



Alcohol effects on hepatic lipid metabolism

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Abstract Alcoholic liver disease (ALD) is the most prevalent type of chronic liver disease with significant morbidity and mortality worldwide. ALD begins with simple hepatic steatosis and progresses to alcoholic steatohepatitis, fibrosis, and cirrhosis. The severity of hepatic steatosis is highly associated with the development of later stages of ALD. This review explores the disturbances of alcohol-induced hepatic lipid metabolism through altered hepatic lipid uptake, de novo lipid synthesis, fatty acid oxidation, hepatic lipid export, and lipid droplet formation and catabolism. In addition, we review emerging data on the contributions of genetics and bioactive lipid metabolism in alcohol-induced hepatic lipid accumulation.—Jeon, S., and R. Carr. Alcohol effects on hepatic lipid metabolism. *J. Lipid Res.* 2020. 61: 470–479.

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Globally, approximately two billion people consume alcoholic beverages, and alcohol abuse is a leading cause of liver-associated morbidity and mortality (1). The spectrum of alcoholic liver disease (ALD) ranges from simple steatosis to alcoholic steatohepatitis, progressive fibrosis, and cirrhosis. Currently, there are no accepted therapeutics to halt or reverse ALD in patients, despite the profound economic and health impacts of ALD. The earliest and most common hepatic response to alcohol is excess fat accumulation (steatosis). Alcoholic fatty liver is diagnosed when alcohol consumption results in hepatic fat exceeding 5% of the liver weight. Nearly all alcohol consumers develop steatosis (2), widely considered to be a less injurious stage than advanced stages of liver disease but whose severity is highly linked to the development of later stages of ALD (3) and that results from alcohol's toxic effects on hepatic lipid metabolism. In contradistinction, alcohol intake alone is insufficient to cause significant liver disease in the majority of consumers, although the amount of alcohol consumed is highly associated with the severity of ALD (4). Consider-

ing the high incidence of steatosis and lack of approved treatments for ALD, a better understanding of the mechanisms by which steatosis develops is essential to identify early biomarkers and develop ALD therapeutics, especially for those who have been unable to achieve abstinence.

The liver is the major organ responsible for metabolizing ingested alcohol to the toxic metabolite acetaldehyde (5), and most data support that it is alcohol's metabolism per se that is required for hepatic lipid dysregulation. Once metabolized, alcohol exerts a myriad of effects on hepatic lipid regulation that promote steatosis (Fig. 1). In this review, we focus predominantly on alcohol's dysregulation of triglyceride metabolism, the predominant lipids implicated in the development of steatosis. From effects on the production of triglyceride to effects on fatty acid uptake and oxidation, lipophagy, triglyceride export, and hepatocellular lipid storage, alcohol impacts cellular lipid homeostasis, which in turn has implications for cellular dysmetabolic stress and inflammation pathways thought to spur liver disease progression. In addition, we explore the role of bioactive lipids in alcohol-induced lipid accumulation, an emerging area of study that has broad implications for the transition from steatosis to more advanced stages of ALD.

EFFECTS OF ALCOHOL ON HEPATIC FATTY ACID UPTAKE

As triglycerides are the predominant hepatic neutral lipids that accumulate in ALD, significant attention has been paid to alcohol's effects on fatty acids whose esterification results in triglyceride synthesis. Exogenous sources of hepatocyte fatty acids include the uptake of circulating NEFAs from adipose tissue lipolysis and intestinally derived

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Abbreviations: ACC, acetyl-CoA carboxylase; ALD, alcoholic liver disease; AMPK, AMP-activated protein kinase; ChREBP, carbohydrate response element-binding protein; CIDE, cell death-inducing DNA fragmentation factor- α -like effector; CPT, carnitine palmitoyltransferase; DGAT, diacylglycerol acyltransferase; FATP, fatty acid transporter protein; LD, lipid droplet; MBOAT7, membrane bound O-acyltransferase domain-containing 7; PAP, phosphatidyl phosphatase; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PEMT, phosphatidylethanolamine methyltransferase; PLIN, perilipin; PNPLA3, patatin-like phospholipase domain-containing protein 3; SAM, S-adenosyl methionine; SCD1, stearoyl-CoA desaturase 1; SIRT1, sirtuin 1; VDAC, voltage-dependent anion channel.

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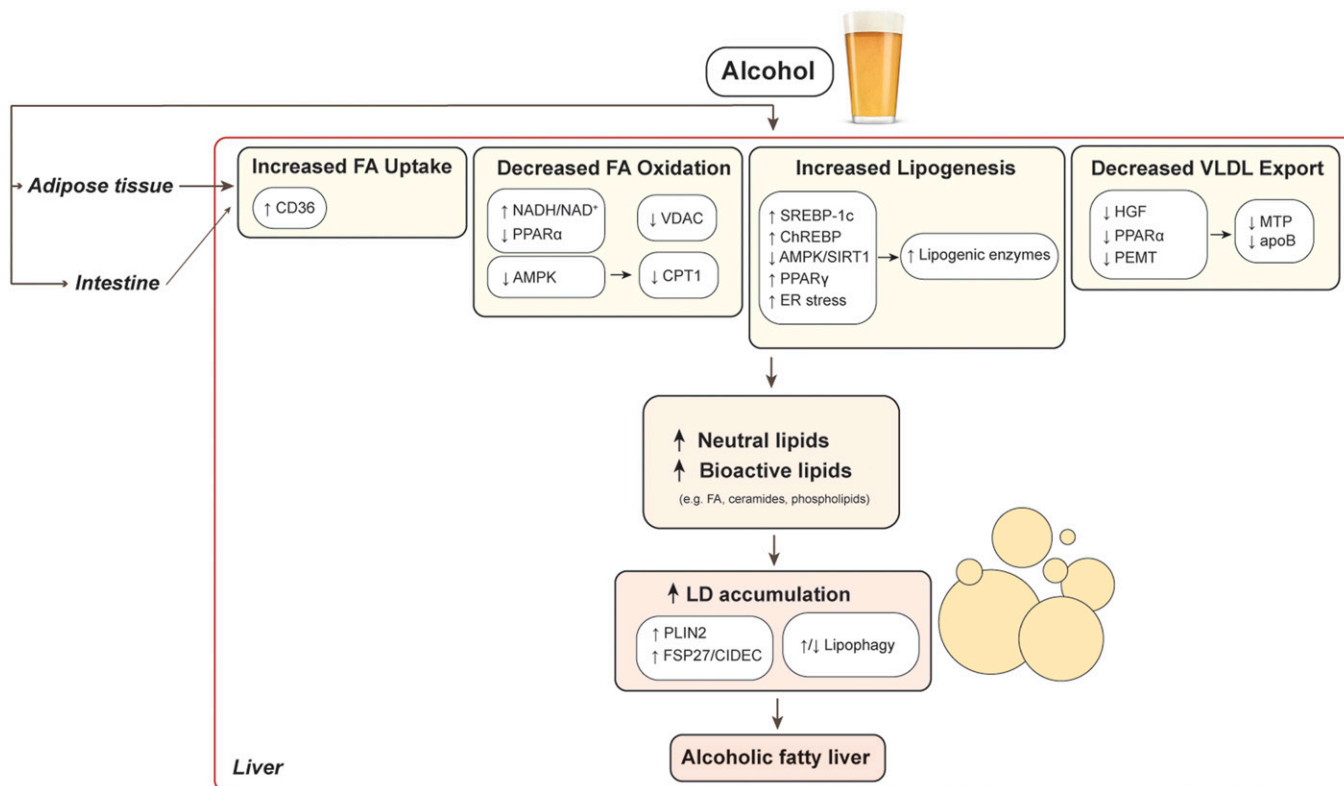


Fig. 1. Mechanisms of alcohol-induced hepatic lipid accumulation.

chylomicrons. Chronic alcohol consumption exacerbates adipose tissue lipolysis in large part through inciting adipose tissue insulin resistance (6), although other mechanisms, such as alcohol-induced catecholamine release, play a role (7, 8). Indeed, in alcohol-fed rodents (9, 10), peripheral adipose depots are reduced compared with controls. This is in line with our own data demonstrating that alcohol worsens insulin sensitivity in part by inhibiting the lipogenic response of adipocytes to insulin (11), a finding that correlates with observations in clinical studies showing that patients with alcohol addiction have less fat mass and increased NEFA levels (12, 13). In rodents, these effects on adipose tissue lipolysis and subsequent hepatic fatty acid uptake take place as early as within 2 weeks of chronic alcohol ingestion based on high resolution mass spectrometry and *in vivo* deuterium labeling (10).

Alcohol consumption can also increase the supply of intestine-derived fatty acids from chylomicrons (14). Chylomicrons are formed by intestinal enterocytes and are taken up by hepatocytes as chylomicron remnants via the LDL receptor and the LDL receptor-related protein after undergoing lipolysis by lipases (15, 16). Although this is an established chylomicron clearance pathway, chylomicron-derived fatty acids are not major sources of alcohol-induced hepatic neutral lipid accumulation (17), unlike what is observed with white adipose tissue-derived fatty acids (18).

Upon circulating to the liver, NEFAs enter hepatocytes via hepatic fatty acid transporters. Hepatic plasma membrane fatty acid transporters include fatty acid transporter proteins (FATPs) and fatty acid translocase/CD36. Among six isoforms of FATPs, FATP2 and FATP5 are highly ex-

pressed in the liver, while CD36 expression is normally low (19). Although CD36 does not play a significant role in fatty acid transport in normal liver, CD36 is highly inducible and contributes to hepatic steatosis under pathological conditions such as excessive alcohol consumption (20). In fact, chronic alcohol upregulates the expression of CD36 while there is no clear consensus on the expression of FATP2 and FATP5 in the liver (9, 21, 22). Furthermore, CD36 ablation alleviates ethanol-induced hepatic lipid accumulation, corroborating the participation of CD36 in the development of alcoholic steatosis (22). As a consequence of the increased expression of hepatic fatty acid transporters, alcohol consumption increases the hepatic capacity for exogenous fatty acid uptake. Indeed, fatty acid uptake is increased in primary cultured hepatocytes from alcohol-fed rats (23). Together, these alcohol-induced alterations in both hepatic fatty acid delivery and capacity for uptake contribute to increased hepatic lipid accumulation and resultant hepatic steatosis.

EFFECTS OF ALCOHOL ON HEPATIC FATTY ACID OXIDATION

Excessive alcohol consumption impairs fatty acid catabolism predominantly through inhibition of mitochondrial β -oxidation, which we and others have determined is the most significant contribution to alcohol-induced hepatic lipid accumulation (24–28). In addition to the direct toxic effects of alcohol on mitochondria (29), alcohol oxidation increases the NADH:NAD⁺ ratio, thus promoting alcohol

oxidation at the expense of fatty acid oxidation (12, 30, 31). Alcohol also inhibits carnitine palmitoyltransferase (CPT1) activity, a rate-limiting step in fatty acid translocation for mitochondrial β -oxidation, by reducing AMP-activated protein kinase (AMPK) activity and increasing acetyl-CoA carboxylase (ACC) activity and malonyl-CoA levels (32). Considering that carnitine is an essential cofactor for CPT1, alcohol-induced carnitine deficiency is another plausible mechanism of mitochondrial β -oxidation inhibition. Indeed, lower concentrations of plasma carnitine have been reported in alcoholic patients and chronically alcohol-fed animals (33, 34). In addition to effects on CPT1, ethanol closes voltage-dependent anion channels (VDACs) in the mitochondrial outer membrane (35–37). VDACs are responsible for outer membrane permeability for metabolites including long-chain fatty acyl-CoA, which can lead to suppressed β -oxidation. In cultured rat hepatocytes, ethanol and acetaldehyde inhibit VDAC conductance (36, 37).

PPAR α

PPAR α is a key transcriptional regulator of many genes involved in mitochondrial oxidation and whose ligands include free fatty acids and fatty acid derivatives (38). On forming a heterodimer with retinoid-X receptor, activated PPAR α binds DNA recognition sites to regulate the transcription of target genes. Even with the increased free fatty acids released from adipose tissue, alcohol is able to suppress PPAR α signaling by decreasing PPAR α binding activity, decreasing protein levels of retinoid-X receptor, and downregulating AMPK. In mice, PPAR α agonists (Wy14643 and clofibrate) override these effects of alcohol on PPAR α signaling and prevent hepatic steatosis (39). Despite the potential utility of PPAR α agonism in alcoholic steatosis, there have been no clinical studies to examine its efficacy in humans with ALD.

SIRT1-AMPK axis

AMPK is a serine-threonine kinase protein complex that helps maintain cellular energy homeostasis. Once activated by allosteric binding of AMP and by phosphorylation via one of its upstream kinases, AMPK rewires metabolism to inhibit anabolic processes and augment catabolism (40). Activated AMPK inhibits lipid synthesis via inhibitory phosphorylation of ACC. Inactivation of ACC leads to decreased levels of malonyl-CoA, an inhibitor of CPT1, which promotes fatty acid oxidation. AMPK also inhibits lipogenesis by inhibiting SREBP-1c and carbohydrate response element-binding protein (ChREBP) as discussed later. In ethanol-fed rodents, AMPK activity is decreased, which increases hepatocellular lipid accumulation through the activation of ACC, SREBP-1c, and ChREBP (41).

Sirtuin 1 (SIRT1) is an NAD⁺-dependent deacetylase that acts as a master sensor of NAD⁺ and modulates cellular metabolism. In the liver, SIRT1 plays a pivotal role in the regulation of lipid metabolism by modifying the acetylation status of various targets (42). The interdependent regulation between SIRT1 and AMPK in hepatic lipid metabolism is well established (43). SIRT1 stimulates AMPK by modulating liver kinase B1 (LKB1), an upstream AMPK kinase;

conversely, AMPK increases cellular NAD⁺ levels, which subsequently activates SIRT1. Hence, the SIRT1-AMPK axis is a central signaling system that controls lipid metabolism. Chronic ethanol administration impairs the hepatic SIRT1-AMPK axis in several animal models (32, 44), thus stimulating lipogenesis. Resveratrol, an agonist of both SIRT1 and AMPK, shows its protective action against hepatic lipid accumulation by stimulating the hepatic SIRT1-AMPK axis in alcohol-fed mice (45).

EFFECTS OF ALCOHOL ON HEPATIC DE NOVO LIPOGENESIS

Besides uptake of fatty acids from extrahepatic sources, hepatocytes can synthesize fatty acids from nonlipid precursors such as sugars and proteins (46). De novo lipogenesis employs several lipogenic enzymes including ACC, FAS, and stearoyl-CoA desaturase 1 (SCD1) that are controlled coordinately by transcriptional factors and metabolic regulators including clock genes (47). In addition, a series of key enzymes including phosphatidyl phosphatases (PAPs) (e.g., lipin) and acyltransferases [e.g., diacylglycerol acyltransferase (DGAT)] are involved in glycerolipid synthesis, which further contribute to triglyceride and phospholipid production (48). Alcohol exposure modulates many of the lipid regulatory factors and lipogenic enzymes and, in doing so, augments hepatic triglyceride accumulation as reviewed below.

SREBP-1c

SREBP-1c is a transcription factor that regulates expression of ACC, FAS, and SCD1 (49). SREBP-1c is implicated in the pathogenesis of alcohol-induced steatosis, as SREBP-1c knockout mice are protected from alcohol-induced hepatic steatosis (50). SREBP-1c protein and its transcripts are increased in the livers of acutely and chronically ethanol-fed mice (51, 52), but unlike the lipogenic genes it regulates (i.e., ACC and FAS), SREBP-1c does not appear to be influenced by alcohol-specific effects on circadian genes (47). The activation of SREBP-1c in ALD is mediated through several pathways. Acetaldehyde directly induces the maturation of SREBP-1c (52). Alcohol also indirectly activates SREBP-1c via multiple factors, including ER stress, adenosine, and endocannabinoid production (53–56). SREBP-1c is also activated by lipopolysaccharide and TNF α , both of which are elevated in ALD patients (57, 58). Finally, alcohol downregulates AMPK, SIRT1, and signal transducer and activator of transcription 3 (STAT3), factors that suppress SREBP-1c expression (32, 44, 59).

ChREBP

ChREBP is a key transcription factor that acts similarly to SREBP-1c, regulating lipogenic target genes (i.e., FAS, SCD1, elongase 6, glycerol phosphate acyltransferase). ChREBP is activated in mice fed chronically with alcohol by a mechanism that involves dephosphorylation of ChREBP through the inhibition of AMPK and protein phosphatase 2A (PP2A) (60). Acute alcohol feeding also increases ChREBP activity

via acetylation by SIRT1 (61). In addition, ChREBP silencing prevents ethanol-induced hepatic steatosis in mice, indicating an important role of ChREBP in steatosis under binge drinking conditions.

PPAR γ

PPAR γ , a member of the nuclear hormone receptor superfamily involved in lipid metabolism (62), also contributes to the pathogenesis of ALD. The role of PPAR γ in ALD is less established than the contributions of other pathways. Namely, ethanol upregulates protein expression of both PPAR γ 1 and PPAR γ 2 isoforms in the steatotic livers of mice (63) and knockdown of hepatic PPAR γ alleviates alcohol-induced lipid accumulation in mouse livers by blunting SREBP-1c and lipogenic genes such as *Fas*, *Dgat1*, and *Dgat2*. However, pioglitazone, a systemic PPAR γ agonist that promotes adipocyte lipogenesis, alleviates hepatic lipid accumulation in alcohol-fed rodents (64, 65). These data demonstrate that there are likely tissue-specific effects of alcohol on PPAR γ that merit further investigation before modulation of this pathway can be recommended as a therapeutic strategy in alcoholic steatosis.

EFFECTS OF ALCOHOL ON GLYCEROLIPID SYNTHESIS

Among a series of enzymes involved in glycerolipid synthesis, lipin-1 has gained attention as one key enzyme explaining the pathogenesis of ALD. Lipin-1 plays dual functions in lipid metabolism as a PAP enzyme and as a transcriptional coactivator depending on its subcellular localization (66). When in the cytoplasm, lipin-1 can transiently translocate to the ER, interact with substrates, and generate diacylglycerol, a precursor of triglycerides and phospholipids. In the nucleus, lipin-1 functions as a transcriptional coactivator to increase fatty acid oxidation by activating PPAR α and PPAR γ coactivator-1 and suppressing SREBP-1c. Lipin-1 subcellular localization can be modulated by an alternative mRNA splicing process that produces two lipin-1 isoforms (α and β) and posttranslational modifications (e.g., phosphorylation, sumoylation, and acetylation) (66).

Accumulating evidence suggests that lipin-1 plays a pivotal role in the development of ALD. Ethanol exposure upregulates total hepatic lipin-1 expression in cultured hepatocytes and mouse livers (67, 68). In particular, ethanol increases cytosolic localization of lipin-1, resultant PAP activity, and triglyceride synthesis, whereas ethanol reduces its nuclear entry and disturbs fatty acid oxidation and VLDL secretion in mouse liver. The expression and localization of lipin-1 are modulated by multiple signaling molecules including miR-217, SIRT1-AMPK, SREBP-1c, and adiponectin in ALD models (67, 69–71). The underlying mechanisms of altered subcellular localization of lipin-1 in response to ethanol involve the increased hepatic ratio of lipin-1 β / α as well as disturbed posttranslational modifications (67, 72).

The final step of triglyceride biosynthesis is catalyzed by DGAT enzymes, whose gene is encoded by *DGAT1* and *DGAT2*. To catalyze the esterification reaction between a fatty-acyl CoA and diacylglycerol, DGAT1 preferentially targets exogenous fatty acids while DGAT2 utilizes mostly de novo synthesized fatty acids (73, 74). Chronic alcohol exposure upregulates hepatic expression of both DGAT1 and DGAT2 in mice (75, 76); however, the specific mechanistic role of DGAT in alcohol-induced triglyceride formation is unknown.

EFFECTS OF ALCOHOL ON HEPATIC LIPID EXPORT

Hepatocytes export neutral lipids by packaging them into VLDL, which prevents intrahepatic triglyceride accumulation. VLDL secretion depends on the availability of hepatic lipids and the hepatocytes' capacity for VLDL assembly. VLDL particles are generated in the ER upon apoB100 lipidation, which is facilitated by microsomal triglyceride transfer protein. Nascent VLDL is then transferred to the Golgi apparatus where mature VLDL is formed.

Alcohol impairs VLDL assembly and secretion. Ethanol-fed rodents have decreased apoB synthesis and hepatic microsomal triglyceride transfer protein expression and activity, effects reversed by pharmacological hepatocyte growth factor administration and PPAR α agonism, respectively (77–79). Alcohol's alteration of methionine metabolism is an additional mechanism by which VLDL secretion is impaired. Namely, ethanol reduces S-adenosyl methionine (SAM) levels. The SAM-dependent enzyme phosphatidylethanolamine methyltransferase (PEMT) is responsible for the production of the VLDL lipid phosphatidylcholine (PC), and PEMT inhibition causes steatosis in mice (80, 81). Consequently, alcohol's inhibitory effects on SAM impair VLDL production indirectly through PEMT inhibition and PC reduction (82).

EFFECTS OF ALCOHOL ON NEUTRAL LIPID STORAGE IN LIPID DROPLETS

In the liver, neutral lipids (triglycerides and cholesterol esters) are stored in lipid droplets (LDs) (83). Compared with the effects on triglyceride metabolism, the effect of alcohol on hepatic cholesterol esters is less well established but may be increased (84). Nascent LDs are thought to form from budding of the outer membrane of the ER, and as such, nascent LDs and outer ER membrane have several membrane proteins in common. The triglyceride synthetic enzyme DGAT is noteworthy (85, 86). The core of hepatocyte LD lipids is enveloped by a phospholipid monolayer of proteins involved in lipid and glucose homeostasis (83, 85). The major hepatocyte LD family of proteins is the perilipin (PLIN) family. In addition to PLINs, LD membranes incorporate a diverse set of surface proteins including proteins involved in LD growth and triglyceride synthesis, the cell death-inducing DNA fragmentation factor- α -like effector (CIDE) protein family (87, 88), and DGAT enzymes (48), respectively. Alcohol's regulation of these LD proteins and

the contribution of LD-associated genetic factors are discussed below.

PLINs

PLIN proteins (formerly PAT proteins, named for perilipin adipocyte differentiation-related protein and tail-interacting protein of 47 kDa) are an ancient family of LD binding proteins whose regulation is important for LD formation and degradation (89). In mammals, there are five isoforms of PLINs (PLIN1–5) with shared sequence homology but with unique tissue-specific roles in LD biology. PLIN1 and PLIN2 are constitutively bound to the LD membrane while PLINs 3–5 are considered exchangeable proteins that can attach to and detach from the LD membrane. Normal liver expresses PLIN2, -3, and -5 (90–94), while there is increased expression of PLIN1, -2, -3, and -5 in fatty liver (93–96). Among the PLIN family proteins, only PLIN2 and PLIN3 have been implicated in alcohol-induced hepatic lipid accumulation. Namely, we and others demonstrated that PLIN2 upregulation occurs in alcohol-fed rodents and humans (93, 97, 98). Our laboratory additionally observed that hepatic PLIN2 upregulation coincides with the onset of steatosis and insulin resistance in an experimental rodent model of chronic alcohol consumption (11) and conversely, that alcohol-fed PLIN2-null mice are refractory to hepatic steatosis and glucose intolerance (98). These findings establish a critical role of PLIN2 in alcoholic steatosis development and alcohol-induced glucose dysregulation, the latter of which is associated with the risk of progressive ALD (6, 99).

In contrast to the constitutively bound PLIN2, PLIN3 is an exchangeable protein, which is stable in the cytosol in the quiescent state and able to be recruited to nascent LDs (100, 101). Although some rodent models have not demonstrated a significant role of PLIN3 in ALD pathogenesis (11, 98), other investigations point to an inhibitory role of alcohol in PLIN3-mediated lipid export from the ER and resultant ER lipotoxic stress (102). These data may suggest a potentially protective role of PLIN3 against ALD, but further studies are warranted to clarify this relationship.

CIDEc

Once nascent LDs form, they can expand via local triglyceride synthesis. So-called macrosteatosis results from the coalescence of smaller LDs, thought to be mediated by the CIDE family of proteins (103). Hepatic expression of *Fsp27/Cidec* (but not *Cidea* and *Cideb*) is upregulated in chronic-plus-binge ethanol-fed mice. Moreover, hepatic expression of FSP27/CIDEc correlates with the degree of steatosis in human alcoholic hepatitis patients (104).

LD-associated genetic factors

Emerging evidence suggests that LD-associated genetic factors account for the susceptibility of alcoholic patients to ALD. Several genome-wide association studies demonstrated that patatin-like phospholipase domain-containing protein 3 (*PNPLA3*), transmembrane 6 superfamily member 2 (*TM6SF2*), and membrane bound *O*-acyltransferase domain-containing 7 (*MBOAT7*) are important genetic de-

terminants of the risk and severity of ALD (105–107). Of these, the *PNPLA3* missense I148M mutation is considered the most important risk allele for the development of ALD and the related condition, nonalcoholic fatty liver disease (108). *PNPLA3* has been shown to have triglyceride hydrolase and transacylase activity, but its physiological role is not completely understood due to discordant findings in mouse models (109–111). The risk variant of *PNPLA3* accumulates on LDs in mice (112) and, in vitro, impairs lipophagy (113).

Like polymorphisms in *PNPLA3*, the rs641738 C>T variant in *MBOAT7* is linked to an increased risk of alcohol-related cirrhosis (108). This *MBOAT7* variant is also associated with an increased risk of steatosis and fibrosis (114). *MBOAT7* is a multi-spanning transmembrane protein and works in the remodeling cycle of phospholipids, involved in the transfer of fatty acids between phospholipids and lysophospholipids (115, 116), but the precise role of *MBOAT7* in hepatic lipid accumulation remains unclear.

A polymorphism of the *PLIN2* gene has been reported (117). A minor allele of this missense polymorphism, Ser251Pro, impairs formation of a *PLIN2* α -helix, causing reduced plasma triglyceride and VLDL levels in humans. Although there is a plausible impact of the *PLIN2* polymorphism on alcoholic fatty liver, its role, like those of the aforementioned polymorphisms, remains unclear.

EFFECTS OF ALCOHOL ON LD CATABOLISM

Catabolism of LDs takes two forms: lipolysis by cytosolic lipases such as *ATGL* and autophagy-mediated mobilization. Autophagy is a recycling process by which cellular components are sequestered within vesicles and fuse with lysosomes for degradation. Autophagic degradation of LDs is referred to as lipophagy (118). Lipophagy has been established to play an important role in experimental alcoholic steatosis (119); however, acute alcohol feeding and chronic alcohol feeding have differential effects on lipophagy. Namely, acute ethanol administration induces autophagy in animal models (120–122), while chronic alcohol inhibits lipophagy. This inhibition of lipophagy by chronic alcohol results from lysosomal damage, suppression of lysosomal proteolytic activity, and inhibition of autolysosome formation (123, 124). These data emphasize the importance of differentiating the acute versus chronic effects of alcohol on lipid metabolism and tailoring potential therapies accordingly.

EFFECTS OF ALCOHOL ON BIOACTIVE LIPIDS

Lipidomics analysis has demonstrated that chronic alcohol consumption promotes the accumulation of both neutral and bioactive lipids within hepatocytes (125–128). Neutral lipids such as cholesterol esters and triglycerides (discussed above) lack a charge, have no signaling properties, and are often enclosed within LDs (129, 130). Bioactive lipids are signaling lipid mediators that effect cellular homeostatic and immune pathways (131). Examples include

fatty acids, ceramides, and phospholipids, all of which have been implicated in ALD pathogenesis.

Fatty acids

Increased hepatic fatty acids are one notable feature in ALD, reported by many lipidomic studies in experimental ALD rodent models (126, 132–135). As mentioned above, there are multiple mechanisms to increase hepatic fatty acids in ALD. Interestingly, alcohol exposure alters not only the quantity but also the composition of fatty acids in the liver. Alcohol shifts the fatty acid composition from saturated fatty acids toward unsaturated (mono- and polyunsaturated) fatty acids in alcohol-fed rodents (126–128, 136). In addition, 18-carbon fatty acids with different degrees of saturation (C18:0, C18:1, C18:2, and C18:3) as well as docosahexaenoic acid (C22:6) are notably elevated in alcohol-exposed livers (126, 132, 133). Even with these consistent findings in hepatic fatty acid composition in ALD animal models and several studies demonstrating efficacy of a saturated fatty acid diet in experimental ALD (137–139), data in humans are lacking and limit progress toward applying dietary strategies to human ALD.

Ceramides


Altered sphingolipid metabolism is an emerging area of ALD pathogenesis. In particular, the bioactive lipid ceramide accumulates in the livers of patients with ALD and in animal models of ALD (140, 141). Ceramides are bioactive lipids that can impair insulin signaling, induce oxidative stress, impair fat oxidation, and increase lipoprotein aggregation (142–144), all of which are linked to the pathogenesis of ALD as discussed above. Ceramide de novo synthesis is modulated by alcohol exposure, and the mRNA levels of three ceramide synthases (*CERS1*, *CERS5*, and *CERS6*) and the major subunit of serine palmitoyl transferase (*SPT*) are increased in human livers with advanced ALD (141). In addition, we have demonstrated that *CERS6* positively regulates LD accumulation and is upregulated in experimental in vivo and in vitro models of ALD as well as in humans with alcoholic steatosis (145). We and others have demonstrated further that pharmacologic inhibition of ceramide synthesis blunts ethanol-induced steatosis and improves glucose tolerance (145–149). Together, these investigations demonstrate that dysregulated sphingolipid metabolism plays a critical role in alcoholic steatosis and ALD pathogenesis and present a novel therapeutic opportunity for ALD management.

Phospholipids

Alcohol exposure disturbs phospholipid metabolism in the livers of ALD patients and animal models (127, 150). Among various phospholipids, PC and phosphatidylethanolamine (PE) are two major phospholipids that are distributed in the plasma membrane. Hepatic PC is made from choline catalyzed by the CDP-choline pathway and is also made by PEMT that converts PE to PC via three sequential steps of methylation (151, 152). Alcohol exposure decreases hepatic PC as well as the ratio of PC/PE in ALD patients and animal models (127, 150). Alcohol-induced

reduction in hepatic PC levels occurs by choline deficiency, decreased PEMT activity, and reduced availability of the methyl groups (153–156). Dietary PC supplementation attenuates ethanol-induced fibrosis in baboons (157, 158); dietary betaine administration also attenuates alcoholic steatosis by promoting PEMT activity in mice (82). Given that the PC/PE ratio determines cellular membrane integrity and plays a role in the development of steatosis and steatohepatitis (159), the ethanol-induced perturbations in phospholipid metabolism are likely to promote the development of ALD.

CONCLUSIONS

In summary, alcohol dysregulates many aspects of hepatic lipid metabolism. Alcohol-induced hepatic fatty acid uptake, impairment of fatty acid oxidation, promotion of de novo lipid synthesis and neutral lipid storage, and inhibition of lipid export and LD catabolism are all pathways that converge to cause hepatocellular LD accumulation. In addition to these relatively well-established mechanisms, several emerging areas of alcohol-induced hepatocellular LD regulation have gained attention. In particular, the role of bioactive lipids, such as sphingolipids, and genetics are likely to unveil novel biomarkers and create opportunities for early therapeutic intervention for those at risk of advanced disease. 

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