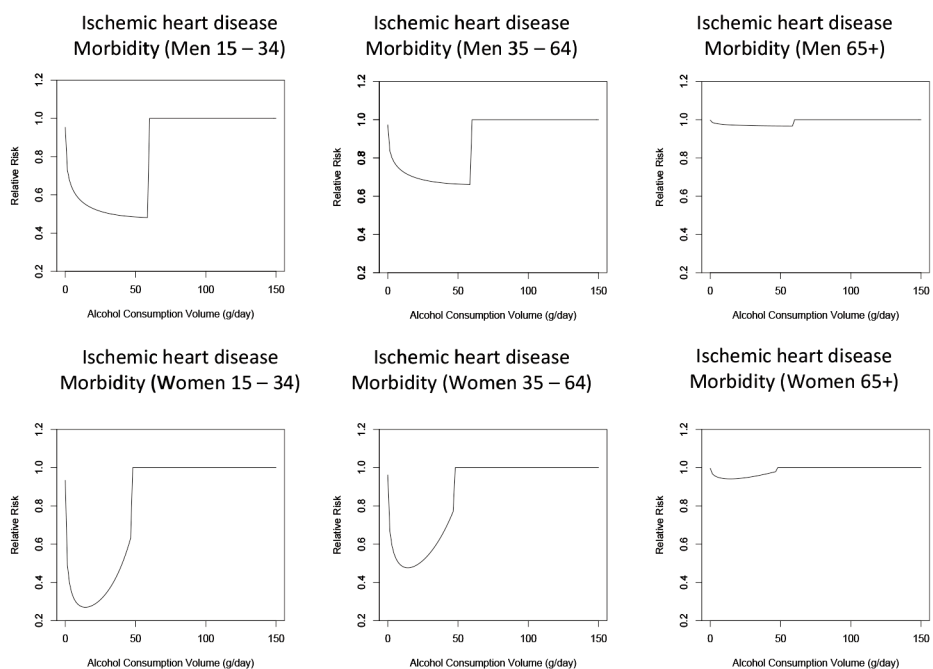


Figure 5 The relationship between increasing amounts of average daily alcohol consumption and the relative risk for ischemic heart disease, with lifetime abstainers serving as the reference group. Low to moderate alcohol consumption has a beneficial effect on both mortality and morbidity from ischemic heart disease. However, the specific effects depend on both the gender and the age of the drinker, with the greatest beneficial effects of low-to-moderate consumption seen on morbidity from ischemic heart disease in women ages 15 to 34.

SOURCE: Lim et al. 2012.

48 grams pure ethanol, or about two standard drinks, per day) leads to a noticeably elevated risk of pancreatitis, whereas consumption below 48 grams per day is associated with a small increase in risk of pancreatitis (see figure 6). Higher levels of alcohol consumption may affect the risk of pancreatitis through the same pathways that cause liver damage, namely the formation of free radicals, acetaldehyde, and fatty acid ethyl esters during the metabolism of alcohol in damaged pancreatic acinar cells (Vonlaufen et al. 2007).

Psoriasis

Psoriasis is a chronic inflammatory skin disease caused by the body's own immune system attacking certain cells in the body (i.e., an autoimmune reaction). Although there is insufficient biological evidence to indicate that alcohol is causally linked with psoriasis, many observational studies have determined a detrimental impact of drinking on psoriasis, especially in male patients. Alcohol is hypothesized to induce immune dysfunction that results in relative immunosuppression. In addition, alcohol may increase the production of inflammatory cytokines and cell cycle

activators, such as cyclin D1 and keratinocyte growth factor, that could lead to excessive multiplication of skin cells (i.e., epidermal hyperproliferation). Finally, alcohol may exacerbate disease progression by interfering with compliance with treatment regimens (Gupta et al. 1993; Zaghoul and Goodfield 2004).

Alcohol's Effects on Other Medication Regimens

Alcohol can affect cognitive capacity, leading to impaired judgment and a decreasing ability to remember important information, including when to take medications for other conditions (Braithwaite et al. 2008; Hendershot et al. 2009; Parsons et al. 2008). Although the relationship between alcohol consumption and adherence to treatment regimens mainly has been studied in regards to adherence to HIV antiretroviral treatment (Braithwaite and Bryant 2010; Hendershot et al. 2009; Neuman et al. 2012), research also has shown that alcohol consumption and alcohol misuse impact adherence to medications for other chronic diseases, with significant or almost-significant effects (Bates et al. 2010; Bryson et al. 2008; Coldham et al. 2002; Verdoux et

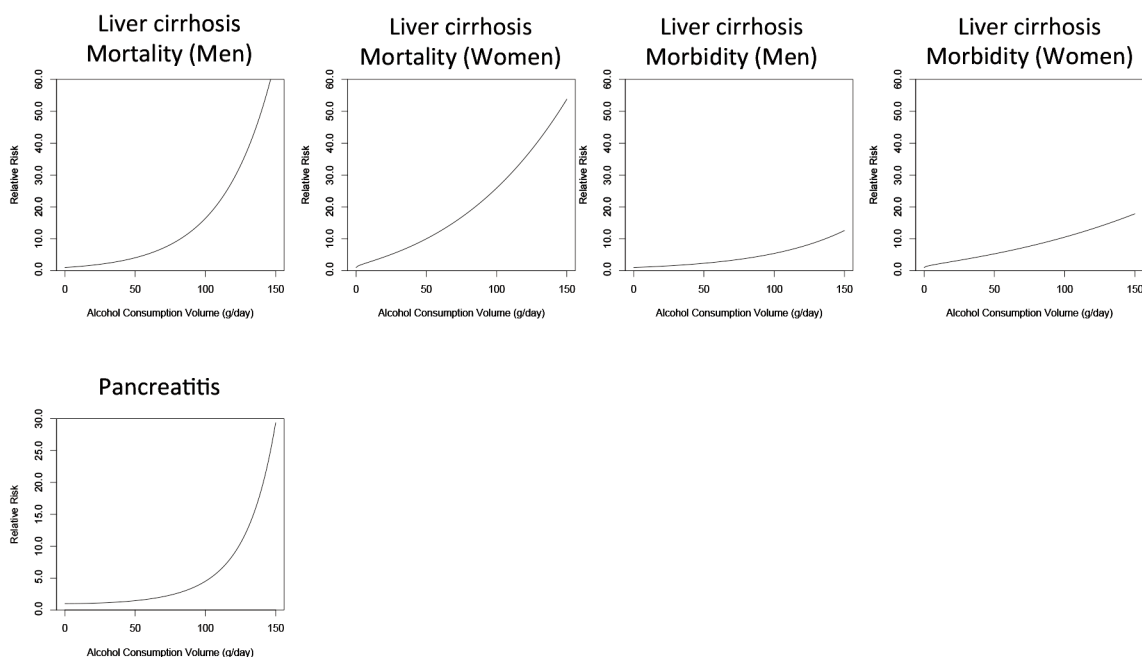


Figure 6 The relationship between increasing amounts of average daily alcohol consumption and the relative risk for digestive diseases (i.e., liver cirrhosis and pancreatitis), with lifetime abstainers serving as the reference group. For liver cirrhosis, alcohol's effects on mortality are greater than those on morbidity, and slight differences exist between the effects in men and women.

SOURCE: Lim et al. 2012.

al. 2000). Thus, for diseases or conditions managed by pharmacotherapy, alcohol consumption likely is associated with increased morbidity and even mortality (if nonadherence to the medication could be fatal) if drinking results in nonadherence to medication regimens.

Impact of Sex, Race, and Age on the Association of Alcohol Consumption with Chronic Diseases

Given the same amount of alcohol consumed, men and women can have differing morbidity and mortality from alcohol-related chronic disease and conditions. These differences may be related to the pharmacokinetics of alcohol in men and women. Women generally have a lower body water content than men with the same body weight, causing women to reach higher blood alcohol concentrations than men after drinking an equivalent amount of alcohol (Frezza et al. 1990; Taylor et al. 1996). Moreover, women appear to eliminate alcohol from the blood faster than do men, possibly because they have a higher liver volume per unit body mass (Kwo et al. 1998; Lieber 2000). In addition to these pharmacokinetic factors, hormonal differences also may play a role because at least in the case of liver disease, alcohol-attributable harm is modified by estrogen. However, hormonal influences on alcohol-related risks are not yet fully understood (Eagon 2010).

As noted previously, a deficiency of the ALDH2 enzyme in people carrying the *ALDH2 Lys487* allele contributes to an elevated risk of cancer from alcohol consumption. Because alcohol metabolism also plays a role in many other chronic diseases, the *ALDH2 Lys487* allele also may increase the risk for digestive diseases. The heterogeneity of risk caused by this allele, which is more prevalent in Asian populations, may lead to incorrect measurements of the risk for cancer and digestive disease outcomes in countries with a small Asian population, and will lead to incorrect results if the RRs from these countries are applied to Asian populations (Lewis and Smith 2005; Oze et al. 2011).

Because the pathology of alcohol-related ischemic heart disease is affected by the age of the drinker (Lazebnik et al. 2011), differences also may exist in the risk of ischemic heart disease in different age groups. Preliminary research assessing this issue across multiple studies has found that the association between alcohol consumption and the resulting risk for ischemic heart disease does indeed differ by age (see figure 5). However, no meta-analyses to date have investigated the effects of alcohol consumption on the risk of morbidity and mortality in different age groups for other chronic diseases and conditions. Accordingly, research is needed to assess if the varying relationship between alcohol consumption and ischemic heart disease in different age groups results from biological differences in pathology or from differences in drinking patterns. Additionally, research is needed to assess if age modifies the risk relationships between alcohol and other diseases.

Estimating the Alcohol-Attributable Fractions of Chronic Diseases and Conditions

When assessing the risk of chronic diseases and conditions that are related to alcohol consumption in some, but not all, cases, one of the variables frequently analyzed is the alcohol-attributable fraction (AAF)—that is, the proportion of cases that can be attributed to the patient's alcohol consumption. Because alcohol consumption can be modeled using a continuous distribution (Kehoe et al. 2012; Rehm et al. 2010b), the calculation of the alcohol-attributable burden of disease uses a continuous RR function.⁷ Thus, the AAFs for chronic diseases and conditions can be calculated using the following formula:

$$AAF = \frac{P_{abs} + P_{form}RR_{form} + \int_{+0}^{max} P(x)RR(x) dx - 1}{P_{abs} + P_{form}RR_{form} + \int_{+0}^{max} P(x)RR(x) dx}$$

In this formula, P_{abs} represents the prevalence of the disease among lifetime abstainers, P_{form} is the prevalence among former drinkers, RR_{form} is the relative risk for former drinkers, $P(x)$ is the prevalence among current drinkers with an average daily alcohol consumption of x , and $RR(x)$ is the relative risk for current drinkers with an average daily alcohol consumption of x . These AAFs vary greatly depending on alcohol exposure levels. (For examples of AAFs and information on the calculation of the 95 percent confidence intervals for chronic diseases and conditions see Gmel and colleagues [2011]).

Limitations of RR Functions for Chronic Diseases and Conditions

The chronic disease RR functions outlined in figures 2 to 6 are derived from the most up-to-date and rigorous meta-analyses in which the risk of a disease (i.e., for mortality and, where possible, morbidity) was provided by alcohol consumption as a continuous function (for more details on the meta-analysis methods used in each study, see the original articles cited in table 2). However, the RR functions and the relationship between alcohol consumption and the risk of chronic diseases and conditions are biased by multiple factors. First, the RRs can be limited by poor measurement of alcohol exposure, outcomes, and confounders. Research on alcohol consumption patterns and disease is scarce, and only few studies have investigated the effects of drinking patterns on chronic diseases and conditions. Thus, the chronic disease and condition RRs presented in this article may be confounded by drinking patterns, which are correlated to overall volume of alcohol consumption. Additionally, the volume of alcohol consumed generally is poorly measured, with many medical epidemiology studies measuring alcohol consumption only at baseline. As a result, these analyses do not include measures

⁷ The exception to this approach is tuberculosis because only data on categorical alcohol exposure risks are available.

of the volume of alcohol consumed during the medically relevant time period, which may encompass several years. For example, in the case of cancer, the cumulative effects of alcohol may take many years before an outcome is observed. Likewise, many of the larger cohort studies only use single-item, semi-quantitative food questionnaires that measure either frequency or volume of consumption.

Second, medical epidemiology studies typically suffer from poorly defined reference groups (Rehm et al. 2008). Thus, such studies typically only measure alcohol consumption at one point or during one time period in a participant's life and classify, for example, all people who do not consume alcohol during the reference period as abstainers, even though it is essential to separate ex-drinkers, lifetime abstainers, and very light drinkers. As a result, these measurements of alcohol consumption may lead to incorrect risk estimates because the groups of nondrinkers in these studies have heterogeneous risks for diseases (Shaper and Wannamethee 1998). The potential significance of this issue is underscored by previous research indicating that more than 50 percent of those participants who identified themselves as lifetime abstainers in medical epidemiology studies also had reported lifetime drinking in previous surveys (Rehm et al. 2008).

Third, chronic disease and condition outcomes in medical epidemiology studies also frequently are poorly measured, most often by means of self-reporting. Additionally, other confounding factors, such as relevant, non-substance use-related confounders, often are not controlled for.

Fourth, RR estimates for chronic diseases and conditions resulting from alcohol consumption frequently are hampered by weak study designs that base estimates of alcohol-related risks on nonexperimental designs (i.e., case-control and cohort studies). These study designs are limited by factors that cannot be controlled for and which may lead to incorrect results. For example, experimental studies on the effects of antioxidants have failed to confirm the protective effects of such agents found in observational studies (Bjelakovic et al. 2008). Furthermore, the sampling methodology of many of the cohort studies that were used in the meta-analyses for the above-presented RRs is problematic, especially when studying the effects of alcohol consumption. Many of the cohorts in these studies were from high-income countries and were chosen based on maximizing follow-up rates. Although the chosen cohorts exhibited variation in average daily alcohol consumption, little variation was observed in drinking patterns and other potential moderating lifestyle factors.

The overall effect of these limitations on the RRs and AAFs, and on the estimated burden of mortality and morbidity calculated using these RRs, currently is unclear. In order to investigate the effect of these biases, studies should be undertaken that combine better exposure measures of alcohol consumption with state-of-the-art outcome measures in countries at all levels of economic development. These studies are important, not only for understanding the etiology of alcohol-related chronic diseases and conditions, but also for formulating prevention measures (Stockwell et al. 1997).

Limitations of AAFs for Chronic Diseases and Conditions

In most studies assessing AAFs for chronic diseases and conditions, the AAF for an outcome is calculated as if the health consequences of alcohol consumption are immediate. Indeed, for most chronic diseases and conditions, including even diseases such as cirrhosis, a large degree of the effects caused by changes in alcohol consumption can be seen immediately at the population level (Holmes et al. 2011; Leon et al. 1997; Zatonski et al. 2010; for a general discussion see Norström and Skog 2001; Skog 1988). For cancer, however, the situation is different. The effects of alcohol consumption on the risk of cancer only can be seen after years, and often as long as two decades. Nevertheless, for the purpose of illustrating the entire alcohol-attributable burden of disease it is important to include cancer deaths, because they account for a substantial burden. For example, a recent large study found that in Europe 1 in 10 cancers in men and 1 in 33 cancers in women were alcohol related (Schütze et al. 2011). Therefore, in the interpretation of alcohol's effect on mortality and burden of disease in this article, the assumption that there has been uniform exposure to alcohol for at least the previous two decades must be kept in mind.

Another limitation to calculating the burden of chronic diseases and conditions attributable to alcohol consumption is the use of mainly unadjusted RRs to determine the AAFs. The RR formulas were developed for risks and were adjusted only for age (see Flegal et al. 2006; Korn and Graubard 1999; Rockhill and Newman 1998), although many other socio-demographic factors are linked with both alcohol consumption and alcohol-related harms (see figure 1). However, two arguments can be made to justify the use of mainly unadjusted RR formulas in the 2005 GBD study. First, in risk analysis studies (Ezzati et al. 2004) almost all of the underlying studies of the different risk factors only report unadjusted risks. Relying on adjusted risks would severely bias the estimated risk functions because only a small proportion of generally older studies could be included. Second, most of the analyses of alcohol and the risk of chronic diseases and conditions show no marked differences after adjustment (see Rehm et al. 2010*b*). However, the need for adjustment to the RRs may change when other dimensions of alcohol consumption, such as irregular heavy-drinking occasions, are considered with respect to ischemic heart disease.

Conclusions

There are limitations to the current ability to estimate the burden of chronic diseases and conditions attributable to alcohol consumption. The comparative risk assessment study within the GBD study only can determine this burden based on current knowledge of alcohol consumption and risk and mortality patterns at a global level. More detailed, country-specific estimates often are limited by the validity of the available consumption and mortality data. As more stud-

ies are published, it is likely that new confounders will be discovered for some of the relationships between alcohol consumption and various chronic diseases and conditions. The results from such new studies then may be used in meta-analyses of the effect of alcohol in diseases where alcohol only plays a small role, such as bladder, endometrial, and ovarian cancer. New studies also may lead to the recognition of a causal link between alcohol consumption and other diseases. Furthermore, new confounders and new studies may disprove the relationship between alcohol consumption and certain diseases that currently are considered to be causally linked.

Although there are limitations to the current methodology used to estimate the alcohol-attributable burden of chronic diseases and conditions, the limitations discussed in this article do not affect the overall conclusion that alcohol consumption is related to a considerable number of chronic diseases and conditions and contributes to a substantial amount of the global burden of chronic diseases and conditions. Therefore, alcohol consumption should be considered in developing intervention strategies aimed at reducing the burden of chronic diseases and conditions. ■

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Economic Costs of Excessive Alcohol Use

This brief sidebar summarizes recent findings on the economic costs of excessive alcohol use and highlights the primary methods used to estimate these costs. The total economic cost of excessive drinking in 2006,¹ including costs for health care, productivity losses, and costs such as property damage and alcohol-related crime, was estimated to be \$223.5 billion (see table) (Bouchery et al. 2011). Prior to this estimate, the last comprehensive analysis reported that the estimated economic costs of excessive drinking were \$148 billion in 1992 (Harwood et al. 1998). Data from that report were used to project a cost estimate of \$185 billion for 1998² (Harwood 2000). For a review of the global burden of alcohol use, see Rehm and colleagues (2009). For a broader examination of both the methods used to reach the current estimates and details on each of the estimated costs, as well as analysis of the significance and limitations of the study, see Bouchery and colleagues (2011, 2013).

Researchers used updated data, new data sources, and new measurement tools to develop the 2006 estimate. For example, the Alcohol-Related Disease Impact (ARDI) software,³ created by the Centers for Disease Control and Prevention, was used to assess the relationship between excessive drinking and various health and social outcomes. This software, originally released in 2004, was designed to allow researchers to calculate alcohol-attributable deaths, years of potential life lost, direct health care costs, indirect morbidity and mortality costs, and non-health-sector costs associated with alcohol misuse.

How the Costs of Excessive Alcohol Use Are Estimated

As in the 1992 and 1998 reports of estimated costs, the most recent report was based on the “cost-of-illness” approach. That is, costs for health care, productivity losses, and other expenses for 2006 were obtained from national databases, and alcohol-attributable fractions (AAFs) were applied to assess the proportion of

costs that could be attributed to excessive alcohol consumption.⁴ For example, researchers would consult a national health care database to determine the cost of liver cirrhosis and multiply this cost by a reasonable AAF to determine the proportion of the total cost that can be attributed to excessive alcohol use. Likewise, costs of lost productivity, crime, and other consequences of excessive alcohol use are estimated based on informa-

Table Total Economic Costs of Excessive Alcohol Consumption in the United States, 2006

Cost Category	Total Cost
Health Care Costs	
Alcohol Abuse and Dependence	\$10,668.457
Primary Diagnoses Attributable to Alcohol	8,526.822
Inpatient Hospital	5,115.568
Physician Office and Hospital Ambulatory Care	1,195.946
Nursing Home Care	1,002.888
Retail Pharmacy and Other Health Professional	1,212.420
Fetal Alcohol Syndrome	2,538.004
Other Health System Costs	2,822.308
Prevention and Research	1,207.120
Training	29.527
Health Insurance Administration	1,585.660
Total Health Care Costs	\$24,555.591
Productivity Losses	
Impaired Productivity	\$83,695.036
Traditional Earnings	74,101.827
Household Productivity	5,355.629
Absenteeism	4,237.580
Institutionalization/Hospitalization	2,053.308
Mortality	65,062.211
Incarcerations	6,328.915
Victims of Crime	2,092.886
Fetal Alcohol Syndrome	2,053.748
Total Productivity Losses	\$161,286.103
Other Effects on Society	
Crime Victim Property Damage	\$439.766
Criminal Justice System	20,972.690
Motor Vehicle Crashes	13,718.406
Fire Losses	2,137.300
FAS Special Education	368.768
Total Other Effects	\$37,636.930
Total	\$223,478.624

¹ The most recent year for which data were available.

² The estimates given for 1992 and 1998 have not been adjusted for inflation.

³ The software is available online at: http://apps.nccd.cdc.gov/DACH_ARDI/Default/Default.aspx.

⁴ Although cost-of-illness studies remain a popular tool for estimating the impact of alcohol abuse, researchers have criticized such studies as an invalid measure of alcohol's impact on society. For a recent review of arguments against this approach, see Mäkelä (2012).

tion gathered from various national surveys and databases.⁵

Defining Excessive Alcohol Consumption

The recent report estimated the costs of excessive alcohol consumption, which includes binge drinking (four or more drinks per occasion for a woman and five or more drinks per occasion for a man), heavy drinking (more than one drink per day on average for a woman and more than two drinks per day on average for a man), any alcohol consumption by persons younger than 21, and any alcohol consumption by pregnant women. The surveys used generally ask respondents about the 30 days prior to the survey. The focus on excessive drinking, rather than alcohol use disorders, allows for a more comprehensive look at the health and social consequences that are associated with harmful drinking patterns, including a wide range of acute and chronic health problems, productivity losses due to absenteeism, and crimes committed while intoxicated. In some cases, a history of alcohol abuse or dependence was used as a specific indicator of excessive drinking (e.g., productivity losses based on lost earnings).

Alcohol-Attributable Fractions

AAFs, or the proportion of a condition or outcome that is attributed to excessive alcohol consumption, were used to estimate both health-related costs, including deaths and health care expenditures related to excessive drinking, and the costs of specific criminal offenses.

Crime-Specific AAFs. Certain alcohol-related crimes (e.g., driving under the influence of alcohol, public drunkenness, and liquor law violations) are fully attributed to alcohol. For other offenses, researchers estimated the proportion attributable to alcohol based on the percentage of offenders intoxicated at the time of their offense (according to self-reported alcohol-consumption data from surveys of inmates). For homicide, researchers used the AAF from ARDI because it takes into account drinking by both the perpetrator and the victim.

The Costs of Excessive Alcohol Use

Of the total estimated cost of excessive alcohol use for 2006, lost productivity represents 72.2 percent,⁶ health

care costs represent 11.0 percent, criminal justice system costs make up 9.4 percent, and other consequences make up 7.5 percent (see figure). ■

Acknowledgement

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⁵ For information on the main data sources used in the report, see Bouchery and colleagues (2011, 2013).

⁶ Although the methods used to estimate productivity losses attributed to premature mortality are consistent with previous cost-of-illness studies, alternative methods with greater support from economists (i.e., the so-called willingness-to-pay approach) would yield much larger cost estimates.

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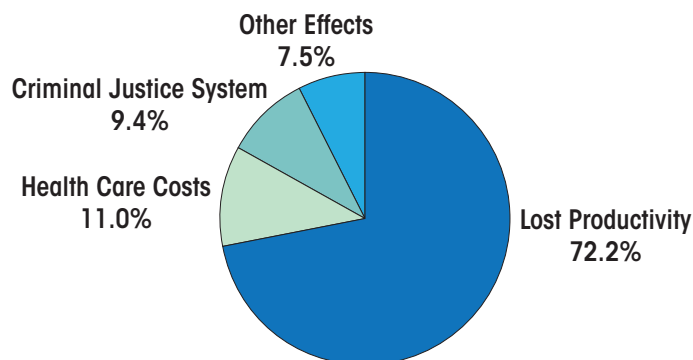
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Figure

Epigenetic Events in Liver Cancer Resulting From Alcoholic Liver Disease

Samuel W. French, M.D.

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Epigenetic mechanisms play an extensive role in the development of liver cancer (i.e., hepatocellular carcinoma [HCC]) associated with alcoholic liver disease (ALD) as well as in liver disease associated with other conditions. For example, epigenetic mechanisms, such as changes in the methylation and/or acetylation pattern of certain DNA regions or of the histone proteins around which the DNA is wrapped, contribute to the reversion of normal liver cells into progenitor and stem cells that can develop into HCC. Chronic exposure to beverage alcohol (i.e., ethanol) can induce all of these epigenetic changes. Thus, ethanol metabolism results in the formation of compounds that can cause changes in DNA methylation and interfere with other components of the normal processes regulating DNA methylation. Alcohol exposure also can alter histone acetylation/deacetylation and methylation patterns through a variety of mechanisms and signaling pathways. Alcohol also acts indirectly on another molecule called toll-like receptor 4 (TLR4) that is a key component in a crucial regulatory pathway in the cells and whose dysregulation is involved in the development of HCC. Finally, alcohol use regulates an epigenetic mechanism involving small molecules called miRNAs that control transcriptional events and the expression of genes important to ALD. **KEY WORDS:** Alcohol consumption; alcohol abuse; chronic alcohol use; alcoholic liver disease; ethanol metabolism; alcoholic liver disease; liver cancer; hepatocellular carcinoma; epigenetics; epigenetic mechanisms; DNA methylation; histone methylation; stem cells; micro RNAs

The molecular pathogenesis of liver cancer (i.e., hepatocellular carcinoma [HCC]) is a multistep process that involves both genetic changes, such as chromosomal abnormalities and mutations of the DNA sequence (i.e., somatic mutations), and epigenetic mechanisms, such as chemical modifications of the DNA and the histone proteins around which the DNA is wrapped to form the chromosomes, microRNA post-transcriptional regulators, and changes in various signaling pathways (Wong et al. 2010). This review will focus on the epigenetic phenomena that contribute to the pathogenesis of HCC resulting from alcoholic liver disease (ALD).

Does ALD Lead to HCC Formation?

According to some studies, ALD is the most common cause of HCC, accounting for approximately one-third of all HCC cases (Morgan et al. 2004). Chronic alcohol use of greater than 80 g/day (or approximately three standard drinks or more per day) for more than 10 years increases the risk for HCC approximately fivefold. In patients with decompensated alcoholic cirrhosis, in whom the liver damage is so extensive that the functional portions of the organ can no longer compensate for the damaged ones, the risk of developing HCC approaches 1 percent per year, and this

risk does not decrease with abstinence (Morgan et al. 2004). However, HCC also can occur in patients with noncirrhotic ALD. Finally, HCC is more likely to develop 1 to 10 years after the cessation of drinking by ALD patients. Therefore, HCC in these patients is not directly caused by alcohol consumption (Donato et al. 2002).

Alcohol abuse also has synergistic effects with other risk factors for the development of HCC, such as infection with hepatitis B virus (HBV) or hepatitis C virus (HCV), diabetes, and obesity (Hassan et al. 2002; Loomb et al. 2010; Morgan et al. 2004). For example, studies in Italy (Tagger et al. 1999) and the United States (Hassan et al.

2002) found that in patients with HCV infection, alcohol consumption over 80 g/day increased the odds ratio of developing HCC by 7.3 and 4.5, respectively. Likewise, a study conducted in Africa (Mohamed et al. 1992) determined a synergism between HBV and alcohol consumption over 80 g/day in the development of HCC (odds ratio of 4.4).

What Do ALD, HCV, and HBV Have in Common?

The livers of patients who develop HCCs commonly are cirrhotic. Moreover, they often contain molecules (i.e., markers) indicating that the cells undergo changes in their structure and function to a less specialized (i.e., less differentiated) state. These progenitor/stem cell markers mainly are found in the cirrhotic portion of the liver and in the regions where the HCC develops. These changes and markers have been observed in the livers of patients developing HCC associated with ALD, HBV, and HCV (Oliva et al. 2010). The reversion of normal liver cells (i.e., hepatocytes) into progenitor and stem cells is caused by epigenetic mechanisms. For example, during the development of the progenitor and stem cells, changes occur in the expression of several genes that result from the addition of too many or fewer-than-normal methyl groups to the DNA (i.e., DNA hyper- and hypomethylation, respectively). This alteration of methylation patterns results in an epigenetic reprogramming of the cells (Alison et al. 2009; Collas 2009; Iacobuzio-Donohue 2009; Ohm and Baylin 2009; Richly et al. 2010; Sasaki 2006; Sawan et al. 2008). In addition, modification (i.e., methylation and the addition of acetyl groups [acetylation]) of the histone proteins play roles in the epigenetic modification of progenitor and stem cells that underlies the transformation into cancer cells (i.e., a carcinoma) (Iacobuzio-Donohue 2009). Alcohol excess can induce all of these epigenetic changes that contribute to

the transformation of hepatocytes into progenitor or stem cells.

How Does Alcohol Generate Epigenetic Changes?

DNA Methylation

One step in the metabolism of beverage alcohol (i.e., ethanol) in the liver is the oxidation of ethanol by a molecule called cytochrome P450 2E1 (CYP2E1). During this reaction, highly reactive, oxygen-containing molecules (i.e., reactive oxygen species [ROS]) are generated (Bardag-Gorce et al. 2006). ROS are among the most potent agents and conditions that can alter methylation patterns in the liver, including DNA methylation. Thus, oxidative DNA damage caused by ROS, such as the formation of an abnormal variant of the DNA building block (i.e., nucleotide) deoxyguanine called 8-oxyguanine (8-OHdG), can result in a decrease in methylated DNA during DNA repair (Weitzman et al. 1994). 8-OHdG can be incorporated into DNA regions rich in the nucleotides cytosine and guanosine (i.e., CpG islands) in which the cytosine residues frequently are methylated. Incorporation of 8-OHdG into such CpG islands inhibits the methylation of adjacent cytosine residues by enzymes called methyl transferases, resulting in hypomethylation. Also, 8-OHdG formation can interfere with the normal function of DNA methyl transferases and prevent DNA re-methylation (Sagaki 2006). The relationship between alcohol, ROS formation, and DNA damage was demonstrated by studies in cultured liver cells (i.e., HepG2 cells) that were genetically modified to produce excessive levels of CYP2E1. When these cells were incubated with ethanol, ROS-induced DNA damage occurred as indicated by the formation of 8-OHdG (Bardag-Gorce 2006).

Ethanol also interferes with the metabolism of the amino acid methionine into a compound called S-adenosyl-

methionine (SAME) by several different methyl transferase reactions. SAME, in turn, is needed as the methyl-group donor for many methylation reactions and is converted into S-adenosyl-homocysteine (SAH). Ethanol inhibits methionine adenosyl transferase, which converts methionine into SAME, as well as enzymes that help regenerate methionine (i.e., betaine homocysteine methyltransferase and methionine synthase) (Seitz and Sticke 2007). This was shown in rodent models of ALD, where ethanol feeding decreased the SAME/SAH ratio in the liver (Esfandari et al. 2010). The net effect of all these alcohol-induced reductions in methyl transferase activity is to reduce the synthesis of SAME, which in turn leads to a decrease in DNA methylation.

The significance of reduced SAME production in the development of HCC is supported by findings that SAME feeding can inhibit tumor formation (Hitchler and Domann 2009). Furthermore, studies found that the SAME content and the SAME/SAH ratio were decreased in tissue regions that showed some damage but had not yet turned into cancer cells (i.e., in preneoplastic lesions). SAME feeding blocked the transformation of these preneoplastic lesions into HCCs because it promoted global DNA methylation. Moreover, SAME administration inhibited the expression of certain cancer-inducing genes (i.e., proto-oncogenes) called *c-myc*, *c-Ha-ras*, and *c-K-ras*, because the SAME supplementation allowed for the methylation (and thus blockage) of the regulatory regions (i.e., promoters) for those genes. The potential role of SAME in preventing tumor formation and survival also was supported by an in vitro study demonstrating that SAME decreased the survival of a type of liver cell tumor Hepa 1-6 in a dose-dependent manner (Oliva et al. 2012). Finally, SAME treatment prevented cultured liver tumor cells (i.e., H411e cells) from forming a tumor in a model of laboratory rats (Lu et al. 2009).

Histone Modifications

Histones, which exist in numerous variants, regulate gene expression, with the level of gene expression depending on the modifications that the histones undergo. These modifications may include methylation, acetylation, the addition of phosphate groups (i.e., phosphorylation), or the addition of a molecule called ubiquitin (i.e., ubiquitination). These modifications also can have an impact on tumor development. For example, the removal of acetyl groups (i.e., deacetylation) as well as hypermethylation is linked to the inactivation (i.e., silencing) of genes that can help repress tumor formation (i.e., tumor suppression genes) and as a result may promote tumor development (i.e., carcinogenesis). Thus, some cancers exhibit CpG island hypermethylation in combination with multiple histone modifications, such as deacetylation of histones H3 and H4, methylation of histone H3K9, trimethylation of histone H3K27, and a loss of trimethylation of histone H3K4 (Hamilton 2010).

Histone Acetylation. Alcohol exposure can alter histone acetylation

and methylation patterns. Researchers have investigated these effects in rats that chronically were fed alcohol through a tube into the stomach (i.e., intragastric tube feeding). These studies identified several alterations in histone methylation and acetylation that correlated with the changes seen in HCCs. Thus, alcohol-treated animals showed increased acetylation of histone H3K18 (Bardag-Gorce et al. 2009) and histone H3K9 (Bardag-Gorce et al. 2007). Furthermore, the levels of several proteins (i.e., phospho c-Jun, phospho AKT threonine 308, p38, pERK, and phospho-SAPK/JNK) in the nucleus of HCC cells were reduced whereas the nuclear levels of a molecule called β -catenin were increased. (For a list of the genes and proteins and their main functions, see table 1.) An increase in β -catenin in the nucleus of hepatocytes indicates activation of a signaling pathway, known as the canonical WNT/ β -catenin pathway,¹ that can be involved in tumor formation. This often is seen in HCCs related to ALD and HBV and HCV infection and leads to abnormal cell proliferation and survival (Hamilton 2010). The chronically alcohol-fed rats also had

increased levels of an enzyme called histone acetyltransferase (HAT) p300, which is responsible for histone acetylation (Bardag-Gorce et al. 2007). This increase could explain the increased histone H3K4 and H3K9 acetylation, which, in turn, globally activates gene expression (Bardag-Gorce 2009). Simultaneously, the levels of a deacetylase (i.e., SIRT1) also were increased in the alcohol-fed animals (Bardag-Gorce et al. 2007; 2009). This change was accompanied by alterations in the levels of several other molecules, including increases in RAR β and peroxisome proliferator-activated receptor (PPAR) C coactivator 1 α (PGC1 α) expression and a decrease in PPAR γ expression.

The increase in HAT p300 levels observed in chronically alcohol-fed rats also could lead to an increase in a signaling molecule called p21WAF1/C, p1 (p21) through several direct and

¹ Among other functions, the WNT/ β -catenin pathway is involved in the fate of stem cells and regulates whether stem cells proliferate or self-renew. Accordingly, there is a strong correlation between WNT/ β -catenin signaling and the onset of cancer. Normally, β -catenin cannot travel to the nucleus and is degraded. Under certain conditions, however, the degradation of β -catenin is prevented and it enters the nucleus. This can lead to excessive stem cell renewal and proliferation, predisposing the cells to the formation of tumors.

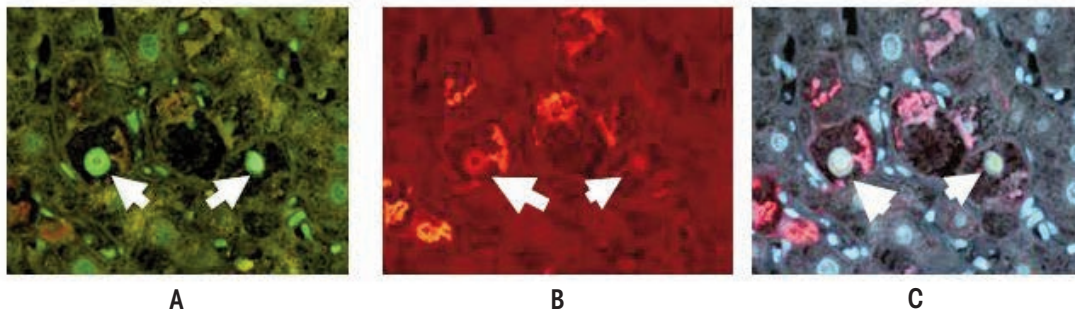


Figure 1 Histone deacetylase 1 (HDAC1) is upregulated in the nuclei of liver cells (i.e., hepatocytes) that form Mallory-Denk bodies (MDBs), which are indicative of liver damage. The image shown is from a liver biopsy from a patient with alcoholic hepatitis. The liver section was IHC double stained for HDAC1 (green nuclei arrows) (A), ubiquitin to identify cells with MDBs (red, arrows) (B), and tricolor (C). Magnification: $\times 350$.

indirect mechanisms² (Fang et al. 2007). p21 and a related protein called p27 are enzyme complexes that can mediate the phosphorylation of certain other proteins (i.e., protein kinase complexes) and which cause delays in the cycle progression at various stages of the cell cycle, thereby preventing the cells from dividing and multiplying normally. This leads to cell-cycle arrest, genetic instability, programmed cell death (i.e., apoptosis), and oncogenic effects (Abbas and Dutta 2011; Serres et al. 2011, 2012). p21 expression is regulated by histone acetylation, with greater acetylation promoting p21 expression. This process is regulated by a protein complex that is associated with the p21 promoter and which includes an enzyme called histone deacetylase-1 (HDAC1) that reduces acetylation (Dokmanovic et al. 2007) and, thus, p21 expression. Agents that promote acetylation by inhibiting deacetylation (i.e., HDAC inhibitors) accordingly also induce p21 expression, causing cell-cycle arrest (Dokmanovic et al. 2007; Gui et al. 2004). For this reason, HDAC inhibitors are used to treat cancers (Drummond et al. 2005). Liver cells that show signs of ALD—that is, which form Mallory-Denk bodies (MDBs)³—show increased HDAC1 levels in their nuclei compared with adjacent normal hepatocytes (see figure 1) (French et al. 2010). The HDAC inhibitor trichostatin A inhibited formation of MDBs in cell cultures from the livers of drug-primed mice (Oliva et al. 2008), indicating that these agents also may be able to prevent the development of liver disease.

The induction of p21 by alcohol abuse may explain why HCC more often only occurs after the patient has stopped drinking. As mentioned above, alcohol consumption induces p21 expression, causing the cell-cycle arrest. After prolonged abstinence, this induction no longer persists in the liver, eliminating the cell cycle arrest and promoting cell multiplication and, thus, tumor formation.

The role of p21 and p27 in HCC also is supported by studies showing

that both proteins are overexpressed in alcoholic hepatitis and in rats chronically fed ethanol (Crary and Albrecht 1998; French et al. 2012; Koteish et al. 2007). For example, immunohistochemical studies of liver samples from patients with alcoholic hepatitis found that many of the cells were positive for p27 (see figure 2), and additional analyses indicated that cell-cycle progression was blocked in these cells as indicated by low numbers of nuclei showing expression of (i.e., positive for) a protein called ki-67 (French et al. 2012). Another study demonstrated that both p21 and p27 overexpression inhibit the regeneration of the liver in rats whose liver had been partially removed (Koteish et al. 2007).

Histone Methylation. The levels of methylated histone H3K4 (H3K4me2), as well as histone H3K27 (H3K27me3), are increased in the nuclei of liver cells from rats fed ethanol intragastrically for 1 month (Bardag-Gorce et al. 2009), as demonstrated by intense nuclear staining in immunohistochemical analyses of liver samples. H3K4me2 is associated with active transcription, which seems to have beneficial effects. In particular, the

² Direct mechanisms would include HAT p300-induced histone acetylation as seen in human HCC cells, whereas indirect mechanisms could include the induction of a molecule called integrin β 17 through regulatory elements called Sp1 sites (Fang et al. 2007).

³ MDBs are inclusions found in the cytoplasm of liver cells and are indicative of liver damage; they are most commonly found in patients with ALD.

Table 1 Abbreviations of the Protein Names Mentioned in This Article, Their Full Names, and Their Main Functions

Acronym	Full Name	Function
DNACD133	Prominin 1	Cancer stem cell marker
CD49f	Integrin α 6	Cell adhesion Cell signaling
ERK	Extracellular signal-regulated kinase	Signaling pathway for growth of cells
EZH2	Enhancer of Zeste homology 2	Methylates DNA
MyD88	Myeloid differentiation response gene	Activates NF κ B
Nanog	Named after Tir NanOg legend	Stem cell renewal
Oct 4	Octamer-binding transcription factor 4	Self-renewal of embryonal cells
p21 Waf1/C.p1	Type of p21 Cip/kip family	Regulates the cell cycle
p27	Type of p27 member Cip/kip	Regulates the cell cycle
pERK	Phosphorylated ERK	Activated ERK
Phospho AKT Threonine 308	AKT-mouse forming thymomas	Regulates cell survival
Phospho cJun	Early response transcription factor	Activates cJun Stimulates cell growth
Phospho-SAPK/JNK	Stress-activated protein kinase Jun-amino kinase	Activates fetal liver formation
PPARGC1 α	PPAR γ coactivator 1 α	Regulates energy metabolism
PPAR γ	Peroxisome proliferator-activated receptor γ	Regulates fatty acid storage
RAR β	Retinoic acid receptor β	Regulates cellular growth
SOX 2	SRY (Sex determination region Y) box 2	Induces pluripotential cells
TLR4	Toll-like receptor 4	Innate immunity
β catenin	Cadherin associated protein	Wnt signaling pathway

combination of H3K4me2 with acetylated histone H3K18, which is seen in the alcohol-fed rats, would correlate with an improved prognosis in cancers. A loss of H3K4me2 impairs the body's ability to control DNA damage in cancer because it increases the risk of mutations and, consequently, cancer development (Lennartsson and Ekwall 2009).

Whereas histone acetylation is a highly dynamic process, modification of histones by methylation of one or more lysine amino acids (i.e., mono-, di-, and trimethylation) is thought to be a more lasting change that forms a "cellular memory." Methylation is performed by enzymes known as methyl transferases, and the activity of these enzymes may be specific to certain histones. The enzymes that generate persistent methylation patterns and other histone modifications are known as histone code writers.⁴ One such enzyme called EZH2 has intrinsic histone H3K27 methyl transferase activity; it assembles into a multiprotein complex called polycomb repressive complex 2 (PRC2) that, together with another protein complex (i.e., PRC1), maintains

a state of transcriptional repression and plays an important role in gene silencing (Muntean and Hess 2009). One role of EZH2/H3K27me3 is to target PRCs to sites of transcriptional regulation and DNA replication. The latter provides the means of perpetuating the characteristics (i.e., phenotypes) of the dividing cell to the daughter cells.

The gene-silencing pathway mediated by H3K27 methylation is linked to the second major silencing pathway (i.e., DNA methylation) via a deacetylase called SIRT 1 that is recruited by the PRC2 complex and contributes to gene silencing (Muntean and Hess 2009). SIRT 1 levels are increased in the alcohol intragastric tube-feeding rat model cited above. In contrast, when SIRT 1 activity is decreased, EZH2 levels increase, which enhances the EZH2-mediated repression of target genes (Lu et al. 2011). Upregulation of EZH2 expression in tumors appears to correlate with disease progression by maintaining a stem cell-like phenotype. Overexpression of EZH2 can lead to cancer progression mediated by deregulation of epigenetic mechanisms (Muntean and Hess 2009). However,

EZH2 levels do not seem to be affected by alcohol and other factors that can induce liver damage. For example, EZH2 levels were not changed in mice that exhibited a precursor stage to HCC (as characterized by the presence of balloon cells and MDBs) after drug treatment, in liver biopsies of patients with alcoholic hepatitis, or in MDB-forming HCCs (French et al. 2012) (see figure 3). However, in all three cases there were increases in a modified form of EZH2 (i.e., phosphorylated EZH2 [pEZH2]), which is degraded more rapidly in the cells than unmodified EZH2 and is located in the MDBs as demonstrated by immunohistochemistry. This degradation of pEZH2 occurs at cell components called proteasomes. However, proteasomes are inhibited by ethanol excess; as a result,

⁴ The term histone code refers to the hypothesis that modifications of different histones have different effects on gene expression—that is, whereas methylation of some histones activates gene expression, methylation of other histones may inhibit it. It is thought that the histone modifications serve to recruit other regulatory proteins that specifically recognize the modified histones. These recruited proteins then act to alter chromatin structure actively or to promote transcription. Enzymes that generate the histone modifications that make up this histone code are referred to as code writers.

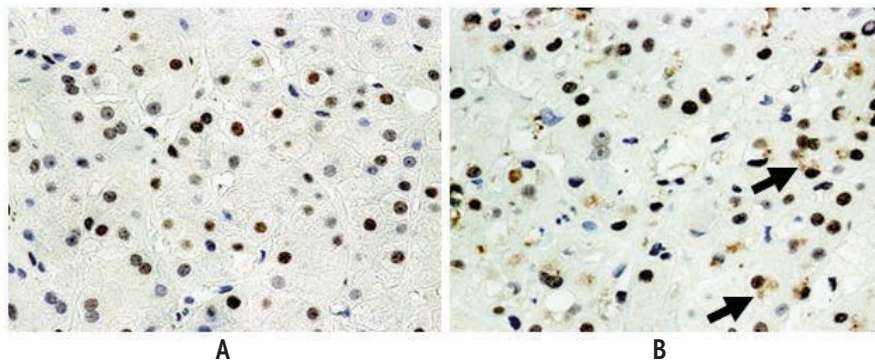


Figure 2 The signaling molecule p27 is upregulated in the nuclei of liver cells (i.e., hepatocytes) in a liver biopsy from two patients with alcoholic hepatitis. The livers were stained with an immunoperoxidase-labeled antibody that recognizes p27. The hepatocyte nuclei positive for p27 appear brown; those that are negative for p27 appear blue. (A and B) Most of the nuclei stained positive. (B) The Mallory-Denk bodies (MDBs) also stained brown (arrows), indicating that p27 also is sequestered in the MDBs. Magnification $\times 520$.

pEZH2 levels are increased in MDBs. Moreover, in all three cases, the levels of H3K27me3 were reduced in the nuclei of the damaged liver cells (i.e., cells that were ballooned or formed MDBs) compared with neighboring normal liver cells as shown by different experimental approaches (Bardag-Gorce et al. 2010; French et al. 2012). Paradoxically, when tumors form, they overexpress EZH2. High expression of EZH2 in tumors is associated with poor survival (Gieni and Hendzel 2009). Thus, EZH2 overexpression represses expression of the product of a tumor suppressor gene called E cadherin that causes cells to stick to each other. Accordingly, loss of E cadherin expression by tumor cells may cause loss of cell cohesion, which would promote metastasis and thus a more unfavorable prognosis.

How Are Stem Cells Converted to Cancer Stem Cells in ALD?

In individuals with HCC associated with ALD, focal progenitor cell/stem cell formation occurs both in portions of the liver that show cirrhosis and in the HCC cells as indicated by the expression of certain proteins (i.e., Nanog,

Yapi-1, Igf2bp, and Sox2) (see figure 4 A, B, C.). This raises the question whether the liver cells that are transformed into progenitor cells/stem cells in the cirrhotic liver subsequently are transformed in a second step into cancer stem cells in HCC. One of the regulatory molecules involved in this process is called Nanog. It is a transcription factor that is thought to play a crucial role in the self-renewal of embryonic stem cells and helps them maintain their ability to subsequently differentiate into numerous other cell types. Cancer stem cells can express both EZH2/H3k27me3 and Nanog, and the epigenetic balance between these factors determines the further fate of the cells. When the levels of Nanog are high and those of EZH2/H3K27me3 are low, the cells exhibit self-renewal activity—that is, they multiply and a tumor can develop. Paradoxically, when the reverse is true, the cancer stem cells differentiate into cells that no longer proliferate (Villasante et al. 2011). Also, EZH2-mediated epigenetic silencing of tumor suppressor genes leads to the activation of the WNT/ β -catenin signaling pathway mentioned earlier, which culminates in the proliferation of HCC cells (Cheng et al. 2011). EZH2 overex-

pression occurs in many different cancers, where it acts as a classical oncogene that can promote tumor formation by silencing several tumor suppressor genes, such as E cadherin. These suppressor genes play a role for both tumor cells and cancer stem cells (Crea 2011).

What Role Do Epigenetic Changes in TLR4 Play in Stem Cell Transformation?

Another molecule that is involved in the epigenetic mechanisms contributing to ALD-related HCC and which helps regulate the activity of Nanog is called toll-like receptor 4 (TLR4). Studies in a certain line of genetically modified mice (i.e., HCV core transgenic mice) that were chronically fed alcohol found that TLR4 activation leads to up regulation of Nanog in stem cells (Machida et al. 2009). This TLR4–Nanog pathway promotes the development of liver tumors induced by a variety of factors, including alcohol, diabetes, and HCV (Machida et al. 2012). The activation of TLR4 is regulated both at the transcriptional level (i.e., via molecules called lipopolysaccharides [LPS]) and at the epigenetic level (i.e., via acetylation of histones and methylation of DNA). For instance,

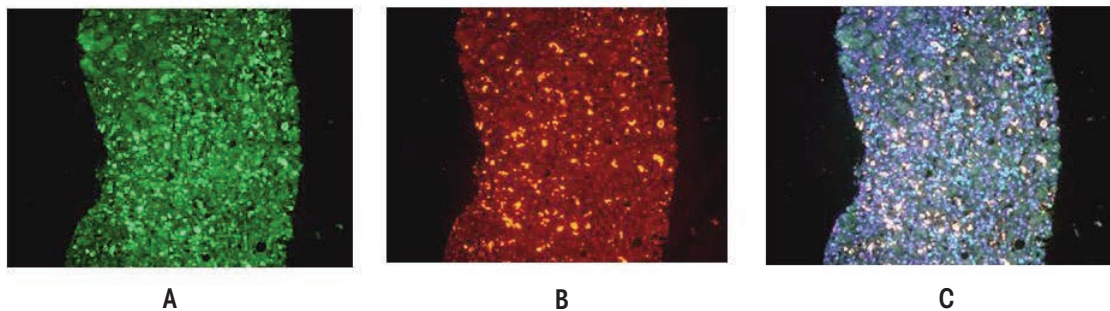


Figure 3 These images show a double-immunostained liver biopsy from a patient with alcoholic hepatitis where most of the hepatocytes had formed Mallory-Denk bodies (MDBs). The MDBs stained positive for (A) pEZH2 (green), (B) ubiquitin (red), and (C) merged (yellow), indicating that the pEZH2 colocalized in the MDBs. Magnification $\times 350$.

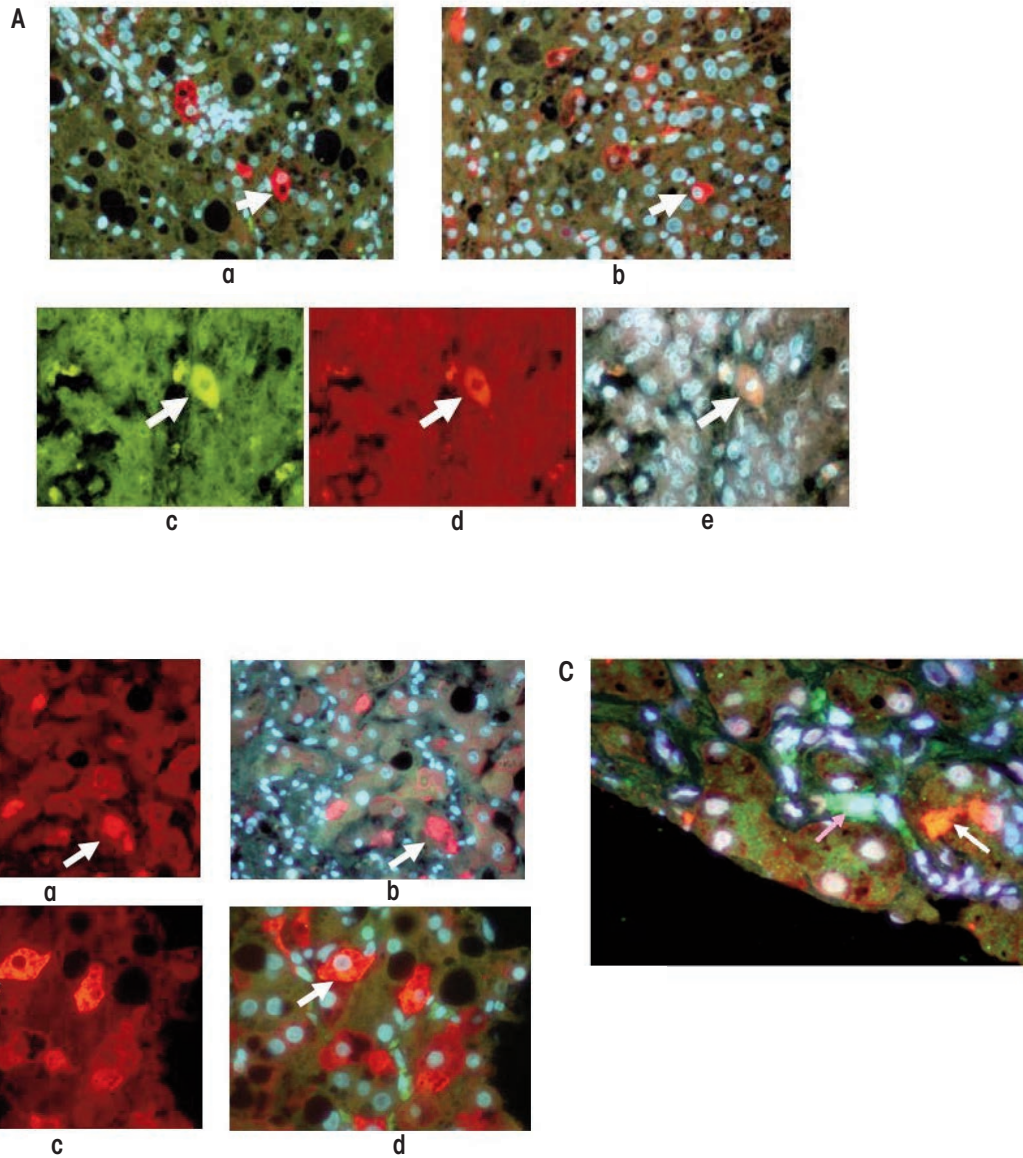


Figure 4 Analysis of different marker proteins in stem cell/progenitor cells located in the livers of patients with alcoholic liver disease with cirrhosis and associated hepatocellular carcinoma (HCC). **(A)** Liver cirrhosis and HCC samples stained for both YAP-1 (green) and IGF2bp3 (red). a) Cirrhosis; b) HCC (magnification $\times 350$); c) HCC; d) HCC; e) Tricolor image merged from c and d (magnification $\times 525$). **(B)**, a and b) Liver cirrhosis sample double stained for Nanog (green) and SOX2 (red). Note the Mallory-Denk bodies (MDBs) (arrow) stain positive for SOX 2. c and d) Liver cells stained for Yap 1 (green) and SOX 2 (red). The liver cells/progenitor cells stain positive for SOX2 (arrows). Magnification $\times 780$. **(C)** Liver sample from a patient with alcoholic hepatitis double stained for the Nanog protein (green) and ubiquitin (red). The stem cell stains positive for Nanog (pink arrow) and an MDB stained positive for ubiquitin (white arrow). Magnification $\times 780$.

increased methylation of regulatory DNA regions in front of the gene encoding TLR4 was found in embryonic stem cells. Moreover, increased methylation suppressed TLR promoter activity in reporter gene assays (Zampetaki et al. 2006). In addition, other assays (i.e., ChIP assays) in embryonic stem cells demonstrated that histones H3 and H4 had lower-than-normal acetylation levels (i.e., were hypoacetylated) in the TLR promoter region. Treatment with inhibitors of DNA methylation or deacetylase partially relieved repression of the TLR4 gene and increased its responsiveness to LPS (Zampetaki et al. 2006). The combined inhibition of DNA methylation and histone deacetylase activity leads to a robust induction of TLR4 with return of LPS responsiveness.

Rats fed ethanol intragastrically for 1 month had increased levels of TLR4 and another molecule called MyD88 in their livers. This effect could be prevented by feeding the animals SAME together with the alcohol, which, as mentioned earlier, is required for methylation. These findings indicate that methylation can prevent the alcohol-induced changes in TLR4 and MyD88 levels (Oliva et al. 2012). Similar changes in TLR4 expression and protein levels were found in mice that developed liver tumors after being fed a compound called diethyl 1,4-dihydro-2,4,6-trimethyl-3,5-pyridinedicarboxylate (DDC). Again, the changes could be prevented by also feeding the animals SAME (Bardag-Gorce et al. 2010). More detailed analyses determined reductions in the levels of H3k27me3 that also could be prevented by SAME feeding. These findings indicate that DDC feeding causes histone demethylation, which in turn results in increased TLR4 expression (Bardag-Gorce et al. 2010). In fact, the mice exhibited numerous epigenetic changes of histone methylation and acetylation (Bardag-Gorce et al. 2008), as well as DNA methylation of the gene encoding interleukin 12A (Oliva and French 2012).

Researchers also have studied the TLR4-Nanog pathway in another line

of genetically modified mice (i.e., HCV Ns5a transgenic mice) that were fed alcohol; under these conditions, liver tumors form in the animals that contain cancer stem cells (Machida et al. 2012). During this process, cells that normally differentiate into hepatocytes (i.e., hepatic stem cells) are transformed into tumor-initiating stem-like cells (TISCs), which then may develop further into cancer cells and cause tumor formation in other tissues. For example, TISC cells isolated from alcoholic patients induced tumor formation in cultured tissues (i.e., in vitro) and after transplantation into laboratory animals (i.e., in a xenograft model). The role of TLR4 and Nanog in this process was demonstrated by findings that when TLR4 or Nanog were silenced, the tumor-initiating properties of the TISCs were attenuated. Further studies found that Nanog upregulated the expression of two genes encoding molecules called Yap 1 and activator Igf2bp3, which in turn inhibited transforming growth factor- β signaling in the TISCs. Transforming growth factor- β signaling inhibits the growth of liver cells; accordingly, its inhibition would favor the proliferation of TISCs to form liver tumors. These observations suggest that TLR4 may be a universal proto-oncogene that is responsible for the development of TLR/Nanog-dependent TISCs. By staining tissue samples with specific markers researchers demonstrated that TISCs can be found in patients with cirrhosis and HCCs caused by alcoholism as well as by nonalcoholic hepatitis and HBV or HCV infection (Bardag-Gorce et al. 2008; French et al. 2011; Oliva et al. 2010) (figures 4A–C).

Machida and colleagues (2012) also found that TISCs isolated from the livers of alcohol-fed HCV Ns5a transgenic mice and from alcoholic patients carried molecules called CD133 and CD49f (i.e., were CD133⁺/CD49f⁺ cells). CD49f enhances the cell's ability to differentiate into different cell types (i.e., multipotency) and maintains the cells' stem-cell-like characteristics by directly controlling the regulatory

molecules OCT4 and SOX2 (Yu et al. 2012). In addition, CD49f activates a signaling pathway called the phosphatidylinositol 3-kinase (PI3K) AKT pathway and suppresses the levels of a protein called p53, which regulates the cell cycle and acts to prevent tumor formation (i.e., is a tumor suppressor gene). Immunohistochemical analyses of liver biopsies from patients with alcoholic hepatitis that contained numerous MDBs found that these cells expressed high levels of CD49f in the cytoplasm and the nuclei (see figure 5). This finding supports the concept that MDB-forming hepatocytes have progenitor and pluripotential properties and eventually may transform into TISC cells. Furthermore, in mice that were fed DDC and subsequently developed MDBs and, ultimately, HCC, CD49f, in combination with other molecules, induced MDB formation. This process could be blocked by inhibiting the phosphorylation of ERK and thus the activation of this protein as well as MDB formation (Wu et al. 2005).

What About MicroRNAs?

MicroRNAs (miRNAs) are a class of small noncoding RNAs that, in general, negatively regulate gene expression at the posttranscriptional level. Each miRNA controls a specific set of target genes. miRNAs have been identified in various tumor types, including HCCs. miRNAs also are encoded by specific genes in the DNA. miRNA genes that harbor CpG islands can undergo methylation-mediated silencing, similar to many tumor suppressor genes. As a result, the miRNAs are not produced and therefore cannot inhibit the expression of their target genes. In one study examining the expression of 11 miRNA genes in HCCs, three of those genes were silenced (i.e., those encoding miRNAs miR-124, miR-203, and miR-375) (Furuta et al. 2010). For miR-124 and miR-203, the methylation frequently was tumor specific and was not found in nontumor tissue.

Thus, these miRNAs were suppressive miRNAs for HCC that could be silenced epigenetically. This silencing resulted in the activation of multiple target genes (i.e., those encoding CDK6, vimentin, SET, and MYNO domain) (Furuta et al. 2010). Conversely, for other miRNAs the levels were increased in HCC, including miR-21, miR-34a, miR-221/222, miR-224, miR-106a, miR-92, miR-17-5 p, miR-20, and miR-18 (Braconi and Patel 2008; Murakami et al. 2006). Finally, one miRNA (i.e., miR-126) was specific to HCC and alcohol use (Ladeiro et al. 2008).

Studies have shown that alcohol use regulates miRNAs that control transcriptional events and the expression of genes important to ALD (Mandrekar 2011). Mice fed alcohol as part of a liquid diet showed decreases in the levels of 1 percent of the total known miRNAs and increases in the levels of 3 percent of the miRNAs (Dolaniuc et al. 2009). For example, the levels of miR-182, miR-183, and miR-199a-3P were decreased, whereas those of miR-705 and miR-122 were increased. So far the miRNAs associated with HCC do not overlap with those associated with experimental ALD. However, there is overlap of changes in miRNA expression

observed in mice fed a methyl-deficient diet for 12 weeks and those identified in HCCs (i.e., in both models the levels of miR-34a and miR-122 are changed) (Pogribny et al. 2009). In the methyl-deficient mice these miRNAs were associated with more extensive liver damage. These data mechanistically link alterations in microRNA expression to the pathogenesis of HCC and strongly suggest that differences in the susceptibility to liver carcinogenesis may be determined by differences in the miRNA expression response to factors such as methyl deficiency.

Summary

ALD is a major cause of HCC, which usually develops long after alcohol abuse has ceased and when cirrhosis has developed. This clinical pattern suggests that changes in epigenetic liver cellular memory occur that affect differentiation and cellular renewal, as well as the transformation to HCC. Progenitor hepatocytes develop during the cirrhotic process from normal cells through epigenetic mechanisms, such as changes in DNA hyper- or hypomethylation, histone acetylation and methylation, and epigenetic repro-

gramming. For example, oxidative DNA damage from ethanol-induced ROSs leads to loss of methylated DNA. Chronic ethanol feeding leads to altered methionine metabolism and reduced DNA methylation because the levels of the major methyl donor SAME are lowered. This process can be prevented by feeding SAME or another compound called betaine together with ethanol.

Likewise, chronic ethanol feeding alters the methylation and acetylation of histones in the liver. Histone acetylation leads to upregulation of p21, causing cell cycle arrest and DNA damage. This, in turn, results in loss of DNA methylation, as demonstrated in experimental rat models as well as in human alcoholic hepatitis and HCCs. Histone H3K27me₃, together with EZH2, regulates stem cell renewal and differentiation of progenitor stem cells in the liver. The balloon cells, which form MDBs in alcoholic hepatitis and HCCs, show a decrease in nuclear H3K27me₃ and an increase in pEZH2 in the MDBs. This supports the role of MDB-forming cells as progenitor cells that give rise to HCC transformation. This concept is supported by findings that the MDB-forming cells also express proteins that are markers of embryonal stem cells (i.e., SOX2 and

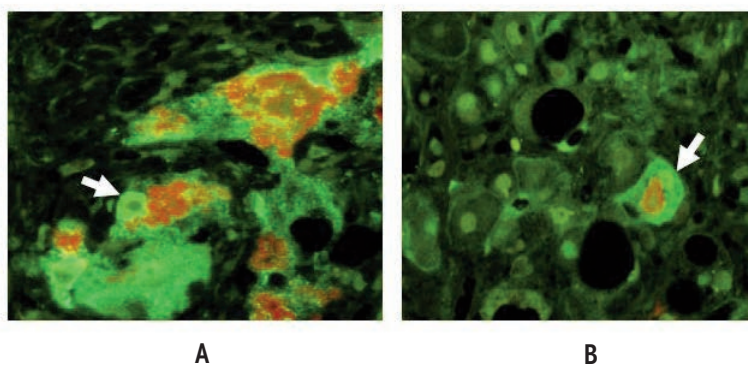


Figure 5 Immunohistochemical analysis of a liver biopsy obtained from a patient with alcoholic hepatitis with Mallory-Denk body (MDB) formation. The samples were stained for the presence of CD49f (integrin subunit $\alpha 6$) (green) and ubiquitin (red). Note that the MDBs stain both red for ubiquitin and green for CD49f. The arrows point to the nuclei that stain green except for the nucleolus. The yellow fringe on the MDB indicates colocalization of both proteins at the interface of the MDBs. The round black holes are macrovesicular fat globules in the hepatocytes. **A**) (magnification $\times 700$) shows a cluster of MDB-forming cells. **B**) (magnification $\times 1,050$) shows a single cell forming an MDB.

CD49f). The transformation of these progenitor cells to HCC is driven by the TLR4 signaling pathway, which is upregulated by increases in LPS levels in the liver that result from alcohol abuse. This upregulation of the TLR4 pathway, which has been demonstrated in rats chronically fed ethanol, can be prevented by SAME supplementation. These and other findings support the concept that TLR4 may be a proto-oncogene responsible for the transformation of progenitor cells into HCC in ALD as well as HCV infection. ■

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Epigenetic Effects of Ethanol on the Liver and Gastrointestinal System

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The widening web of epigenetic regulatory mechanisms also encompasses ethanol-induced changes in the gastrointestinal (GI)–hepatic system. In the past few years, increasing evidence has firmly established that alcohol modifies several epigenetic parameters in the GI tract and liver. The major pathways affected include DNA methylation, different site-specific modifications in histone proteins, and microRNAs. Ethanol metabolism, cell-signaling cascades, and oxidative stress have been implicated in these responses. Furthermore, ethanol-induced fatty liver (i.e., steatohepatitis) and progression of liver cancer (i.e., hepatic carcinoma) may be consequences of the altered epigenetics. Modification of gene and/or protein expression via epigenetic changes also may contribute to the cross-talk among the GI tract and the liver as well as to systemic changes involving other organs. Thus, epigenetic effects of ethanol may have a central role in the various pathophysiological responses induced by ethanol in multiple organs and mediated via the liver–GI axis.

KEY WORDS: Ethanol; ethanol metabolism; alcohol consumption; epigenetics; epigenetic effects; epigenetic mechanisms; alcohol-induced epigenetic alterations; liver; gastrointestinal system; immune system; DNA methylation; histone acetylation; microRNAs (miRNAs); cell-signaling; oxidative stress; alcoholic liver disease; steatohepatitis; liver cancer; hepatic carcinoma

Epigenetic modifications are emerging as important dynamic mechanisms contributing to both transient and sustained changes in gene expression. In some cases, epigenetic changes even can be inherited, although the mechanism for this remains elusive. Several types of epigenetic modifications have been studied in recent years. For example, several laboratories have actively examined modifications, of one end (i.e., the N-terminus) of the histone proteins around which the DNA is wrapped in the cell nucleus to form the chromatin. After their initial synthesis (i.e., after translation), histones can undergo a variety of modifications, such as acetylation, methylation, or phosphorylation, at different sites and under different conditions with diverse consequences. Another frequently studied type of epigenetic modification is the methylations of DNA at regions rich in cytosine and guanosine nucleotides

(i.e., CpG islands), which has been found to affect, for example, cancer genes. Small RNA molecules called micro-RNAs (miRNAs) that cause inhibition of the first step of gene expression (i.e., transcription) or degradation of RNA also are considered to be master regulators involved in the modification of gene expression in abnormal conditions or disease states. Furthermore, all of these epigenetic mechanisms are influenced by foreign substances to which the body is exposed (i.e., xenobiotics) and environmental conditions.

The accumulation of all these findings has led to a dramatic shift from a genetic to an epigenetic basis in the conceptual thinking about the causes of disease. This also applies to the causes underlying ethanol-induced conditions, and new developments particularly have highlighted the importance of epigenetic mechanisms in mediating ethanol's

actions in the liver and gastrointestinal (GI) tract (see figure 1). These developments are the focus of this review.

Alcohol-Induced Epigenetic Alterations in the Liver and GI Tract

Histone Acetylation, Methylation, and Phosphorylation

Evidence for the ethanol-induced epigenetic modifications of histone H3 first was obtained by Park and colleagues (2003) who demonstrated H3 acetylation in primary cultures of rat liver cells (i.e., hepatocytes). Other researchers subsequently determined that ethanol altered methylation of histone H3 at two lysine residues (i.e., lys-4 and lys-9) (Pal-Bhadra et al. 2007) and that phosphorylation of histone H3 at two serine residues (i.e.,

ser-10 and ser-28) was increased in ethanol-exposed hepatocytes (Lee and Shukla 2007). Additional studies have established that these changes occur not only in cultured hepatocytes but also in vivo in the liver and other organs (see Kim and Shukla 2006; Shukla and Aroor 2006) as well as in other liver cell types (e.g., hepatic stellate cells) (Kim and Shukla 2005). Alcohols other than ethanol that can be found as contaminants in adulterated alcoholic drinks also can modify histones (Choudhury et al. 2008). Finally, by interfering with single-carbon metabolism, ethanol may potentiate the epigenetic effects of toxins released by certain bacteria in the GI tract (i.e., lipopolysaccharide or endotoxin). These toxins promote methylation of histone H3 at lys-4 (Ara et al. 2008), which could in turn contribute to the progression of alcoholic liver disease (ALD).

The histone proteins form larger complexes called nucleosomes around which the DNA is wound in the cell

nucleus. Modifications at different sites in histone H3 (e.g., lys-4, lys-9, ser-10, ser-28, etc.) may occur on nucleosomes located in the same or different domains of the chromatin (James et al. 2012). These site-specific modifications, in turn, will be associated with changes in the expression of different genes with diverse effects. Thus, ethanol can influence an intricate network of epigenetic modifications.

It should be noted that although the observed global ethanol-induced changes in histone modifications suggest that they would result in large-scale, perhaps genome-wide, alterations in gene expression, epigenetic changes also can be limited to selected subsets of genes, depending to some degree on the method and mode of ethanol administration. Indeed, a gene-specific increase in H3K9 acetylation has been observed in rat liver in response to chronic ethanol feeding even in the absence of obvious global changes in histone acetylation (Park et al. 2012).

Ethanol-induced histone modification is associated with altered expression of several genes, including those encoding the ethanol-metabolizing enzyme alcohol dehydrogenase (ADH), the cancer-promoting gene (i.e., oncogene) *c-jun*, and the gene encoding a protein called plasminogen activator inhibitor 1 (PAI-1), which is involved in the dissolution of blood clots and in various diseases (e.g., fibrosis and certain types of cancer) (see table 1).

Changes in miRNAs

miRNAs are RNA molecules that do not serve as templates for protein production but have regulatory functions (for more information on miRNAs, see the article by Balamaran et al., pp. 18–24). To date, hundreds of miRNAs have been identified (Miranda et al. 2010) whose expression may be altered by various stimuli and as a result of changes in internal or environmental conditions. For example, chronic

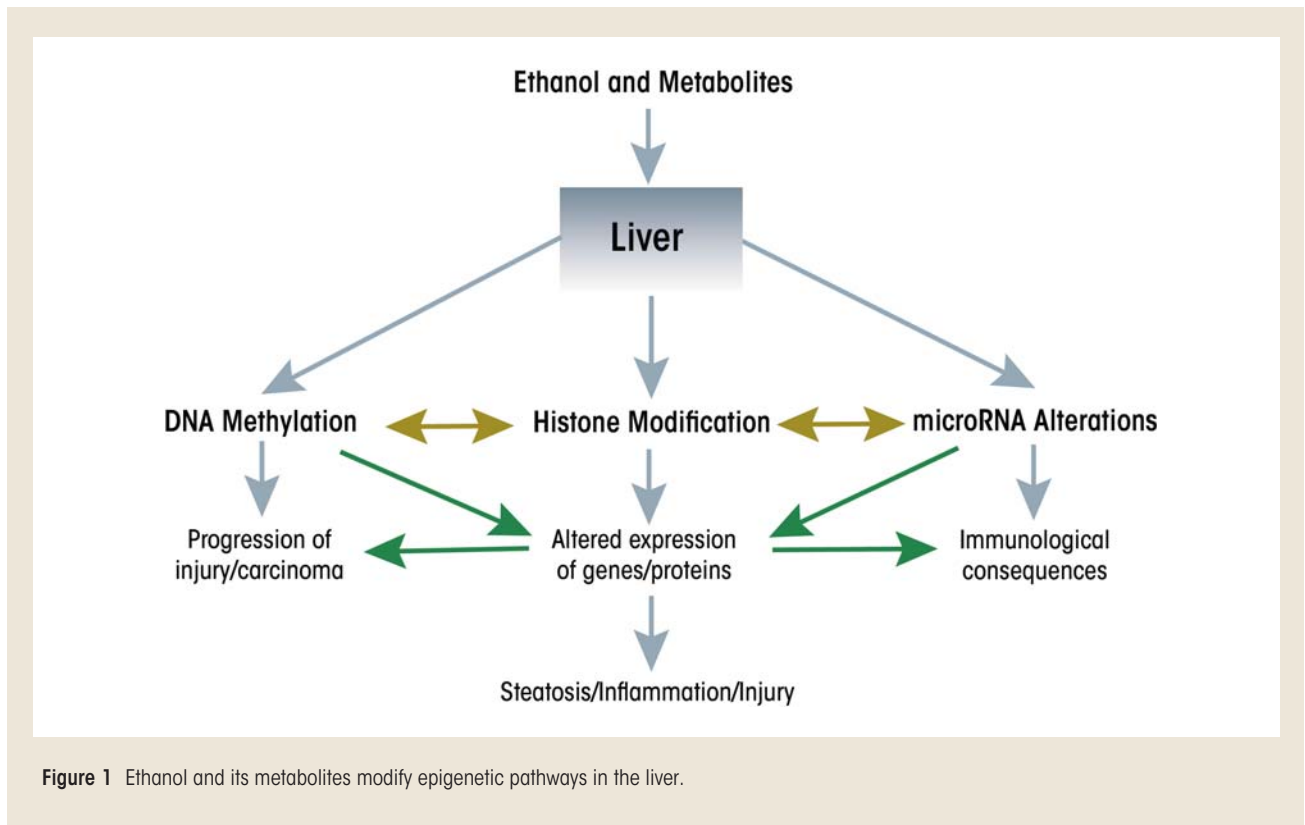


Figure 1 Ethanol and its metabolites modify epigenetic pathways in the liver.

ethanol feeding results in up- or down-regulation of 1 percent or more of known miRNAs in the liver of mice (Dolganiuc et al. 2009) and rats (Dippold et al. 2013). Among those that were upregulated in rat liver by ethanol exposure were miR-34a, miR-103, miR-107, and miR-122 (Dippold et al. 2013), which have been implicated in the regulation of lipid metabolism (Esau et al. 2006; Lee et al. 2010), iron (Castoldi et al. 2011), and maintenance of glucose levels (i.e., glucose homeostasis) (Trajkovski et al. 2011). Conversely, the levels of miR-200b and miR-19b were downregulated under the same experimental conditions (Dippold et al. 2013). Similar results were observed in mice, where chronic ethanol feeding with a liquid Lieber-DeCarli diet led to upregulation of miR-705 and miR-1224 and

downregulation of miR-182, miR-183, and miR-199a-3p in the liver. However, the biological targets of these miRNAs in the context of alcohol consumption still need to be determined (Dolganiuc et al. 2009; see table 1).

Ethanol exposure also influences miRNA expression in response to other changes in the organism. For example, the levels of a miRNA called miR-21 normally increase after a part of the animal's liver is removed (i.e., after partial hepatectomy), which had been thought to contribute to the regeneration of the liver. Ethanol enhances this increase in miR-21 but paradoxically interferes with the regenerative process (Dippold et al. 2012). The significance of the miR-21 increase therefore remains to be elucidated.

Chronic ethanol feeding of mice and exposure of mouse hepatocytes to

ethanol *in vitro* also induces miR-217 (Yin et al. 2012), which has been proposed to be linked to excess fat accumulation in the liver. Interestingly, this effect on fat metabolism seems to be correlated with reduced expression of an enzyme involved in histone modification (i.e., the class IV histone deacetylase [HDAC], SIRT-1). SIRT-1 is a molecular target not only of miR-217 but also of miR-34a (Lee et al. 2010) which, as indicated above, also is upregulated by ethanol (Dippold et al. 2013). Likewise, expression of another miRNA, miR-101, can downregulate the level of another enzyme involved in histone modification called histone methyltransferase Ezh2 (Cao et al. 2010). Although it is not known if miR-101 expression is affected by ethanol, these studies point to the intriguing possibility that change, in miRNA levels also could indirectly affect other epigenetic changes such as histone acetylation and methylation.

Changes in miRNA levels in response to ethanol are not limited to the hepatocytes but also affect other types of cells found in the liver and GI tract. For example, ethanol feeding leads to up-regulation of miR-20 and miR-203 as well as down-regulation of miR-135 and miR-199 in liver sinusoidal endothelial cells (Yeligar et al. 2009), and increases the levels of miR-132 and miR-155 in Kupffer cells (Bala et al. 2011). In addition, elevated levels of miR-212 have been detected in intestinal epithelial cells of patients with ALD (Tang et al. 2008). These changes in miRNA levels are correlated with altered expression of certain proteins in these cells, including increased expression of endothelin-1 (ET-1) and ET-1 receptor (ET-BR) in endothelial cells (Yeligar et al. 2009), increased expression of the proinflammatory cytokine tumor necrosis factor- α (TNF α) in Kupffer cells (Bala et al. 2011), and reduced expression of a protein called zonula occludens 1 (ZO1), which helps ensure the tight connection between intestinal epithelial cells (Tang et al. 2008). As will be discussed later in this article, these

Table 1 Epigenetic Parameters Altered by Ethanol in the Liver and Gastrointestinal System

Component	Molecular Alterations/ Entity	Possible Effect On
DNA	DNA methylation via DNA methyltransferase (DNMT) enzymes DNMT1, DNMT3a, and DNMT3b	Alcohol dehydrogenase (ADH), genes for folate metabolism
Histone	<p>Type of modification</p> Acetylation Methylation Phosphorylation	ADH, LSD LSD C-jun, plasminogen activatory inhibitor 1 (PAI-1)
	<p>Modifying enzymes</p> Histone acetyl transferases (HATs) GCN5 p300 MOZ Histone deacetylases (HDACs) HDAC 1,3,5,6,7,9,10,11 SIRT-1	
micro-RNA	<p>Upregulation</p> miR 03,20,21,29A,34a,101,103 miR107, 122, 132,148, 152, 155 miR 212, 217, 349, 705, 1224 miR 1256	Lipogenesis
	<p>Downregulation</p> miR 19b, 135, 182, 183, 200b miR 199a-3P	Immune response

changes in turn may contribute to the cross-talk between the liver and the GI and immune systems that ultimately may be responsible for the development of ALD.

Although changes in miRNA levels can affect expression of enzymes involved in other epigenetic modifications, it is equally clear that expression of miRNAs themselves can be subject to regulation by histone modifications and/or DNA methylation at the DNA regions that regulate miRNA expression (i.e., at their promoters). For example, the ethanol-induced expression of miR-155 seems to be regulated by the recruitment of a regulatory protein called nuclear factor κ B (NF κ B) to the miR-155 promoter (Bala et al. 2011), presumably accompanied by epigenetic changes associated with gene activation. In other studies, removal of methyl groups from (i.e., demethylation of) cytosine nucleotides at the promoters of miR-29a and miR-1256 correlated with upregulation of these miRNAs in prostate cancer cells (Li et al. 2012). Although it is not yet known whether miRNAs regulated by ethanol also may be regulated by DNA methylation, these studies clearly point to the intriguing possibility of cross-talk among molecular components involved in different types of epigenetic modifications (see figure 1).

Changes in DNA Methylation Patterns

Ethanol also can alter the methylation patterns of DNA in liver, thereby influencing gene expression. For example, genes encoding enzymes involved in ethanol metabolism (e.g., ADH) are regulated by DNA methylation (Dannenberg et al. 2006). It therefore is likely that reduced levels of DNA methylation (i.e., hypomethylation) in response to ethanol will modulate the transcription of these genes. This effect is particularly relevant in patients with late-stage ALD, where ethanol is involved in the promotion of hepatic carcinoma. Like changes in miRNA expression, alcohol-induced changes in DNA methylation also have been observed

in organs other than the liver. For example, chronic ethanol feeding in rats affects methylation of genes regulating absorption of the vitamin folate in the intestine (Wani et al. 2012). Folate is an important cofactor in single-carbon metabolism; therefore, its deficiency in turn could affect methylation reactions in various other organs, including the liver.

Kutay and colleagues (2012) found that ethanol affects methylation patterns by reducing the levels and activity of key DNA methylation enzymes, DNA methyl transferase (DNMT) 1 and 3b, without altering their mRNA levels. However, chronic ethanol feeding did not reveal any detectable methylation at the CpG islands in the promoters of several genes examined in liver (e.g., genes called *Acpat 9*, *Lepr*, and *Ppar α*), suggesting that promoter methylation may not be involved in regulating the expression of these genes. Instead, transcriptional activation or chromatin modification may be the predominant mechanism involved in ethanol-induced gene expression. This possibility has yet to be confirmed in additional studies, including studies in human liver.

Several observations suggest that changes in DNA methylation induced by diet, folate deficiency, or alcohol exposure may represent important epigenetic mechanisms. For example, chronic exposure to ethanol has been shown to produce DNA hypomethylation throughout the genome in the colonic mucosa in rats, and this hypomethylation may constitute a pathway by which carcinogenesis is enhanced (Choi et al. 1999). Other studies have focused on the role of a compound known as S-adenosylmethionine (SAME), which acts as a methyl donor, in liver injury. Ethanol-induced alterations in SAME levels can affect the methylation of histones or DNA, which in turn can modify gene expression, thereby contributing to liver injury (Lu and Mato 2012).

Role of Ethanol Metabolism and Oxidative Stress in Ethanol-Related Epigenetic Mechanisms

The actions of ethanol in the liver are complex because it is metabolized via both oxidative and nonoxidative pathways that result in the generation of several metabolites, such as acetaldehyde and acetate. Interestingly, both of these metabolites, as well as ethanol itself, increase histone H3 acetylation. This observation is supported by studies investigating the effects of inhibitors of ADH (i.e., 4-methyl pyrazole) and of another alcohol-metabolizing enzyme called aldehyde dehydrogenase (i.e., methyl cyanamide). These inhibitors prevented acetaldehyde and acetate formation and also reduced ethanol-induced increases in histone acetylation (Park et al. 2003), suggesting that ethanol metabolism has a role in this effect. Other findings suggest that ethanol-derived acetate may increase histone acetylation by increasing the available levels of acetyl groups for these reactions. Thus, studies in a cultured macrophage cell line found that downregulation of an enzyme that converts acetate into acetyl CoA, which then is used for histone acetylation, ameliorates the acetate effect on histone modification (Kendrick et al. 2010). However, the significance of this observation in vivo is unclear because the changes in acetyl-CoA levels following alcohol consumption are rather modest and transient.

Another important consequence of ethanol metabolism in the liver is the production of reactive oxygen species (ROS), leading to oxidative stress. ROS have been shown to play a role in ethanol-induced histone acetylation. Antioxidants that selectively interfere with different steps of ROS production affect this response. For example, general antioxidants (e.g., resveratrol or quercetin) inhibit histone acetylation. Conversely, inhibitors of certain enzyme complexes that are involved in ROS productions, such as rotenone (which inhibits mitochondrial complex 1) and antimycin (which inhibits

mitochondrial complex 3) increase histone acetylation (Choudhury et al. 2010). These observations are consistent with the view that ROS contribute to the epigenetic effects of alcohol consumption.

Role of Cell-Signaling Pathways in Ethanol-Related Epigenetic Mechanisms

The cellular actions of ethanol, including its epigenetic effects, are mediated via several signaling pathways (Mandarekar and Szabo 2009). One of these involves several enzymes called mitogen-activated protein (MAP) kinases (MAPKs) and therefore is known as the MAP kinase cascade. There are several different MAP kinase pathways that involve different MAPKs and which differentially affect ethanol-induced epigenetic modifications. For example, histone H3 phosphorylation is dependent on p38 MAPK (Lee and Shukla 2007), whereas histone H3 acetylation is regulated by a MAP kinase cascade involving MAPKs called ERK1/2 and JNK (Park et al. 2005). Even more intriguing is the finding that acetate-induced acetylation of histone H3 is MAPK independent (Park et al. 2005; Aroor et al. 2010). Thus, the involvement of different signaling pathways likely adds another level of regulatory control on histone modifications by ethanol and its metabolites (Shukla et al. 2013). These remarkable differences in signaling pathways utilized by ethanol and acetate may underlie the different modes of histone modifications and consequences of ethanol and its metabolites. This issue remains to be addressed in future studies.

Role of Epigenetic Mechanisms in Ethanol-Induced Steatosis, Steatohepatitis, and Carcinoma

Excessive alcohol consumption can lead to a range of liver disorders, including fatty liver (i.e., steatosis), steatosis

accompanied by inflammation of the liver (i.e., steatohepatitis), and progressing in some cases to liver cancer (i.e., carcinoma). Histone modifications, DNA methylation, and miRNA expression may all play roles in ethanol-related steatosis and inflammatory responses. For example, ethanol affects the activity of enzymes called histone acetyl transferases (HATs) that mediate

Histone modifications, DNA methylation, and miRNA expression may all play roles in ethanol-related steatosis and inflammatory responses.

histone acetylation. One of these ethanol-regulated HATs is called GCN5 (Choudhury et al. 2011); it modulates the expression of a protein called PGC1 β , which is involved in fat metabolism in the liver (Kelly et al. 2009). Furthermore, chronic intragastric ethanol feeding of rats leads to an increase in the levels of another HAT called p300 in the cell nuclei at peak blood alcohol level, which is correlated with increased acetylation of H3-lys-9 (Bardag-Gorce et al. 2007).

Another type of histone-modifying enzyme are the HDACs. Chronic feeding of mice with an ethanol liquid diet downregulates the activity of the HDAC SIRT-1 and increases the expression of lipin-1, an important regulator of lipid synthesis in the liver (Yin et al. 2012). In contrast, other studies indicated that the transcription levels of SIRT-1 and PGC1 β —another regulatory protein involved in lipid metabolism—are increased by chronic intragastric ethanol feeding in rats (Oliva et al. 2008). Recent studies also have shown that liver-specific knockout of the gene encoding HDAC3 in mice leads to severe hepatic steatosis and increased expression of lipogenic genes, although

whether HDAC3 expression or function is altered by ethanol has yet to be elucidated (Sun et al. 2011). Increasing evidence thus suggest that both HATs and HDACs are likely to play a role in ethanol-induced liver injury (see Kirpich et al. 2012; Park et al. 2005; Pochareddy et al. 2012; Shepard et al. 2008; Yin et al. 2012). In addition to changes in lipid metabolism two molecules involved in inflammatory reactions (i.e., interleukin [IL] 8 and PAI-1) also are influenced by ethanol-induced histone modifications. Finally, ethanol-induced DNA hypomethylation has been implicated in the development of steatosis (Kutay et al. 2012) as well as hepatic carcinoma, an end consequence of ALD (Lambert et al. 2011).

miRNAs also mediate some of ethanol's effects in causing liver disorders. For example, the down regulation of SIRT-1 in mice in response to ethanol feeding described above appears to be mediated by miR217 (Yin et al. 2012). A high-content screening of 327 human miRNAs identified 11 that when over-expressed in human hepatocytes lead to either increased or decreased intracellular lipid droplets, with miR-181d being the most efficacious inhibitor of lipid droplet formation (Whittaker et al. 2010). As discussed above, the immunological responses of liver macrophages are thought to involve miR-155 (Bala et al. 2012). Moreover, several miRNAs have been postulated to play a role in ethanol-induced intestinal defects (Tang et al. 2008) which could also indirectly exacerbate liver injury (see further discussion below).

Time Dependence and Persistence of Alcohol-Induced Epigenetic Changes

Interestingly, the various epigenetic modifications observed in cultured hepatocytes in response to ethanol follow different time courses. For example, phosphorylation of H3 starts before acetylation and methylation of this histone (see figure 2). Furthermore, although the global changes

more, although the global changes in histone modifications appear to be transient, with the effect peaking at about 24 hours following initial ethanol exposure, it is likely that these changes may trigger secondary changes in gene expression (including those of miRNA) or DNA modification that are much longer lasting. To date, little is known about the time course and sustainability of these other epigenetic modifications. It also is possible that even when the overall global changes in histone modification have subsided, some of the secondary changes may persist in nucleosomes associated with specific genes and may continue to influence expression of these genes.

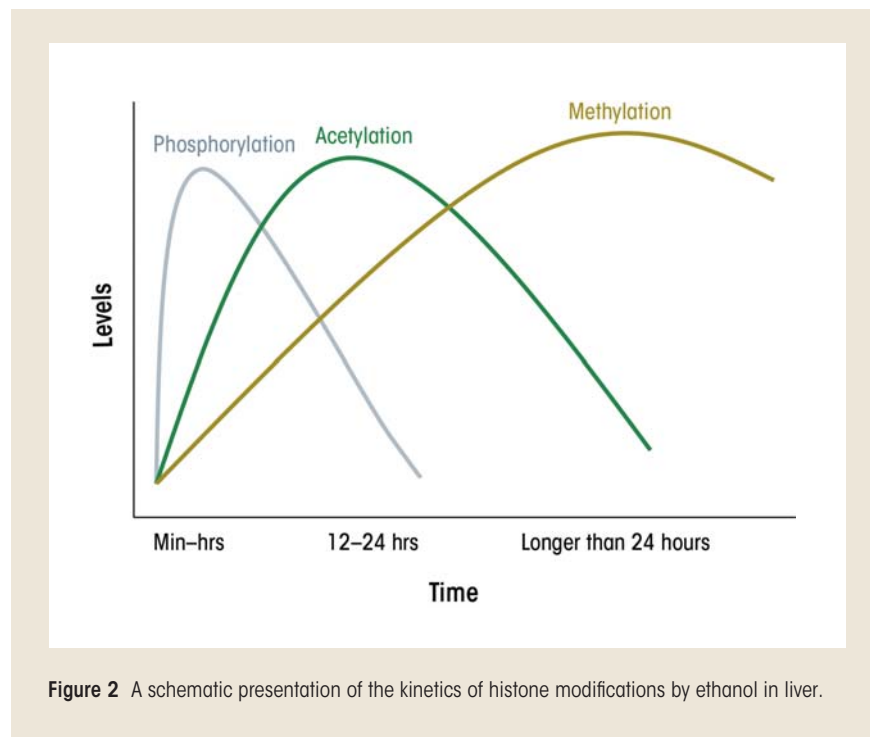
The responses to ethanol consumption in vivo also have not yet been fully elucidated. Chronic ethanol treatment definitely results in abundant epigenetic changes months after the ethanol feeding began. How long these changes remain after withdrawal of alcohol has not been carefully evaluated with respect to the GI tract and liver. Studies in other organ systems, however, suggest that some of these changes could indeed persist for a long time. For example, prenatal exposure of rat fetuses to ethanol resulted in the development of hepatic insulin resistance in the offspring 3 months after birth, which was correlated with an increase in HDAC activity and decrease in HAT activity in the liver (Yao and Nyomba 2008). Furthermore, exposure of males to ethanol was correlated with hypomethylation of normally hypermethylated regions in the DNA of the sperm corresponding to various paternally imprinted genes (Ouko et al. 2009). Epigenetic changes in these imprinted genes could be transmitted to the progeny following fertilization and thus affect the development and perhaps physiological functions of different organs, including the liver. Epigenetic effects of alcohol thus might even be able to exert long-lasting transgenerational effects in the offspring.

Relationship to the Immune System

Evidence gathered in the past decade has clearly shown that ethanol alters several immunological parameters. One important participant in ethanol's actions is a group of regulatory molecules called macrophage toll-like receptors (TLRs), particularly TLR 4. Ethanol's effects on TLRs likely are mediated via miRNAs because, as mentioned earlier, ethanol increases the levels of several of these noncoding RNAs. Other studies have shown that ethanol influences the activities of different classes of TLR-regulated genes through distinct epigenetic histone modifications (Foster et al. 2007). Specifically, several pro-inflammatory genes are selectively deacetylated during the development of immune tolerance and are no longer inducible in the tolerant macrophages. It is tempting to speculate that by affecting histone modifications, ethanol could interfere with the development of tolerance and thus promote a chronic inflammatory state. Consistent with this idea, exposure of cultured

macrophages to ethanol or ethanol metabolites resulted in increased production of TNF- α (Shen et al. 2009), although whether this involves increased histone modification at the TNF- α promoter remains to be established. In addition to the involvement of Kupffer cells, it is likely that interactions between activated hepatic stellate cells and hepatocytes also contribute to a pro-inflammatory environment by increasing the production of cytokines. This cross-talk between stellate cells and hepatocytes appears to be inhibited by deacetylase inhibitors, such as trichostatin (Coulouarn et al. 2012).

It should be pointed out that ethanol's effects on the immune system likely are rather complex. In contrast to the enhanced inflammatory response seen during steatohepatitis following chronic ethanol administration, acute exposure to ethanol in vivo suppresses various inflammatory responses (e.g., leukocyte recruitment and endothelial cell activation) (Saeed et al. 2004). It is not completely clear if this anti-inflammatory effect is related to epigenetic changes; however, other studies have shown that



treatment with HDAC inhibitors likewise inhibits the migration of macrophages in response to an inflammation-inducing stimulus (i.e., exposure to lipopolysaccharide) (Maa et al. 2010). Thus, it appears that ethanol may exert potent effects on the immune system, which likely are related to its epigenetic action, and that chronic and acute ethanol treatment could elicit different outcomes (Shukla et al. 2013).

Cross-Organ Talk Between the Liver and GI Tract

The nutrients and xenobiotics taken up orally pass through the intestinal system and then to the liver, the major metabolic organ in the body. Ethanol can alter the permeability of the intestine, a condition known as leaky gut. This alcohol-induced gut leakiness is an important factor in ALD because it allows endotoxin to enter the circulation and initiate liver damage (Keshavarzian et al. 2009). The alcohol-induced gut leakiness may in part be caused by epigenetic changes to genes coding for proteins involved in joining epithelial cells to each other (i.e.,

epithelial cell junction proteins) (Tang et al. 2008). For example, alcohol induced overexpression of miR-212 and downregulated expression of the ZO1 protein. A decrease in ZO1 disrupts intestinal permeability and integrity, resulting in gut leakiness (Tang et al. 2008).

The response of the liver to ethanol and endotoxin is a complex process involving macrophage-like Kupffer cells, hepatocytes, and stellate cells. Alcohol's effects on the activities of these cells may lead to liver injury and ultimately carcinoma. Ethanol causes epigenetic alterations in these cells that could result in changes in expression of genes associated with modified histones, including genes coding for various cytokines. Increases in the expression of these cytokines may occur in the liver, resulting in increased cytokine levels that then are circulated through the blood to other organs (e.g., heart or kidney) and in turn affect the functions of these organs. Thus, alcohol-induced epigenetic effects in the liver eventually may influence the cross-talk among these organs (see figure 3). This will be a fruitful topic for future studies to fully comprehend the

role of ethanol-induced epigenetic alterations in the GI–hepatic system and its link to the responses of other organs.

Conclusions and Future Strategy

The consequences of ethanol-induced epigenetic alterations can be positive or negative, depending on the type and duration of the epigenetic changes. Furthermore, the epigenetic responses to ethanol and its metabolites (e.g., acetate) also can differ with a variety of consequences. This diversity remains to be examined thoroughly. Additionally, modifications in DNA and histones located in specific nucleosomes or chromatin domains may differ in their transcriptional effects on various genes, consequently exhibiting varying effects. Alterations in the expression levels of a plethora of miRNAs will add another level of regulatory control over these responses. Finally, it is fair to assume that the diverse epigenetic pathways cross-influence each other, leading to a highly complex regulatory network. The consequences of these epigenetic alterations in the GI

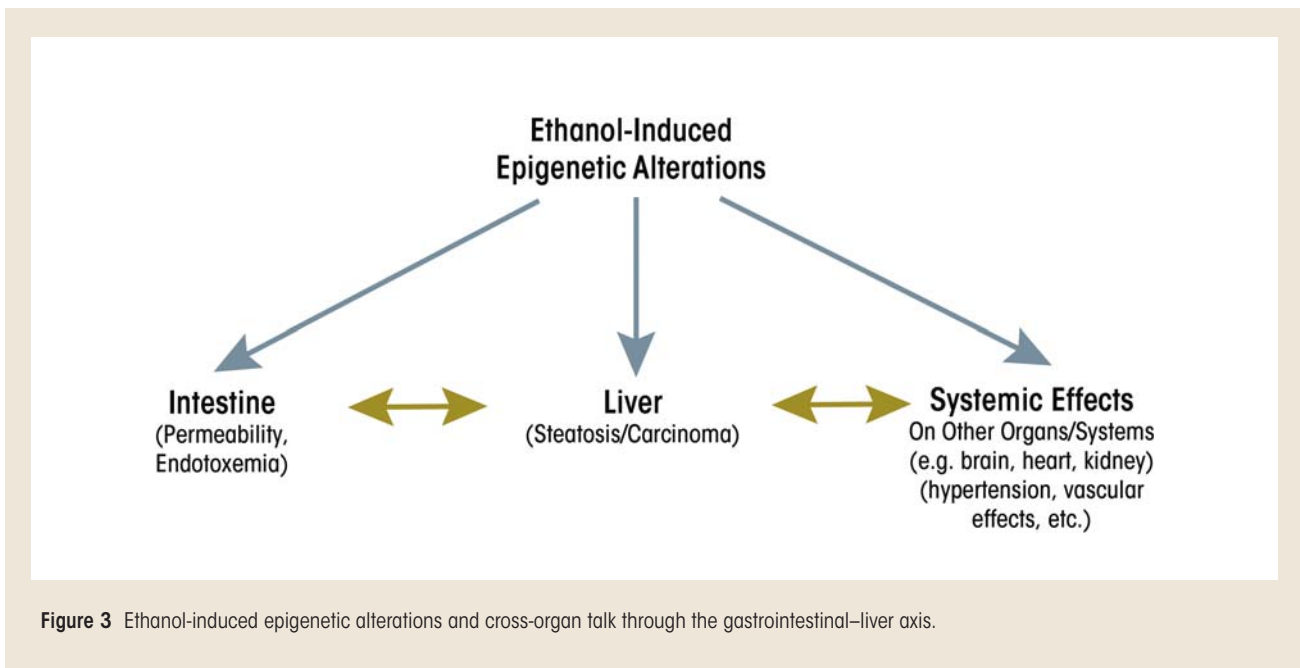


Figure 3 Ethanol-induced epigenetic alterations and cross-organ talk through the gastrointestinal–liver axis.

tract and liver likely have a systemic impact, influencing other organs and their functions as well, although these interactions are as yet relatively unexplored. Thus, many questions remain that need to be addressed by future research into this area. ■

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The authors declare that they have no competing financial interest.

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Stress and the HPA Axis

Role of Glucocorticoids in Alcohol Dependence

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Stress has long been suggested to be an important correlate of uncontrolled drinking and relapse. An important hormonal response system to stress—the hypothalamic–pituitary–adrenal (HPA) axis—may be involved in this process, particularly stress hormones known as glucocorticoids and primarily cortisol. The actions of this hormone system normally are tightly regulated to ensure that the body can respond quickly to stressful events and return to a normal state just as rapidly. The main determinants of HPA axis activity are genetic background, early-life environment, and current life stress. Alterations in HPA axis regulation are associated with problematic alcohol use and dependence; however, the nature of this dysregulation appears to vary with respect to stage of alcohol dependence. Much of this research has focused specifically on the role of cortisol in the risk for, development of, and relapse to chronic alcohol use. These studies found that cortisol can interact with the brain’s reward system, which may contribute to alcohol’s reinforcing effects. Cortisol also can influence a person’s cognitive processes, promoting habit-based learning, which may contribute to habit formation and risk of relapse. Finally, cortisol levels during abstinence may be useful clinical indicators of relapse vulnerability in alcohol-dependent people. **KEY WORDS:** Alcohol dependence; problematic alcohol use; alcohol use disorders; alcohol abstinence; relapse; stress; stress response; stress hormones; hypothalamic–pituitary–adrenal axis; glucocorticoids; cortisol; brain reward pathway

Stress, generally defined as any stimulus that disrupts the body’s internal balance (i.e., physiological homeostasis), has long been suggested to be an important correlate of uncontrolled alcohol consumption or relapse to drinking following a period of abstinence. Large epidemiological studies have reported that a variety of stressors are associated with increased alcohol consumption and binge drinking. These include hazardous and demanding work environments, legal stress, family stress (e.g., unhappy marriage and divorce), and low income (Richman et al. 1996; Rospenda et al. 2000; San Jose et al. 2000; Vasse et al. 1998). Likewise, the Health and Retirement Study found an association between stress from retirement and divorce and increased alcohol intake (Perreira and Sloan 2001). Studies also have shown that people experiencing more severe or highly threatening social

stress following alcoholism treatment have higher rates of relapse compared with people not experiencing such stress (Brown et al. 1990; Noone et al. 1999). On the other hand, prospective and human laboratory studies exploring the relationship between stress, alcohol craving, and relapse have found mixed results, with more recent research suggesting that several factors moderate the effects of stress on alcohol consumption (e.g., Breese et al. 2011; Brennan et al. 1999; Fox et al. 2008; Helzer et al. 2006; Sinha 2007; Sinha and Li 2007; Thomas et al. 2011).

It remains uncertain how stress, per se, might influence vulnerability to alcohol use disorders (AUDs). However, production of the stress hormone cortisol, which is triggered by stress-induced activation of a hormonal system known as the hypothalamic–pituitary–adrenal (HPA) axis, is thought to be involved.

The HPA axis is one of the main stress response pathways and has been studied extensively in relation to alcohol use (Wand 2008). Over 20 years of research has demonstrated that altered HPA axis regulation is associated with problematic alcohol use and dependence and that the nature of this dysregulation varies with respect to the stages of progression toward alcohol dependence. The finding that HPA axis dysregulation and alcohol misuse tend to co-vary has implied a “guilt-by-association” relationship—that is, that abnormal variations in stress-related cortisol production are a risk factor for developing alcoholism in the first place (Wand et al. 1993). A recent review of studies on youth and adolescents similarly suggests that HPA axis dysfunction and exposure to stress are critical components that interact to convey risk for developing AUDs (Scheepis et al. 2011).

As with mood and affective disorders, many researchers consider alterations in HPA axis function crucial for understanding the underlying brain mechanisms of substance use disorders. In contrast to mood and affective disorders, however, alcohol dependence has a biphasic effect on HPA axis dynamics as a person traverses through the various phases of heavy hazardous drinking, including dependent drinking, withdrawal, abstinence, and relapse. Generally speaking, these developmental stages seem to be mirrored by a shift between hyper- and hyposponsiveness of the HPA axis to stressful events (Rose et al. 2010). For example, hyperresponsiveness has been identified in people with a family history of alcoholism (Uhart et al. 2006; Zimmermann et al. 2004a,b), a population that is at increased risk for alcohol dependence (Windle 1997). This observation raises the question whether heightened stress responsivity is clinically meaningful to the development of alcoholism. This view is supported by studies showing that cortisol responsivity correlates with the activity of a brain system, the mesolimbic dopaminergic pathway, which is a central neural reward pathway (Oswald et al. 2005; Wand et al. 2007). With transition to alcohol dependence, compensatory allostatic mechanisms result in injury to HPA axis function and elevation of stress peptide levels (e.g., corticotropin-releasing factor [CRF]) in brain regions outside the hypothalamus. The term allostasis refers to the process through which various biological processes attempt to restore homeostasis when an organism is threatened by various types of stress in the internal or external environment. Allostatic responses can involve alterations in HPA axis function, the nervous system, various signaling molecules in the body, or other systems. Allostatic alterations in HPA axis function have been posited to, among other things, injure brain reward pathways, contribute to depressed mood (i.e., dysphoria) and craving, and further contribute to the maintenance of problem drinking behavior.

This article provides an overview of the clinical evidence for HPA axis and glucocorticoid dysfunction across the developmental phases of alcoholism and explores whether this dysfunction is causally related to, or a consequence of, alcohol dependence. The article describes behavioral and physiological pathogenesis resulting from dysregulation of basal and reactive HPA axis activity. This discussion primarily focuses on human studies and studies that specifically address the glucocorticoid activation component of the stress response. The article also discusses whether these findings have potential predictive value and whether altered glucocorticoid function, regardless of etiology, may serve as a useful clinical marker for the progression of alcohol dependence and treatment prognosis. The review will not address the important role that extrahypothalamic CRF pathways play in mediating the relationship of stress and reward dysfunction (for a review of this issue, see Koob 2010).

Physiology of the HPA Axis

The body responds to stress with self-regulating, allostatic processes aimed at returning critical systems to a set point within a narrow range of operation that ensures survival. These self-regulating processes include multiple behavioral and physiological components. Perhaps the best-studied component of the stress response in humans and mammals is activation of the HPA axis (see figure 1). Neurons in the paraventricular nucleus (PVN) of the hypothalamus release two neurohormones—CRF and arginine vasopressin (AVP)—into the blood vessels connecting the hypothalamus and the pituitary gland (i.e., hypophysial portal blood). Both hormones stimulate the anterior pituitary gland to produce and secrete adrenocorticotrophic hormone (ACTH) into the general circulation. The ACTH, in turn, induces glucocorticoid synthesis and release from the adrenal glands, which are located atop the kidneys.

The main glucocorticoid in humans is cortisol; the main glucocorticoid in rodents, which frequently are used as model systems to investigate the relationship between stress and alcohol use, is corticosterone. Hypothalamic activation of the HPA axis is modulated by a variety of brain signaling (i.e., neurotransmitter) systems. Some of these systems have inhibitory effects (e.g., γ -aminobutyric acid [GABA] and opioids), whereas others have excitatory effects (e.g., norepinephrine and serotonin) on the PVN. Thus, the central nervous system (CNS) and the hormone (i.e., endocrine) system are tightly interconnected to coordinate glucocorticoid activity.

To protect against prolonged activity, the HPA system is carefully modulated through negative-feedback loops designed to maintain predetermined hormone levels (i.e., set points) and homeostasis. To this end, secretion of CRF, AVP, and ACTH in part are controlled by sensitive negative feedback exerted by cortisol at the level of the anterior pituitary gland, PVN, and hippocampus. There are two types of receptors for cortisol—mineralocorticoid (type-I) and glucocorticoid (type-II) receptors—both of which participate in the negative-feedback mechanisms. Cortisol binds more strongly (i.e., has higher binding affinity) for the mineralocorticoid receptors (MRs)¹ than the glucocorticoid receptors (GRs). Because of this difference in binding affinity, the MRs help maintain the relatively low cortisol levels circulating in the blood during the normal daily (i.e., circadian) rhythm. Only when the cortisol concentration is high (e.g., during a stressful situation) does it bind to the GRs with lower affinity; the resulting activation of the GRs terminates the stress response. This delicate negative feedback control mechanism maintains the secretion of ACTH and cortisol within a relatively narrow bandwidth. This is an extremely important homeostatic mechanism because too much or too little exposure

¹Cortisol has similar affinity to the MR as does the mineralocorticoid aldosterone, which helps regulate kidney function.

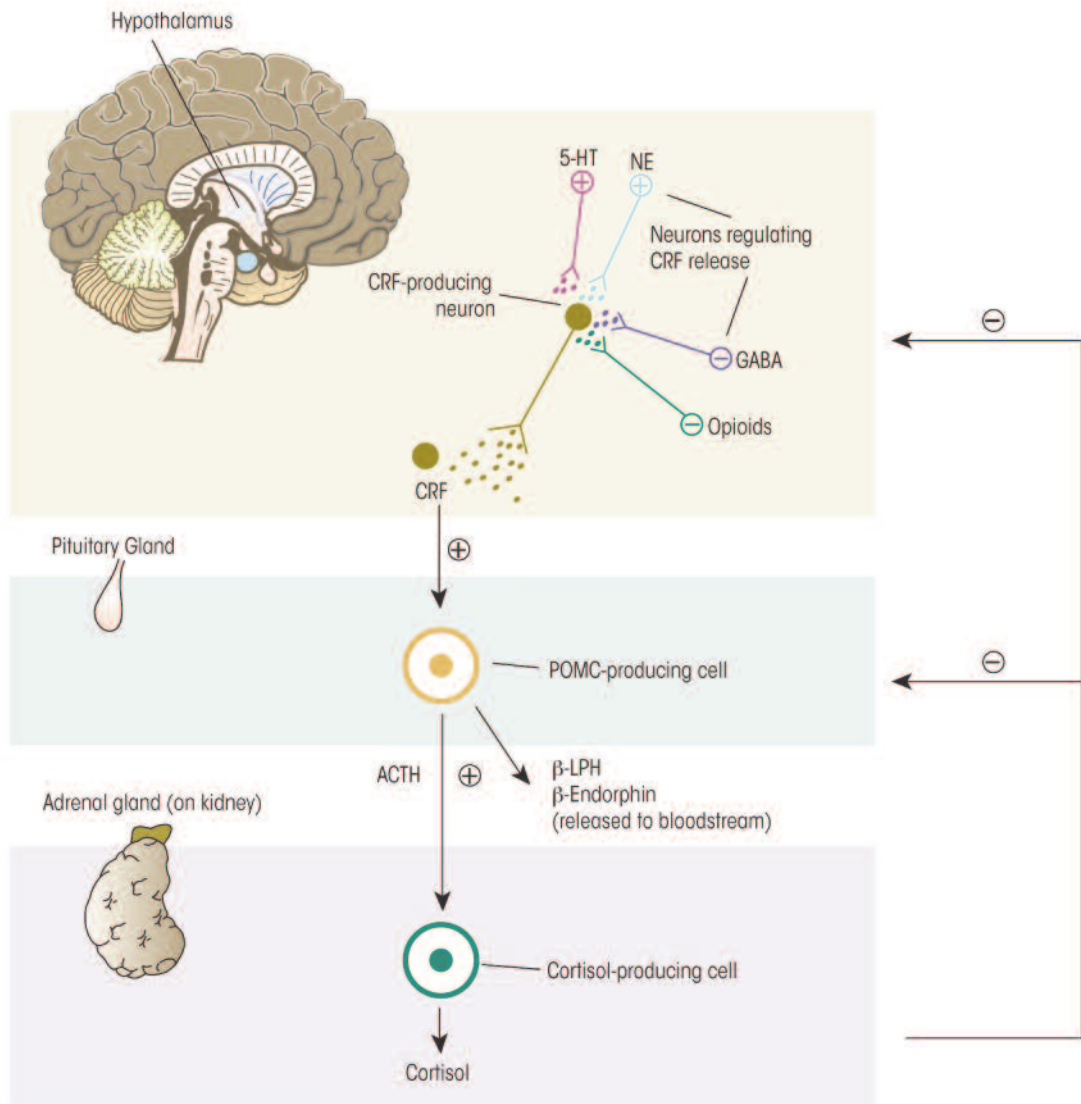


Figure 1 The major components of the stress response mediated by the hypothalamic–pituitary–adrenal (HPA) axis. Both alcohol and stress can induce nerve cells in one brain region (i.e., the hypothalamus) to produce and release corticotropin-releasing factor (CRF). Within the hypothalamus, CRF stimulates the release of a hormone that produces morphine-like effects (i.e., β -endorphin). CRF also is transported to a key endocrine gland, the anterior pituitary gland. There, CRF stimulates production of a protein proopiomelanocortin (POMC). POMC serves as the basis for a number of stress-related hormones, including adrenocorticotrophic hormone (ACTH), β -lipotropin (β -LPH), and β -endorphin. ACTH stimulates cells of the adrenal glands to produce and release the stress hormone cortisol. When cortisol levels reach a certain level, CRF and ACTH release diminishes. Other neurons releasing serotonin (5-HT), norepinephrine (NE), γ -aminobutyric acid (GABA), or endogenous opioids also regulate CRH release.

NOTE: \oplus = excites; \ominus = inhibits.

to cortisol can have adverse consequences to health and well being.

Growing evidence suggests that a protein, FK506 binding protein 5 (FKBP5), regulates GR sensitivity. Binding of this protein to the GR reduces the receptor's affinity for cortisol and its movement (i.e., translocation) to the nucleus. A genetic variation in FKBP5 is associated with enhanced expression of the protein following GR activation. This leads to more GR resistance, diminished negative feedback, and prolonged stress hormone activation following a stressor (Binder et al. 2004; Wochnik et al. 2005).

Physiological Actions of Glucocorticoids

Glucocorticoids are a class of steroid hormones that are essential for the organism to survive. Cortisol, the main glucocorticoid in humans, has been placed in this class because of its effects on the metabolism of the sugar glucose, where its primary function is to increase blood glucose levels by inducing production of additional glucose molecules (i.e., gluconeogenesis). Cortisol also modifies fat and protein metabolism to support the nutrient requirements of the CNS during stress. However, cortisol also has many other wide-ranging effects when it binds to GRs. For example, it influences cardiovascular function, immunologic status (i.e., inflammatory reactions), arousal, and learning and memory; all of these systems therefore are affected when the HPA axis is activated in response to stress.² Thus, cortisol helps maintain or can increase blood pressure by increasing the sensitivity of the blood vessels to signaling molecules, catecholamines. In the absence of cortisol, widening of the blood vessels (i.e., vasodilation) and hypotension occurs. The anti-inflammatory effects of cortisol are brought about by reducing proinflammatory cytokine and histamine secretion and stabilizing the membranes of cell components, lysosomes.

One of the most important actions of cortisol in the context of alcohol use

and the stress response is its role in modifying learning and memory. Both stress and exposure to cortisol can transiently block memory retrieval (van Stegeren 2009), with retrieval of emotional memory more strongly affected than that of neutral memory. Of interest, both cortisol and stress also enhance memory consolidation; this process generally favors consolidation of emotionally arousing information, facilitating habit-based learning. Consistent with the multiple-systems theory to memory organization in the mammalian CNS, studies have identified unique roles for various brain regions in learning and memory. For example, "cognitive" learning and memory is associated with activation of brain circuits in the hippocampus, whereas "habit" learning and memory is associated with activation of the dorsal striatum and the basolateral amygdala (BLA). In addition, nerve fibers projecting from the BLA modulate memory processes occurring in other brain structures. The implications of the fact that cortisol selectively affects emotionally charged memory and habit learning are discussed below.

Determinants of HPA Axis Activity and Cortisol Exposure

Correct regulation of cortisol levels is necessary for survival, and too little or too much cortisol exposure can result in serious harm. Therefore, both basal and stress-induced cortisol levels are maintained carefully. A healthy stress response is characterized by a quick rise in cortisol levels, followed by a rapid decline with the termination of the stressful event. When the organism is burdened by cumulative stress, however, the cortisol burden increases. This results in wear and tear on the organism from excessive exposure to the catabolic properties of glucocorticoids, stress peptides, and proinflammatory cytokines. This burden taxes the organism and

² Certain tissues, however, need to be protected from cortisol, such as the kidneys, colon, and placenta. In these tissues, an enzyme, 11 β -hydroxysteroid dehydrogenase type II, mediates the conversion of glucocorticoids to 11-dehydro metabolites, which are inactive.

can influence the development of neuropsychiatric and metabolic disorders. It therefore is essential to understand the systems that regulate cortisol production.

Three main determinants of HPA axis activity control the amount of cortisol a person is exposed to during adulthood: genetic background, early-life environment, and current life stress. In addition, studies found that post-traumatic stress disorder (PTSD) can contribute to HPA axis disturbances.

Genetic Factors. Differences among individuals in cortisol responses to stress result from a complex interplay between genetic and environmental factors. The genetic contribution to the variability in HPA axis reactivity is believed to arise from DNA variations (i.e., polymorphisms) in the genes encoding neurotransmitters involved in HPA axis regulation. Overall, heritable influences account for approximately 62 percent of the etiological variance in basal glucocorticoid levels (Bartels et al. 2003). Recent candidate gene association studies using laboratory-based stress procedures also have implicated multiple gene variants in explaining some of the variance in cortisol responses to stress, including polymorphisms in the following genes:

- *Nr3c1*, which encodes a glucocorticoid receptor protein (Wust et al. 2004);
- *Nr3c2*, which encodes a mineralocorticoid receptor protein (DeRijk et al. 2006);
- *FKBP5* (Ising et al. 2008);
- *CRFR1*, which encodes the CRF receptor 1 protein (Clarke and Schumann 2009);
- *CRF-BP*, which encodes CRF binding protein (Wang et al. 2007);

- *GABRA6*, which encodes the GABA receptor subunit alpha-6 protein (Uhart et al. 2004);
- *OPRM1*, which encodes the mu opioid receptor protein (Chong et al. 2006); and
- *SLC6A4*, which encodes a serotonin transporter protein (Way and Taylor 2010).

It is certain that additional genes and polymorphisms will be identified in the future.

Early-Life Environment. Pre- and postnatal processes contribute to the lifelong responsiveness of the HPA axis to stressors. In animal models, prenatal ethanol exposure is associated with impaired HPA axis responsivity in adulthood (Hellemans et al. 2010; Weinberg et al. 2008), and emerging evidence suggests that these effects also occur in human infants and toddlers (Haley et al. 2006; Ouellet-Morin et al. 2010). Maternal stress during gestation also modifies HPA axis responsivity of infant and adult offspring (see Charil et al. 2010; Harris and Seckl 2010 for reviews). More recently, studies have focused on the consequences of early-childhood events on the stress response. Childhood trauma is a significant problem in the United States and is associated with mental and physical health problems in adulthood as well as with alterations in HPA axis function (Heim et al. 2009, 2010; Dong et al. 2004; Mangold et al. 2010). For example, it has been hypothesized that exposure to sexual and physical abuse in childhood during critical periods of brain development (i.e., during periods of neural plasticity) may permanently alter stress responsivity (Gillespie et al. 2009; Heim and Nemeroff 2001; Heim et al. 2001). Animal models that have studied this phenomenon have shown that certain forms of neonatal stress results in a modification (i.e., epigenetic methylation) of the glucocorticoid gene that has long-lasting effects on gluco-

corticoid responsivity (Weaver 2009). This alteration in stress responsivity may explain the observation that childhood adversity is a risk factor for the development of alcohol and other drug abuse (Epstein et al. 1998) as well as anxiety and depressive disorders in adulthood (Kessler et al. 1997; Safren et al. 2002).

Glucocorticoids also can alter the methylation patterns of other genes. For example, glucocorticoid administration to adolescent mice reduces methylation of the *FKBP5* gene in the hippocampus, hypothalamus, and blood, which is associated with enhanced expression of *FKBP5* and increased anxiety-like behavior (Lee et al. 2010). The investigators proposed that in addition to altering behaviors, methylation of the gene may be a marker of cortisol burden. Polymorphisms in *FKBP5* also have been associated with psychiatric disorders, such as depression and PTSD, that are characterized by alterations in HPA dynamics (Binder et al. 2004; Yehuda et al. 2009).

An emerging literature also addresses the role of early-childhood adversity on the development of AUDs (for a review, see Enoch 2010). For example, Schmid and colleagues (2010) found an interaction between stressful early-life events and a variant in the *CRFR1* gene that influenced age of drinking initiation and drinking progression in a population of 19-year-olds. Other studies demonstrated that certain variants of the *CRFR1* gene influenced cortisol responses to CRF and the synthetic glucocorticoid dexamethasone (Binder et al. 2010; Tyrka et al. 2009) and were associated with binge drinking in adolescents and total lifetime alcohol consumption in adults (Clarke and Schumann 2009; Hansson et al. 2006; Pastor et al. 2008; Treutlein et al. 2006). Thus, it seems that an interaction between the *CRFR1* gene and early-life events can modify HPA axis dynamics and risk for AUDs. It is certain that other stress gene variants also will be found to interact with environmental factors to increase the risk of AUDs.

Current Stress. Independent of prenatal and childhood stressors, periods of severe, chronic stress in adulthood, such as family- and work-related problems, combat exposure, neighborhood violence, chronic illness, or the development of neuropsychiatric disorders, alter HPA axis dynamics and increase the cortisol burden. Chronic stress triggers an allostatic shift in the normal circadian rhythm of cortisol release as well as in stress-induced cortisol levels. Thus, after chronic stress baseline cortisol levels are elevated, the body's cortisol response to acute stress is blunted, and it takes longer for stress-induced cortisol levels to return to pre-stress levels (e.g., Juster et al. 2010; McEwen 2000; Wingenfeld et al. 2009). This allostatic injury makes the HPA axis more sensitive, resulting in higher cortisol exposure or greater cortisol burden following each stressful episode (McEwen and Gianaros 2010).

PTSD Symptomatology. A fourth potential determinant of HPA axis activity is the presence of PTSD symptoms. The HPA axis has been the main focus of neuroendocrine research in PTSD. In a meta-analysis of 37 studies involving people with PTSD, Meewisse and colleagues (2007) examined cortisol levels in people with PTSD and control subjects. These analyses found no differences in basal cortisol levels between the two groups; however, differences did exist under certain conditions or among certain subgroups of subjects. For example, people with PTSD had lower afternoon levels of cortisol than did control subjects, and women with PTSD had significantly lower cortisol levels than women without PTSD. The specific type of trauma experienced by a person also mattered. Thus, only people who had experienced physical or sexual abuse had significantly lower cortisol levels than control subjects. These findings highlight the complexity of the relationship between HPA axis activity and PTSD pathophysiology.

People with AUDs have a high prevalence of PTSD (Kessler et al. 1997); conversely, women with PTSD were 3.5 times more likely to develop alcoholism than women who did not report past trauma (Sartor et al. 2010). It is difficult to define whether the alterations in the HPA axis seen in people with PTSD by themselves modulate risk for alcoholism because, as discussed above, a history of childhood trauma also increases risk for developing PTSD as well as alcoholism (Binder et al. 2008; Epstein et al. 1998). Therefore, it is possible that exposure to trauma in early childhood may confer the initial insult to HPA axis regulation that later influences the interaction between PTSD and alcohol use (Yehuda et al. 2010). This view is consistent with the finding that people with a flattened cortisol response following trauma had a higher risk of developing PTSD symptoms than did those with normal cortisol levels (e.g., Aardal-Eriksson et al. 2001; Anisman et al. 2001). It remains unclear, however, whether the lower levels of circulating cortisol preceded the traumatic event (Yehuda et al. 2010).

Regardless of whether an underlying HPA axis dysregulation precedes PTSD symptomatology, evidence suggests that dysregulation occurs through increased sensitivity of the negative feedback mechanisms regulating the HPA axis, resulting in lower circulating cortisol levels. Yehuda and coworkers (2009) examined the expression of all genes active in whole-blood samples as well as cortisol levels in people with and without PTSD. This analysis identified 17 genes whose expression differed between people with and without PTSD. Several of the uniquely expressed genes are involved in HPA axis function. For example, the *FKBP5* gene, which serves as a modulator of GR sensitivity, showed reduced expression in people with PTSD, consistent with enhanced GR responsiveness. Moreover, statistical analyses found that *FKBP5* expression was predicted by cortisol levels when PTSD severity also was taken into consideration (Yehuda et al. 2009). Of interest, this profile of HPA axis

dysregulation is distinct from that seen with other psychiatric disorders, such as depression (Handwerker 2009). Taken together, it seems likely that dysregulation of the HPA axis associated with PTSD interacts with epigenetic and environmental influences (Yehuda et al. 2010) and that this interaction translates into increased risk for the development of AUDs.

The HPA Axis and Alcoholism

HPA Axis Dynamics in People at Risk for AUDs

Altered HPA axis responsiveness may be present before alcohol exerts its toxic effects on the CNS and may contribute to initial vulnerability to alcoholism. This vulnerability risk likely is a result of gene–environment interaction (Clarke et al. 2008; Schepis et al. 2011). The current state of knowledge stems from an early and large body of research suggesting that people who have alcoholic family members (i.e., who are family-history positive [FHP] for alcoholism) may be more likely to develop the disorder than those with no such family history (i.e., who are family-history negative [FHN] for alcoholism) (Windle 1997). This risk seems to be linked to abnormal HPA activity (e.g., Dai et al. 2002; King et al. 2002; Sorocco et al. 2006; Uhart et al. 2006; Wand et al. 1998, 1999*a,b*), although the relationships appear complex. Laboratory findings have been mixed and may depend on several factors, such as which type of stressor is used, whether basal or reactive HPA response is measured, and how cortisol is stimulated. The first studies comparing HPA axis responsiveness in FHP and FHN people assessed cortisol levels in response to an agent that can block the opioid receptors (i.e., the opioid receptor antagonist, naloxone). These studies identified stronger cortisol responses to naloxone in FHP subjects than in FHN subjects (Wand et al. 1998, 1999*a,b*, 2001). These findings were replicated using another opioid receptor antagonist,

naltrexone (King et al. 2002). These observations are particularly interesting because they implicate the endogenous opioid system in the interaction between HPA axis activity and alcoholism risk. This signaling system not only modulates the HPA axis but also is a pharmacological target for the treatment of alcohol dependence. Other studies using a psychosocial stressor rather than a pharmacologic stimulator such as naloxone also found a stronger HPA response in FHP than in FHN subjects (Uhart et al. 2006; Zimmermann et al. 2004*a,b*). More recent studies among infants and toddlers with prenatal alcohol exposure who also are believed to be at increased risk for alcoholism have corroborated these latter findings in male but not female children (Haley et al. 2006; Ouellet-Morin et al. 2010). Other studies, however, found blunted HPA axis function in FHP individuals (e.g., Dai et al. 2002; Sorocco et al. 2006).

HPA Axis Dynamics During Intoxication and Withdrawal

As with stress, acute alcohol consumption also directly and indirectly activates the HPA axis by resulting in elevated levels of glucocorticoids (Richardson et al. 2008). In fact, alcohol and other drugs of abuse have been described as a physiological stressor because they can activate the HPA axis. In social drinkers, acute doses of alcohol usually increase cortisol levels, particularly if blood alcohol levels exceed 100 mg percent (Waltman et al. 1993). At some point during the transition from social drinking to alcohol dependence and abstinence, however, the HPA axis becomes dysregulated. For example, King and colleagues (2006) found that cortisol reactivity to acute alcohol administration is attenuated in heavy, hazardous drinkers compared with light, social drinkers. This observation may be related to the general process of tolerance that emerges during heavy hazardous drinking. It is important to note that the subjects in this study were binge drinkers—which reflects a pattern of drinking frequently associated with adverse consequences—but

were not alcohol dependent, suggesting that alterations in the HPA axis may begin even before dependence develops.

The onset of alcohol dependence, however, is accompanied by bouts of elevated cortisol levels in the blood (i.e., hypercortisolism) as the drinker cycles through repeated episodes of alcohol intoxication and the stress of withdrawal (Adinoff et al. 1998; Wand and Dobs 1991). This transition to alcohol dependence is accompanied by an allostatic shift in HPA axis functioning, resulting in abnormally low cortisol responsivity (Koob and Le Moal 2001). Under conditions of alcohol dependence, the allostatic load—a hypothetical measure of cumulative stress—increases and burdens the organism with excessive exposure to stress hormones and peptides as well as pro-inflammatory cytokines (McEwen 2007). Increased allostatic load has been implicated not only in AUDs and other drug use disorders but also in the development of psychiatric disorders (e.g., depression), metabolic syndrome, and systemic hypertension. In the context of drug use, allostatic load not only impacts the stress response via the HPA axis but also encompasses a state of reward dysregulation. At this point, the organism constantly seeks the initial rewarding effects of the drug while tolerance to those effects develops through repeated drug self-administration. This results in a dysfunctional reward system and a maladaptive response to stress. Specifically, the allostatic alterations in cortisol responsivity may have a detrimental effect on the reward systems (Wand 2008).

HPA Axis Dynamics During Abstinence

Wand and Dobs (1991) studied HPA axis function in alcohol-dependent subjects during the first week of abstinence following supervised alcohol withdrawal on a clinical research unit. Although the participants had modestly to highly significantly elevated cortisol levels in the urine during the withdrawal period, they also demonstrated blunted HPA axis responses to CRF, a medica-

tion that blocks cortisol production (i.e., metyrapone), and the ACTH analog cosyntropin immediately following alcohol detoxification. In fact, many of the alcohol-dependent subjects met diagnostic criteria for adrenal insufficiency. Other studies have corroborated these findings of elevated cortisol during the first week of withdrawal and also showed that cortisol levels decreased significantly over time, even plunging below the normal range (Esel et al. 2001; Keedwell et al. 2001; Majumdar et al. 1989).

Later in abstinence (i.e., at 2 to 6 weeks), alcoholics generally regain normal diurnal patterns of cortisol levels (e.g., Leggio et al. 2008). However, they may continue to exhibit a deficient cortisol response to psychosocial and pharmacological HPA axis stimulation for several months (Adinoff et al. 1998, 2005*a,b*; Anthenelli et al. 2001; Bernardy et al. 1996). Junghanns and colleagues (2007) compared HPA axis activity in early abstainers (i.e., mean abstinence 22 days) and long-term abstainers (i.e., mean abstinence 117 days). These investigators found that longer-abstaining people showed a stronger cortisol awakening response, another indicator of HPA axis function, implying that diurnal patterns of cortisol may begin to normalize over longer periods of abstinence. Whether regulation of the HPA axis returns completely to normal, and under what conditions, remains unknown.

Several factors may impact and moderate HPA axis recovery, including severity of withdrawal symptoms (Bernardy et al. 1996), severity and duration of dependence, comorbid childhood trauma (Schafer et al. 2010), and genetic factors underlying the individual stress response. The exact role of cortisol in HPA axis recovery is unclear. Coiro and colleagues (2007) examined the effect of exercise as a biobehavioral stressor in control subjects and alcoholics over an 8-week period. Consistent with other studies, ACTH and cortisol levels were significantly lower in alcoholics in the first month of withdrawal; by 8 weeks,

however, the hormonal response had returned to normal. Interestingly, exercise itself can induce cortisol release (Beaven et al. 2010; Coiro et al. 2007; Usui et al. 2011) and has been investigated as an adjunct for smoking cessation with somewhat promising findings (Williams et al. 2010). This suggests that manipulation of cortisol levels may have therapeutic potential (see below). Indeed, determining the nature, extent, and time course of the attenuated HPA axis response during abstinence may have significant clinical relevance because low levels of basal cortisol and of the ACTH response may predict relapse to alcohol use during early abstinence (Adinoff et al. 1998; Junghanns et al. 2003, 2005; Kiefer et al. 2002).

No prospective longitudinal studies have examined HPA axis changes over longer periods of abstinence. One study of alcoholics who had been abstinent for a mean of 3.5 years found similar ACTH and cortisol responses compared with healthy controls in response to both psychological and pharmacological (i.e., opioid challenge) stressors (Munro et al. 2005). However, the study did not determine whether the alcoholics had recovered a normal level of HPA response with prolonged abstinence, whether they had had a normal response all along, or whether their lack of psychological comorbidity indicated that they were less affected by secondary characteristics related to a hyporesponsive HPA axis. Another study compared alcoholics who had relapsed with abstainers after one year and found that, contrary to findings during short-term abstinence, 1-year abstainers had significantly lower levels of cortisol (Walter et al. 2006). This suggests that the relationship between HPA axis activity and alcohol recovery is dynamic and changes as abstinence persists over time.

One major limitation of these studies is that most of the work has been conducted with male alcoholics; therefore, less is known regarding the HPA hyporesponsiveness during abstinence in females. Adinoff and colleagues (2010) focused on female alcoholics

and found no differences in HPA axis activity between women who had been abstinent for 4 to 8 weeks and age-matched healthy control women. Thus, HPA axis functioning over the long term and its relationship to alcohol use and recovery remains unclear and warrants further investigation.

Possible Roles of Cortisol in the Risk and Development of AUDs

Cortisol's Interaction with Dopaminergic Reward Systems

Studies in animal models have demonstrated that mesocorticolimbic dopamine pathways are involved in the brain's reward system and that the nucleus accumbens in the ventral striatum is a critical region for mediating the rewarding effects of drugs. Virtually all drugs of abuse, including alcohol, have an impact on dopaminergic activity within this brain region (Pierce and Kumaresan 2006). Imaging studies using positron emission tomography (PET) in humans have corroborated the animal findings that drugs of abuse alter mesolimbic dopaminergic activity and have helped elucidate potential neurobiological underpinnings of drug addiction (for a review, see Martinez and Narendran 2009). These and other studies in humans have shown that mesolimbic dopamine release is correlated with the positive subjective effects of the drug (Drevets et al. 2001; Hamidovic et al. 2010; Oswald et al. 2005; Volkow et al. 2002; Wand et al. 2007). However, whereas acute alcohol administration increases synaptic dopamine activity and accumulation, chronic alcohol consumption can lead to lower-than-normal dopamine levels (i.e., a hypodopaminergic state) that may motivate the drinker to seek alcohol in order to restore the normal levels of the neurotransmitter (Volkow et al. 2007). It has been postulated that elevated levels of glucocorticoids contribute to alcohol's reinforcing effects by enhancing modulation of

the dopaminergic and subjective response to alcohol (e.g., Melis et al. 2009).

Glucocorticoids and stress interact with the dopamine reward system in ways that may increase vulnerability for developing addiction (Marinelli and Piazza 2002). For example, glucocorticoids play a critical role in the reinforcing effects of psychostimulants because surgical removal of the adrenal glands (i.e., adrenalectomy), which prevents cortisol production, decreases drug self-administration. Moreover, re-introduction of glucocorticoids at levels similar to those induced by stress reverses this effect (Deroche et al. 1997). In fact, acute stress and drugs of abuse, through different mechanisms, appear to converge upon a common pathway that modifies dopamine neuron output by enhancing long-term potentiation (LTP) of excitatory synapses (Saal et al. 2003) and long-term depression (LTD) of inhibitory synapses (Niehaus et al. 2010). However, these studies did not demonstrate that this effect directly was attributable to cortisol. Another study found that the magnitude of stress-induced cortisol release significantly correlates with mesolimbic dopamine release in the ventral striatum (Pruessner et al. 2004). Taken together, these studies suggest that cortisol may facilitate firing of dopaminergic neurons and, consequently, the reward circuitry and that this process is common with and specific to many drugs of abuse (Saal et al. 2003).

Glucocorticoids themselves also are believed to have reinforcing properties in rats as they seem to modulate self-administration of alcohol and increase brain sensitivity to other addictive drugs (e.g., stimulants and opioids) in the animals. A review by Piazza and Le Moal (1997) concluded that glucocorticoid administration at levels similar to those found in physiological stress responses had positive reinforcing effects. The investigators proposed that under natural conditions (e.g., during conflicts with other animals) the rewarding effects of the glucocorticoids might counteract the aversive effects of external aggressions, thereby allowing the ani-

mal to better cope with threatening situations. Such a mechanism may play a key role in fine-tuning an individual's adaptation to stress and in determining reward-related behavioral pathologies. Thus, increased levels of cortisol may have reinforcing effects, acting on the brain to perpetuate behaviors (e.g., alcohol consumption) that maintain high cortisol levels.

The interactions of the stress response and the rewarding effects of drugs also have been investigated in humans. Imaging studies using PET found that higher cortisol levels in response to amphetamine administration (Oswald et al. 2005) or to a psychosocial stressor (Wand et al. 2007) were positively associated with amphetamine-induced dopamine release in the ventral striatum. Furthermore, subjects with a high cortisol response to these stimuli reported more positive subjective drug effects after amphetamine administration than did subjects with a low cortisol response (Hamidovic et al. 2010; Oswald et al. 2005; Wand et al. 2007). These studies provide evidence that cortisol may play a role in drug reinforcement through its interactions with the dopaminergic reward pathway, which may, in turn, influence vulnerability for and maintenance of alcohol and other drug use.

Cortisol's Effect on Cognitive Processes

LTP is a process that ultimately enhances signal transmission at the synapse. This enhanced synaptic transmission, which has been observed in a variety of neural structures, is widely considered one of the leading cellular mechanisms that underlie learning and memory (Goosens and Maren 2002). As mentioned above, LTP is enhanced by stress. Cortisol has been implicated in this phenomenon because a widespread system of glucocorticoid receptors is found above the hypothalamus, for example, in the limbic system, notably the hippocampus and amygdala, and in the prefrontal cortex. This section discusses the impact of glucocorticoids

on some of the basic (e.g., learning, acquisition, and memory) and higher (e.g., decision-making) cognitive processes that may potentially underlie development of addictive behaviors. This discussion focuses on the regulatory actions of glucocorticoids on neural structures critically involved in cognitive processes related to alcoholism but does not cover the equally important reciprocal effects these structures have on regulating HPA axis function (e.g., Dedovic et al. 2009).

Optimal levels of cortisol are needed not only to meet the body's physical needs but also for learning, memory, and cognitive performance. Both too little and too much cortisol may be damaging and disruptive to memory formation, whereas normal levels of glucocorticoids protect the brain against adverse events and are essential for cognitive processes. Several studies partly may explain this paradox by describing the roles of MRs and GRs in the various stages of information processing and the context in which glucocorticoid-receptor activation takes place. The effects of glucocorticoids on brain tissue as well as cognition can turn from adaptive into maladaptive when actions via both receptor types are imbalanced for a prolonged time (Joels et al. 2008; de Kloet et al. 2007).

The secretion of cortisol and norepinephrine in response to acute stress is known to affect learning and memory (Smeets et al. 2011; van Stegeren et al. 2010). The mammalian brain does not house a solitary brain region mediating the acquisition, consolidation, and retrieval of all types of learned information. Instead, memory and learning are organized in multiple brain systems. Certain brain regions (e.g., the prefrontal cortex) govern goal-directed learning, whereas others (e.g., the dorsal striatum) are responsible for habit formation. Stress can induce a bias by promoting habit-based forms of learning and memory in lieu of goal-directed performance. Specifically, studies in rodents have determined that corticosterone and norepinephrine promote habit-based memory forma-

tion by acting on the amygdala, hippocampus, dorsal striatum, and prefrontal cortex—all of which also are involved in alcohol dependence. The relationship between cortisol and the vulnerability to alcohol dependence as well as to relapse after abstinence could involve cortisol's effects on habit-based learning. In view of the habit-like nature of addictive behaviors, it is fascinating that recent evidence indicates a role for the habit memory system located in the dorsal striatum in the maintenance and expression of drug-seeking and drug-taking behaviors (Everitt et al. 2008). For example, anxiety-inducing (i.e., anxiogenic) drugs can promote the use of dorsal striatal-dependent habit memory in rats (Packard 2009).

Research in humans also has shown that stress is associated with decreased use of cognitive behavioral strategies, which involve the hippocampus, and increased use of stimulus-response strategies, which involve the caudate nucleus (Kim et al. 2001; Schwabe et al. 2007). It is possible that the heightened cortisol responsivity in people at increased risk for alcohol dependence may promote the transition to heavy, hazardous drinking through cortisol's ability to promote habit-based memory formation and learning during alcohol intoxication, especially during states of heightened arousal (Smeets et al. 2009). Furthermore, the wide fluctuations in cortisol secretion observed in alcohol-dependent people could help maintain these habit-based addictive behaviors. Additionally, the hypercortisolism associated with alcohol dependence may in part promote relapse by favoring the use of habit-based memory to guide the expression of maladaptive behaviors. Finally, persistent hypercortisolism observed during repeated episodes of acute alcohol intoxication and withdrawal may be toxic to neurons in the hippocampus. Hippocampal damage, in turn, may result in alcohol-related symptoms such as personality changes, memory loss, and depression.

Chronic exposure to elevated glucocorticoid levels also can have a detrimental effect on prefrontal cortex function

with concomitant neuronal degeneration (Bennett 2008). As mentioned earlier, the prefrontal cortex is involved in complex cognitive operations, including assessing likelihood of reward or punishment during critical decision-making situations as well as assessing internal and external affective cues and responding adaptively, particularly in stressful situations. Psychosocial stress can disrupt prefrontal cortex function in humans (e.g., Liston et al. 2009). However, the specific effects of glucocorticoids in this process remain to be determined (Het et al. 2005) because other physiological changes that occur as part of the overall stress response, such as increased catecholamine levels, also alter prefrontal cortex function (Qin et al. 2009). Animal studies have suggested that glucocorticoids play a role in the cognitive deficits observed after withdrawal from chronic alcohol consumption (Rose et al. 2010). In mice, the glucocorticoid receptor antagonist mifepristone reduced memory deficits during the first and second week after alcohol withdrawal, suggesting that heightened glucocorticoid levels during withdrawal directly contribute to these cognitive deficits (Jacquot et al. 2008). Studies in humans found that cognitive impairment in abstinent alcoholics was related to an attenuated cortisol response to a psychosocial stressor (Errico et al. 2002). Poorer cognitive performance also was related to more withdrawal episodes, heavier alcohol consumption, and higher cortisol levels during withdrawal (Errico et al. 2002; Keedwell et al. 2001). Thus, further studies should investigate the mechanism through which altered stress regulation of the HPA axis impairs cognitive function and relates to poor prognosis in recovering alcoholics.

The amygdala is another limbic structure that is affected by cortisol in ways that might contribute to alcohol dependence. The amygdala is a major extrahypothalamic source of CRF-containing neurons that carry large numbers of CRF-1 and CRF-2 receptors; it has a primary role in the processing and memory of emotional reactions.

Thus, the extended amygdala is crucial for the expression of anxiety, and the central amygdala is a major extrahypothalamic site where CRF is produced and plays a role in mediating fear and anxiety (Gray and Bingaman 1996; Heilig et al. 1994). Whereas the hypothalamic CRF system is important for modulating neuroendocrine responses to stress, the extrahypothalamic CRF system manifests the behavioral response to stress via the amygdala and other limbic regions. In rats with high alcohol preference and anxiety levels, CRF gene expression is reduced in the central nucleus of the amygdala (Hwang et al. 2004); moreover, the extracellular levels of CRF in the central amygdala are increased during acute alcohol withdrawal and during exposure to various forms of stress (Merlo-Pich et al. 1995). Chronically elevated corticosterone levels also increase CRF expression in the central amygdala (Shepard et al. 2000; Schulkin et al. 1998). This enhanced CRF production may contribute to anxiety-like behaviors. The heightened or exaggerated emotional and fearful reactivity to perceived stress, in turn, may drive alcohol consumption observed during heavy, hazardous drinking and alcohol dependence. Consistent with this theory, administration of CRF antagonists reverses anxiety-like behaviors and excessive alcohol drinking associated with alcohol withdrawal (Valdez et al. 2003). These observations suggest that heightened cortisol exposure influences alcohol consumption by inducing anxiety and dysphoria via CRF-mediated activation of the amygdala.

Early Abstinence and Relapse

As mentioned earlier, a blunted hormonal response to stress during early abstinence is related to increased risk for relapse (Junghanns et al. 2003, 2005; Kiefer et al. 2002). The mechanism underlying this relationship is not clear. Because cortisol levels in alcohol-dependent people negatively correlate with self-reported alcohol craving (Bohn et al. 1995), it is possible that relapse to alcohol consumption during early

abstinence partly is driven by alcohol's ability to induce cortisol elevation (Junghanns et al. 2005). If this is the case, cortisol may influence the motivation to drink and relapse via a potential negative-reinforcement pathway. Several observations support this hypothesis. For example, several studies evaluating pharmacological treatments for relapse prevention during early abstinence have examined the relationships among HPA activity, craving, and alcohol intake during early abstinence, based on the hypothesis that risk for relapse may be attenuated through mechanisms that reduce craving and increase cortisol. For example, O'Malley and colleagues (2002) administered naltrexone or placebo for 6 days to alcohol-dependent, non-treatment seekers who then participated in an alcohol self-administration session. Naltrexone treatment resulted in higher cortisol levels, which were associated with lower levels of craving and less alcohol consumption. Similarly, Kiefer and colleagues (2006) studied the efficacy of naltrexone and/or an agent that can block receptors for the neurotransmitter GABA (i.e., acamprosate), both of which are used in alcoholism treatment to reduce craving. The study found that without an active treatment, both ACTH and cortisol levels decreased during early abstinence; conversely, treatment with naltrexone and acamprosate prevented these declines. Moreover, increased ACTH and cortisol during treatment was associated with reduced risk of relapse. Finally, Sinha and colleagues (2009) found that alcohol-dependent patients who had been abstinent for 28 days showed significantly elevated basal cortisol levels as well as a blunted cortisol response to a psychological stressor and to exposure to an alcohol-related cue. Further, stress and cue exposure resulted in significantly enhanced and persistent craving. Although some studies have not been able to demonstrate correlations between changes in cortisol and craving (e.g., Pratt and Davidson 2009), decreased cortisol levels in general have been accompanied by increased craving

during early abstinence, which may underlie risk for relapse to alcohol use. Taken together, these studies suggest that cortisol levels and HPA axis reactivity may be useful clinical indicators in the management of relapse risk and that manipulating HPA axis regulation through either pharmacological or psychosocial intervention is a viable avenue of research for developing new alcoholism treatments.

Summary

The HPA axis, an important physiological stress pathway, may play a significant role in the risk and development of AUDs, and the glucocorticoid cortisol may be useful as a biomarker for HPA axis homeostatic regulation. The hormones of the HPA axis act to maintain homeostasis in the presence of stress through a variety of mechanisms. When the HPA axis becomes dysregulated, regardless of cause, deviations in cortisol reactivity result that have been associated with the progressive stages of alcoholism risk, dependence, and abstinence (see figure 2). Considerable research has been devoted to identifying potential underlying mechanisms of the HPA axis dynamics that contribute to progressive stages of alcohol dependence, and the available evidence support several of these potential mechanisms.

First, non-alcohol-dependent drinkers believed to be at risk for developing an AUD, either because of their family history or because of their hazardous drinking patterns, clearly have altered HPA axis function compared with low-risk individuals. The findings regarding the exact nature of this dysregulation (i.e., whether the HPA axis shows hyper- or hyporesponsivity) are mixed, particularly within the family-history literature. However, the equivocal results most likely are related to differences in experimental strategies used and in the levels of alcohol consumption in these drinkers (e.g., tolerance level). Nevertheless, this body of literature generally has established that

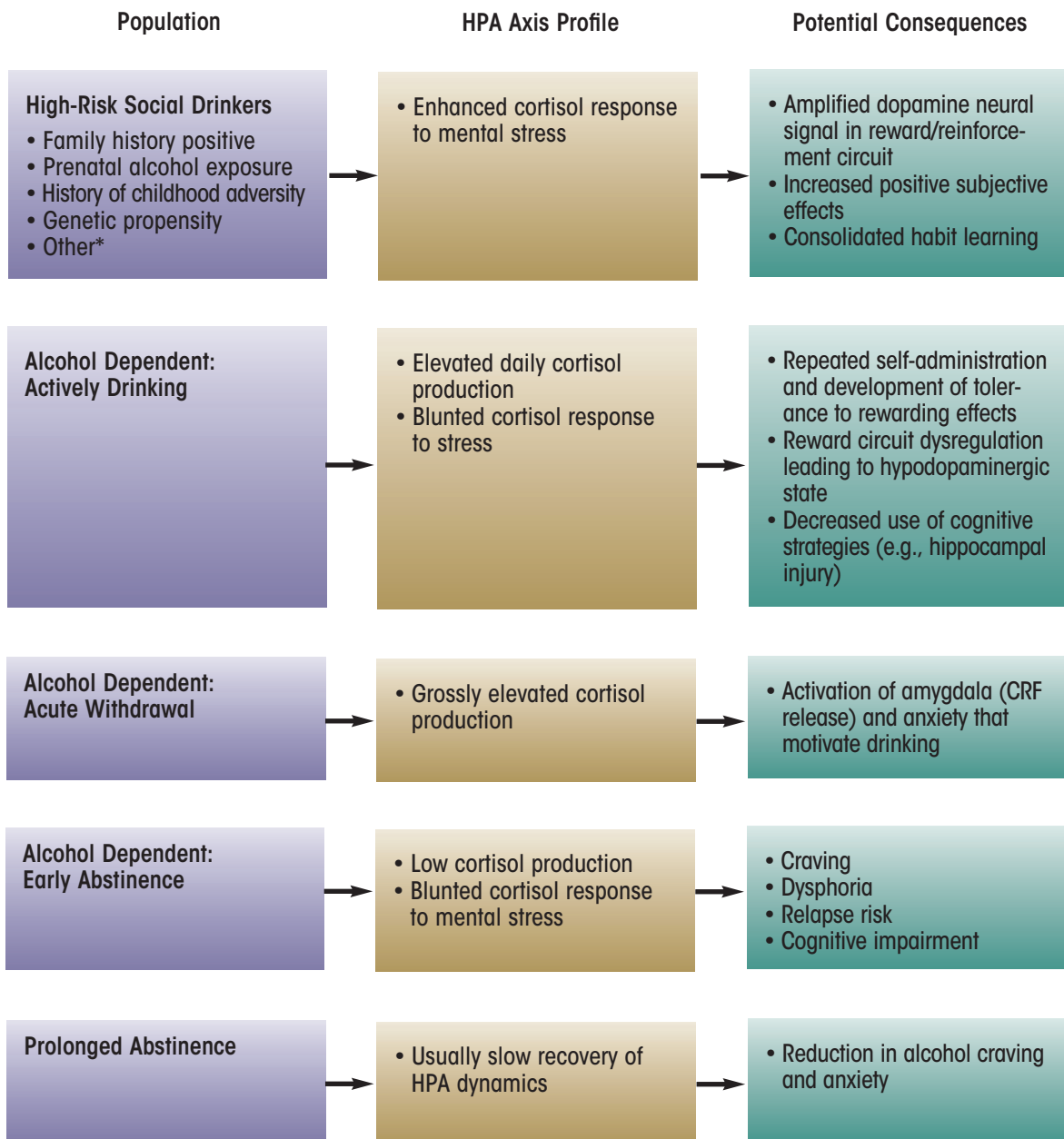


Figure 2 Summary of the activity of the hypothalamic–pituitary–adrenal (HPA) axis during different stages of alcoholism development and their potential consequences.

NOTE: *Low level of response (LR) to alcohol is a phenotype that predicts higher risk for alcohol-related problems (Hu et al. 2005); currently, there are no data characterizing HPA axis response to mental stress in this high-risk group. Posttraumatic stress disorder (PTSD) is a complicated disorder with multiple subtypes and comorbidities; the HPA axis profile of individuals with PTSD symptomatology generally is not thought to react to mental stress with enhanced responsiveness and therefore does not fit the model depicted above for other high-risk social drinkers.

cortisol responsivity serves as a risk marker for the propensity for abuse or dependence.

Second, considerable evidence supports the effect of glucocorticoids in facilitating dopamine-mediated signal transmission in the brain, which has been linked to reward pathways involved in almost all drugs of abuse. Moreover, glucocorticoids themselves have positive reinforcing properties. Conversely, reduced glucocorticoid activity seems to suppress acquisition and self-administration of drugs of abuse (Fahlke et al. 1996; Goeders and Guerin 1996). Thus, glucocorticoids appear to play a critical mediating role in the dopamine reward circuit.

Third, cortisol plays a key role in brain regions that are important for cognitive learning and memory retrieval, encoding, and consolidation. These are central processes affected by shifting hyper- and hypocortisolism throughout alcohol dependence as well as by cortisol responses to stress. It is possible that such perturbations in the HPA axis consolidate the type of habit-based learning (rather than goal-directed learning) that sustains maladaptive behaviors related to alcohol use.

Finally, deficiency in cortisol response during early abstinence is predictive of relapse to alcohol and may modulate conditions that often accompany relapse episodes, such as craving, dysphoria, and severe withdrawal symptoms. Thus, cortisol levels during abstinence may be useful clinical indicators of relapse vulnerability, and interventions that increase cortisol and decrease craving might be useful to prevent relapse.

Taken together, HPA axis function may serve as a predictor of risk for alcohol dependence in alcohol-naïve or social drinkers, facilitate initiation and maintenance of alcohol use, or serve as a predictor for risk of relapse in abstinent alcohol-dependent individuals. Using HPA axis reactivity as a predictive marker may help to identify individuals at risk for dependence or relapse prior to development of those conditions, which would allow the

individuals and their treatment providers to take action and improve overall prevention and treatment efforts for AUDs. ■

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