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Recent Advances in Understanding Alcohol-Induced Organ Damage Theme Issue

REVIEW

Lipid Droplet Dynamics in Alcoholic Steatohepatitis

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Alcohol-associated liver disease poses a significant global health burden, with alcoholic steatohepatitis (ASH) representing a severe subtype driven by chronic alcohol consumption, hepatic inflammation, and limited treatment options. Central to ASH pathogenesis is the dysregulation of lipid droplet (LD) dynamics in hepatocytes. This review explores the critical role of LDs, focusing on alcohol-induced disruptions in LD biogenesis and catabolism. Chronic ethanol exposure enhances LD biogenesis from lipid import and *de novo* lipogenesis, while impairing LD catabolism by inhibiting lipolysis and lipophagy. Also, the review article examines alcohol's effect on remodeling the LD proteome and lipidome, including post-translational modifications. Additionally, LDs emerge as morphologic markers in hepatic stellate cells, where their loss drives fibrosis. Recent advances highlight potential therapeutic targets, such as restoring lipophagy or modulating LD biogenesis, offering hope for effective ASH treatments. This review underlines LDs as pivotal organelles in ASH progression and therapeutic innovation. *(Am J Pathol 2026, 196: 20–34; https://doi.org/10.1016/j.ajpath.2025.07.010)*

Alcohol-associated liver disease (ALD) is a global public health concern, varying from simple steatosis to more severe steatohepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma.[1,2] These conditions can overlap and coexist simultaneously.[1] Alcoholic steatohepatitis (ASH), a form of ALD, is the most critical and life-threatening form of acute-on-chronic liver failure that develops in individuals with prolonged heavy alcohol consumption.[3–5] In Western countries, particularly the United States and Europe, trends in early-age drinking, binge consumption, and increasing alcohol sales have contributed to a growing incidence of ASH. Mortality related to ALD has escalated, especially among younger adults, with severe ASH carrying a 20% to 50% short-term mortality rate. Geographic disparities are notable, where the European region bears the highest per capita alcohol consumption and related mortality, followed by the Americas.[3,6] Although the precise prevalence of ASH is difficult to determine, it is estimated to occur in 10% to 35% of heavy drinkers with advanced fibrosis. The burden is amplified by comorbid conditions, such as malnutrition, viral hepatitis, obesity, and metabolic syndrome, all of which accelerate disease progression and worsen prognosis.[3,6]

Over 90% of heavy drinkers develop hepatic steatosis, characterized by excessive accumulation of lipid droplets (LDs) with hepatocytes.[7,8] LDs are dynamic organelles responsible for neutral lipid storage in hepatocytes and all mammalian cells.[7,9] They serve critical roles in various essential cellular functions, ranging from energy production, protection against lipotoxicity, and the supply of lipid biomass for the synthesis of cellular membranes.[10] Central to the development of ASH is LD biogenesis, which begins

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at the endoplasmic reticulum (ER), where a host of enzymes catalyze the production of neutral lipids, including triglycerides and cholesterol esters, which collect in a lipid lens between the two leaflets of the ER bilayer, forming the LD neutral lipid core.[7,10] This accumulation leads to the budding of LDs into the cytoplasm, a process facilitated by phase separation and LD assembly proteins like Seipin, which spatially arranges this process.[10] The phospholipid composition and membrane asymmetry of the ER influence the formation of these LDs[7] and the recruitment of LD-resident proteins, such as phospholipase domain containing protein 3 (PNPLA3), adipose triglyceride lipase (ATGL), perilipins (PLINs), and cell death−inducing DNA fragmentation factor α−like effector B (CIDEB) proteins, influence lipid metabolism and accessibility.[7,10] Disruption in the balance between LD formation and degradation leads to hepatic steatosis, which is a major factor in the progression of ASH.[7,10] Below, this review covers the central role of LDs in the pathogenesis of ASH, the role of liver hepatocytes and nonparenchymal cells, liver-organ crosstalk, and potential therapies.

Pathogenesis of Alcoholic Steatohepatitis

The incidence of ALD has been rapidly increasing worldwide, particularly in the United States.[11] It is attributed to 50% of all deaths due to liver diseases in 2022. Studies showed that >20 million Americans, approximately 6% of the US population, have alcohol-use disorders.[1] Patients in the late stages of the disease typically do not respond to pharmacotherapy, which is limited to corticosteroids. Liver transplant is the only treatment option for end stages of ALD; moreover, the procedure is highly selective and limited based on post-transplant survival rate and donor availability, respectively.[2,5] In severe cases, ASH could be life threatening, where prognosis is poor, having mortality rates ranging from 20% to 50% at 28 days and can reach up to 70% at 90 days.[5] ASH typically develops in individuals with prolonged excessive alcohol consumption and is characterized by distinct histopathologic features.[3,5] Steatosis, along with hepatocellular ballooning, indicates significant liver injury.[2,3] Furthermore, oxidative stress can impair hepatocyte secretory functions by disrupting bile formation, resulting in cholestasis.[12,13] A hallmark of ASH is the presence of Mallory-Denk bodies, which are abnormal cytoplasmic inclusions in the hepatocytes, composed of insoluble, misfolded protein aggregates that accumulate primarily in the perinuclear region. They contain eosinophilic material and exhibit immunoreactivity to keratin 8, 18, and p62 due to keratin filament misfolding.[1,3] In ASH, macrovesicular steatosis involves large LDs displacing the nucleus peripherally, whereas microvesicular steatosis features small LDs with a centrally located nucleus. Microvesicular steatosis is linked to a worse prognosis because of greater mitochondrial dysfunction and oxidative stress. On the other hand, neutrophil infiltration plays a critical role in driving inflammation and differentiates ASH from nonalcoholic liver conditions.[10]

Alcoholic steatohepatitis is commonly associated with hepatic steatosis, driven by excessive LD accumulation within hepatocytes because of disruptions in lipid biogenesis and metabolism.[10,14] Chronic alcohol consumption enhances *de novo* lipogenesis (DNL) by increasing NADH/NAD^+ ratios, which suppresses fatty acid oxidation by upregulating sterol regulatory element−binding protein 1c (SREBP-1c) and carbohydrate response element−binding protein (CHREBP), key transcription factors that promote fatty acid synthesis.[14] In addition, ethanol metabolism generates acetate, which promotes triglyceride production.[1,2,7] Alcohol also enhances lipid import from adipose tissue lipolysis via fibroblast growth factor 21 (FGF21), ghrelin, and insulin resistance, resulting in elevated free fatty acid flux to the liver, where uptake via fatty acid transport proteins (FATPs) and CD36 exacerbate lipid accumulation.[7,15] Ethanol also impairs lipid export by disrupting Golgi vesicular trafficking and suppressing microsomal triglyceride-transfer protein (MTP), essential for very low-density lipoprotein (VLDL) assembly, ultimately trapping triglycerides within hepatocytes.[15] Additionally, alcohol suppresses LD catabolism by inhibiting both lipolysis and lipophagy. Alcohol reduces ATGL and hormone-sensitive lipase activity in hepatocytes, impairing triglyceride breakdown (lipolysis), while also inhibiting autophagy via mammalian target of rapamycin (mTOR) complex 1 activation and Ras-related protein Rab-7a (RAB7) dysfunction, preventing lysosomal degradation of LDs (lipophagy)[7,15] (Figure 1). These effects are exponentially worsened in obese drinkers with metabolic dysfunction and alcohol-associated liver disease (MetALD), which amplify DNL due to insulin resistance, increased adipose lipolysis, ER stress response, and further suppression of autophagy.[10,14−17] These mechanisms disrupt metabolic balance, leading to LD accumulation and accelerating the transition from steatosis to steatohepatitis and fibrosis in ALD. In summary, LDs are active organelles, not inert fat blobs. In the alcohol-stressed liver, LDs become oversized, protein locked, autophagy resistant, and poorly connected to mitochondria. This dysfunctional state turns LDs from a safe-storage depot into hubs of lipotoxicity and inflammatory signaling, representing the molecular bridge between hepatic steatosis and the full picture of ASH. A more detailed discussion of these processes and their impact on disease progression are presented below.

Lipid Droplet Biogenesis in Alcoholic Steatohepatitis

Overview of Lipid Droplet Biogenesis

Lipid droplets are cytoplasmic organelles predominantly generated via a process initiated at the ER. Fatty acids

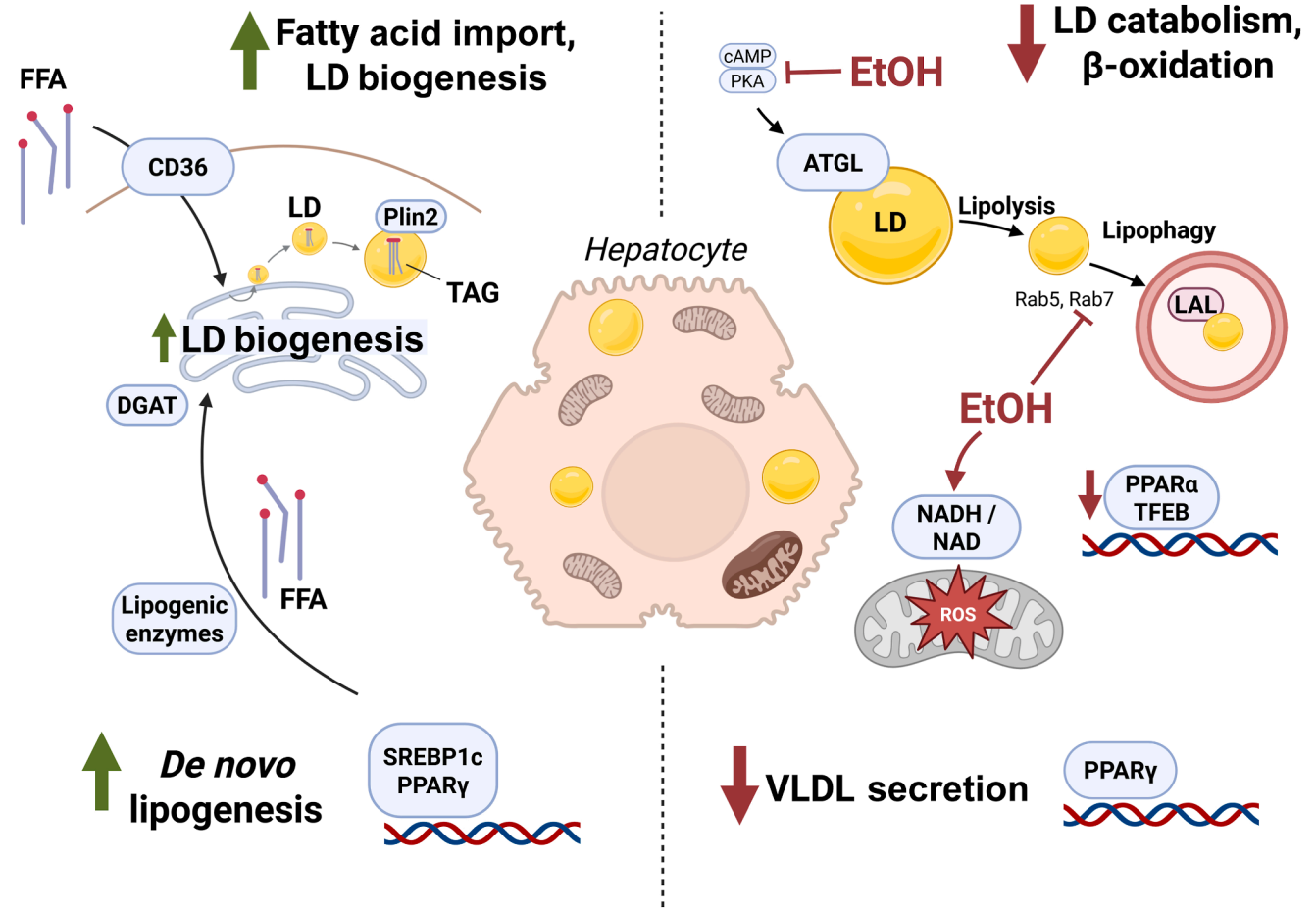

Figure 1 Cellular mechanisms affecting extreme hepatocellular lipid droplet (LD) accumulation in alcohol-associated liver disease. Four distinct mechanisms drive LD accumulation in hepatocytes following alcohol consumption. Fatty acid import by CD36 is accelerated on the basis of increased lipid supply from adipose lipolysis. *De novo* lipogenesis also synthesizes new lipids intracellularly via sterol regulatory element–binding protein 1c (SREBP1c) and peroxisome proliferator-activated receptor (PPAR)-γ transcriptional networks. Both of these pathways result in fatty acid esterificaiton into triacylglycerol in the endoplasmic reticulum during LD biogenesis. At the same time, ethanol (EtOH) decreases LD catabolism by targeting lipolysis, lipophagy, and mitochondrial respiration both directly and through transcriptional down-regulation of mitochondrial and lysosomal gene networks. Finally, lipoprotein assembly and secretion is impaired by alcohol, leading to increased lipid retention within hepatocytes. The figure was created with BioRender.com (Toronto, ON, Canada). ATGL, adipose triglyceride lipase; DGAT, diacylglycerol acyltransferase; FFA, free fatty acid; LAL, lysosomal acid lipase; PKA, protein kinase A; PLIN2, perilipin 2; Rab, Ras-related protein in brain; ROS, reactive oxygen species; TAG, triacylglycerol; TFEB, transcription factor EB; VLDL, very low-density lipoprotein.

sourced from either DNL or extracellular uptake are directed to the ER, where they are converted into neutral lipids consisting mainly of triacylglycerols (TAGs) and cholesteryl esters. The synthesis of TAGs commences with glycerol-3-phosphate acyltransferase and is further catalyzed by diacylglycerol O-acyltransferase (1 and 2), whereas acetyl-CoA acetyltransferase (1 and 2) facilitates the formation of cholesteryl esters. Neutral lipids accumulate between the two leaflets of the ER bilayer, where they eventually exceed a critical concentration that triggers phase separation and the nucleation of a neutral lipid lens (structures typically measuring 30 to 60 nm in diameter in yeast).[18] ER-resident proteins, particularly Seipin and its yeast orthologue SEI1 (FLD1), play pivotal roles during this nucleation phase by forming a decameric, cage-like assembly that promotes lipid demixing.[19,20] Their interacting partner promethin lipid droplet assembly factor 1 (LDAF1), along with factors such as Achaete-Scute family of basic helix-loop-helix (bHLH) transcription factor and peroxisomal biogenesis factor, further delineates nucleation sites by modulating local membrane curvature and lipid composition.[21–23]

Beyond nucleation, LD biogenesis advances through distinct phases of growth and cytoplasmic budding.[24] The bidirectional budding of LDs toward the cytoplasm or the ER lumen (where they will become secreted lipoproteins) is orchestrated by an interplay of membrane tension, phospholipid asymmetry, and curvature conditions that favor the directionality of emergence.[25–27] Proteins, such as lipid phosphate phosphatase (FIT2), potentially acting as a lipid phosphatase, help regulate cytoplasmic budding by converting phosphatidic acid to diacylglycerol, thereby offsetting local phospholipid depletion.[28] Additional mechanisms contribute to LD growth, including Ostwald ripening and protein-mediated fusion via the cell death-inducing DFF45-like

effector (CIDE) family, alongside the formation of membrane bridges through ADP-ribosylation factor 1 and coat protein complex I (ARF1-COPI) complexes that facilitate the transfer of lipid-synthesizing enzymes [eg, glycerol-3-phosphate acyltransferase 4 (GPAT4)] from the ER to the LD surface.[29–32] Recent studies also underline that membrane curvature itself can catalyze LD assembly, highlighting the critical influence of biophysical membrane properties on LD formation.[33]

De Novo Lipogenesis in Alcoholic Steatohepatitis

DNL is the metabolic pathway through which acetyl-CoA is converted into fatty acids from nonlipid precursors, such as carbohydrates, proteins, or even alcohol itself.[34–37] In the context of ASH, acceleration of DNL is a key pathologic feature contributing significantly to hepatic steatosis and liver injury.[38] Central to this process are transcription factors, such as sterol regulatory element–binding protein 1c (SREBP1c) and carbohydrate response element–binding protein (CHREBP), which on activation, enhance the expression of lipogenic enzymes, including acetyl-CoA carboxylase and stearoyl-CoA desaturase 1.[39–41] These enzymes play critical roles in promoting lipid synthesis and accumulation within hepatocytes. In ASH, several factors exacerbate the up-regulation of DNL. Concurrently, hepatic cytochrome P450 2E1 enzyme activity increases reactive oxygen species production, which further stimulates DNL. Mitochondrial dysfunction also plays a role, as silencing mitochondrial carrier homolog 1 rescues mitochondrial activity, lipid β-oxidation, and reduces DNL, offering a potential therapeutic target for ASH.[42] Inhibition of acyl-CoA synthetase short-chain family member 2 has been shown to attenuate alcoholic liver steatosis by epigenetically regulating DNL.[43]

Dietary factors also modulate DNL in ASH. Studies indicate that carbohydrate intake influences DNL more than alcohol itself, with sugar-rich diets enhancing DNL and hepatic triglyceride accumulation.[44] Conversely, omega-3 fatty acids suppress hepatic DNL while promoting fatty acid β-oxidation.[20] Hepatic DNL is further modulated by circadian regulation; loss of nuclear receptor corepressor 1 gene (*NCOR1*) promotes fatty acid β-oxidation but exacerbates alcohol-induced liver injury through monocyte infiltration.[45] Additionally, molecular interventions targeting lipid metabolism show promise in mitigating ASH. For instance, inhibiting the DEP domain-containing mTOR-interacting (DEPTOR) protein, a protein involved in the mTOR signaling pathway, alleviates hepatic steatosis by reducing lipogenesis in ASH.[46] Liver-specific ceramide reduction has demonstrated efficacy in reducing steatosis and improving insulin sensitivity in alcohol-fed mice.[47]

Epigenetic factors are also critical for lipogenesis during ASH pathogenesis, as alcohol dysregulates various histone deacetylases. For example, sirtuin1 (SIRT1) is an NAD^+-dependent histone deacetylase and is inhibited by alcohol-induced decreases in NAD^+/NADH ratios.[48] This results in hyperacetylation and activation of SREBP1c and CHREBP to stimulate lipogenesis. Furthermore, SIRT1 deficiency also contributes to hepatic inflammation during ALD.[49]

Contribution of Adipose Lipolysis in Alcoholic Steatohepatitis

Although the liver is the primary site of ethanol metabolism and ethanol-induced injury, it is well established that adipose tissue is significantly affected by ethanol.[50] ALD progression marked by hepatic steatosis has been related to increased lipolysis in adipose tissue, from which 60% of hepatic fatty acids have been traced.[51] Adipocytes catabolize TAG via lipolysis and distribute the resulting glycerol and nonesterified fatty acids to peripheral tissues in response to catecholamine stimulation. Thus, ethanol activation of adipose lipolysis is a major driver of hepatic steatosis and liver injury in ALD.

One of the mechanisms linking ethanol consumption to adipose lipolysis is FGF21, which is secreted from the liver and is markedly up-regulated following ethanol consumption. FGF21 targets several tissues, one of which is the brain, where it activates sympathetic neuronal circuits that release catecholamines, thereby activating adipose β-adrenergic receptors.[52] This stimulates cAMP production and activation of protein kinase A (PKA) to phosphorylate and activate lipolytic enzymes PLIN1 and hormone-sensitive lipase.[52] PLIN1 phosphorylation activates lipolysis by inhibiting the interaction between PLIN1 and comparative gene identification-58 (CGI-58) protein. Dissociation of this complex allows CGI-58 to interact with and activate the cytosolic lipase ATGL.[53,54] Notably, the absence of FGF21 attenuates alcohol-induced lipolysis in adipose tissue.[52] Although FGF21 exacerbates adipose tissue lipolysis contributing to hepatic steatosis, other reports have observed FGF21 can also serve a protective role when acting on hepatocytes, mitigating ethanol-induced hepatic damage.[55] In addition to FGF21, other endocrine factors are known to activate lipolysis in adipose tissue during ALD. For example, adipose insulin receptors negatively regulate lipolysis, but in the context of insulin resistance, insulin sensitivity is diminished, leading to higher lipolysis activity in adipose tissue.[56] The pancreatic hormone ghrelin also increases adipose lipolysis via insulin signaling, as ghrelin levels are markedly increased by ethanol to reduce pancreatic insulin secretion.[57] Chronically high levels of ghrelin decrease insulin sensitivity in adipose tissue, exacerbating adipose lipolysis and alcohol-associated steatosis (Figure 2).

Epigenetic mechanisms also influence adipose lipolysis in ALD. Ethanol consumption affects methionine metabolism characterized by increased S-adenosylhomocysteine (SAH) levels and a consequent decrease in S-adenosylmethionine/SAH ratio and methylation portential.[58] This contradicts previous studies where hypermethylation was observed in patients with ASH and details that alcohol-induced changes in the liver may affect methylation

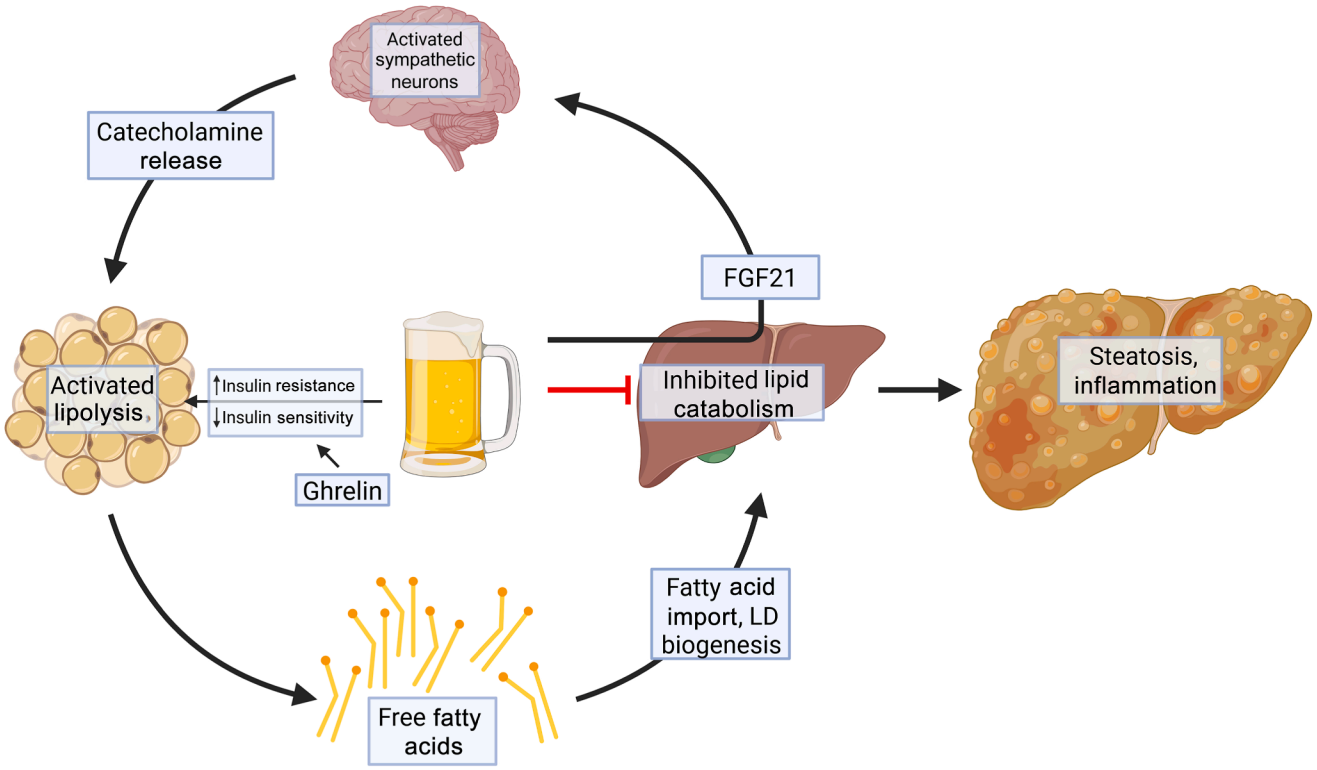

Figure 2 Overview of adipose-liver lipid trafficking driving steatosis in alcohol-associated liver disease. Alcohol consumption leads to lipid accumulation in liver through the activation of lipolysis in adipose tissues. Both fibroblast growth factor 21 (FGF21) and ghrelin facilitate this through two distinct mechanisms: FGF21 stimulates catacholamin release via sympathetic neurons, whereas ghrelin decreases insulin sensitivity in adipose tissues. Insulin resistance, especially in metabolic dysfunction and alcohol-associated liver disease (MetALD), also contributes to activated lipolysis during alcohol exposure. Lipolysis in adipose tissues releases free fatty acids into the bloodstream, which are taken up by the liver, thus increasing hepatocellular lipid import. Simultaneously, alcohol directly inhibits lipid catabolism in liver, leading to excessive lipid storage, which propogates steatohepatitis. The figure was created with BioRender.com (Toronto, ON, Canada). LD, lipid droplet.

processes beyond the availability of methyl groups. For example, alcohol-induced oxidative stress could alter patterns in DNA methylation. Alternatively, increases in DNA methylation may be gene specific or genome-region specific rather than a global increase despite having an overall lower S-adenosylmethionine/SAH ratio. Regardless, lowered methylation potential has been shown to increase hormone-sensitive lipase activity to promote lipolysis in adipose tissue, leading to liver steatosis.[59,60] Indeed, an increase in SAH levels alone can mimic the deleterious pathologic changes in adipose tissue seen with alcohol consumption (increased adipocyte lipolysis, decreased intracellular triglycerides, and reduced LD size).[61] Increased SAH levels also mimicked steatosis changes observed in hepatocytes, like those seen following alcohol exposure (increased hepatocellular triglyceride accumulation, larger LD size, and increased protein expression of genes involved in lipogenesis and fatty acid mobilization).[61]

Decreased Lipoprotein Secretion in Alcoholic Steatohepatitis

In ASH, aberrant lipoprotein assembly and secretion play a central role in the development of hepatic steatosis by blocking lipid exports. Alcohol impairs the secretion of VLDL, a crucial pathway for exporting neutral lipids, and down-regulates microsomal triglyceride transfer protein activity.[62] This inefficiency leads to the intracellular accumulation of cytoplasmic LDs, thereby exacerbating fat deposition within the liver.[63] Although hepatic steatosis is generally linked to enhanced VLDL lipidation and the formation of larger VLDL particles,[64] the compensatory mechanism appears to be compromised in ASH, further contributing to lipid overload and liver injury.[63]

Concomitantly, ASH is also characterized by a marked reduction in high-density lipoprotein (HDL) levels, likely stemming from impaired production of apolipoprotein A1.[63,65] Under physiological conditions, the liver secretes lipid-poor apolipoprotein A1 and immature HDL particles, which undergo lipidation and maturation in the circulation, a process critically dependent on lecithin-cholesterol acyltransferase to convert free cholesterol into cholesteryl esters, thereby forming large, mature HDL particles. In ASH, a shift toward smaller HDL particle size, because of a disproportionate reduction in large- and medium-sized HDL particles, suggests that HDL lipidation or lecithin-cholesterol acyltransferase—mediated maturation is impaired. Moreover, the accumulation of lipoprotein Z, a

Table 1 LD-Associated Phosphopeptides in AH versus Normal Liver

LD protein*	Proposed LD role	Phosphorylated protein site†	Total protein (AH vs normal)†	Citation
Higher phosphorylation in AH vs normal liver ($P < 0.05$)				
ACSL3	LD biogenesis	T688	No change	100
ANXA2	Lipid import, HCV assembly	T19, Y24	Higher	101
ATG2A	Autophagy, LD biogenesis	S403	Lower	102
CAV1	LD biogenesis	S37	Higher	103
NDE1	LD trafficking	S306	No change	104
PRKDC	Unclear	T2671, S2672	No change	
SQSTM	Lipophagy, autophagy	S24, S28, Y148, S152, S226, S233, S249, T269, S403	Higher	105,106
Lower phosphorylation in AH vs normal liver ($P < 0.05$)				
ACACA	*De novo* lipogenesis	S29, S80	Lower	107,108
ALDOA	*De novo* lipogenesis?	S36	Higher	109
GPAT3	LD biogenesis	S66	No change	110
LASP1	Cholesterol trafficking	S61, S146	Higher	111
PLIN1	Lipolysis	S130, S382	Lower	112
PLIN5	Lipolysis, LD-Mito tethering	S277	Not reported	113
RAB13	Unclear	S178	Higher	
RAB18	LD fusion, autophagy	S144	Lower	114
RAB7A	Lipophagy	S72	Lower	80,82
SQSTM	Lipophagy, autophagy	S272	Higher	105,106
WIPI2	Autophagy, LD biogenesis	S413	Higher	102

*Curated list of LD proteins based on Bersuker et al.[99]
†Proteomics data from Hardesty et al.[98]
AH, alcoholic hepatitis; HCV, hepatitis C virus; LD, lipid droplet.

hepatotoxic lipoprotein enriched with free cholesterol, further aggravates liver injury and correlates with poor 90-day clinical outcomes.[66] Together, these disruptions in VLDL and HDL metabolism underline the pathogenic link between decreased lipoprotein secretion and the progression of hepatic steatosis into ASH.

Lipid Droplet Catabolism in Alcoholic Steatohepatitis

Overview of Lipid Droplet Catabolism

Lipid droplet catabolism is accomplished by two synergistic pathways in hepatocytes and other cell types. First is lipolysis, which is induced by activation of the cAMP-PKA pathway, typically via G-protein–coupled receptor (GPCRs; ie, β-adrenergic or other $G\alpha_s$-linked GPCRs). Through a series of phosphorylation events, cytoplasmic lipases, such as ATGL, are recruited to the LD surface and bind with stimulatory cofactors, like abhydrolase domain-containing 5 and lysophosphatidic acid acyltransferase, also known as CGI-58. This action liberates fatty acids from TAGs that are effluxed and transported to mitochondria of neighboring cells for β-oxidation.[67] The second major pathway of LD catabolism is lipophagy, which is the autophagic degradation of LDs by lysosomal acid lipase within acidic vesicles, such as lysosomes, autolysosomes, multivesicular bodies, and late endosomes. Lipophagy can be categorized into two subtypes—macrolipophagy, which uses autophagosomes that target LDs and are acidified by lysosomal fusion; and microlipophagy, which is autophagosome independent and uses direct LD interactions with endolysosomes. As discussed below, alcohol consumption drastically alters each of these pathways uniquely to advance steatosis and steatohepatitis.[68–70]

Alcohol Impairs Hepatocellular Lipolysis

Lipolysis is inducible through GPCRs that activate the cAMP/PKA pathway. As discussed in *Contribution of Adipose Lipolysis in Alcoholic Steatohepatitis*, alcohol consumption increases circulating levels of catecholamines (the ligand for β-adrenergic GPCRs) to activate lipolysis in adipose tissue. Despite this, alcohol paradoxically inhibits lipolysis in hepatocytes by reducing GPCR downstream signaling events. For example, ethanol increases hepatocellular levels of the enzyme phosphodiesterase to stimulate cAMP hydrolysis to AMP, thereby significantly decreasing

Table 2 LD-Associated Phosphopeptides in AC versus Normal Liver

LD protein*	Proposed LD role	Phosphorylated protein site†	Total protein (AC vs normal)†	Citation
Significantly higher phosphorylation in AC vs normal liver ($P < 0.05$)				
ACSL3	LD biogenesis	T688	Higher	100
ANXA2	Lipid import, possibly HCV assembly	T19	Higher	101
CAV1	LD biogenesis	S37	Higher	103
LASP1	Cholesterol trafficking	S146	Higher	111
NDE1	LD trafficking	S306	No change	104
RAB7A	Lipophagy	S72	No change	80,82
RAB8A	LD fusion, LD-mitochondria tethering	S185	No change	115,116
Significantly lower phosphorylation in AC vs normal liver ($P < 0.05$)				
ACACA	*De novo* lipogenesis	S29	Lower	117
PLIN1	Lipolysis	S130, S382	Lower	112
PLIN5	Lipolysis, LD-mitochondria tethering	S277	Not reported	113
SQSTM	Lipophagy, autophagy	S403	No change	105,106
WIPI2	Autophagy, LD biogenesis	S413	Higher	102

*Curated list of LD proteins based on Bersuker et al.[99]
†Proteomics data from Hardesty et al.[98]
AC, alcoholic cirrhosis; HCV, hepatitis C virus; LD, lipid droplet.

cAMP levels.[71] In addition, ethanol consumption seems to have a direct effect on PKA enzymatic activity. In a previous study, PKA phosphorylation events were measured in primary hepatocytes from rats fed a 6-week control versus ethanol diet.[72] PKA phosphorylation was diminished greatly in ethanol-fed hepatocytes, even when treated with 8-Br-cAMP, a nonhydrolyzable analogue of cAMP.[72] It is noteworthy that the direct effects of ethanol on PKA activity have also been observed in lung epithelial cells, where longer treatment with ethanol inhibits PKA-dependent ciliary beating.[73] In total, ethanol inhibition of the cAMP/PKA pathway impacts phosphorylation of lipolytic enzymes and inhibits the trafficking of ATGL to the LD surface[72] (Figure 1). In addition to disrupting lipolysis, alcohol further exacerbates LD accumulation by impairing lipophagy, another critical pathway for lipid degradation in hepatocytes.

Alcohol Impairs Hepatocellular Lipophagy

Ethanol consumption seems to inhibit both macrolipophagy and microlipophagy in hepatocytes. Macrolipophagy uses double-membrane autophagosomes to target LDs for degradation, likely through selective autophagy receptors, such as sequestosome 1 (SQSTM1/p62), which bind to ubiquitinated cargo on the LD surface, thereby recruiting microtubule-associated protein 1A/1B light chain 3−positive autophagosomes. Chronic ethanol exposure is generally accepted to inhibit autophagic degradation of cargo in hepatocytes, but the mechanism seems to differ somewhat depending on the model system. For example, a net decrease in autophagic flux was observed in hepatocytes from rats fed a 6-week ethanol diet versus isocaloric control diet.[74] In the National Institute on Alcohol Abuse and Alcoholism model of 10-day ethanol + 1 binge, autophagic flux appeared to be accelerated, but was deemed as insufficient autophagy because of a severe block in lysosome biogenesis via transcription factor EB[75,76] (Figure 1). Mechanistically, ethanol activates mTOR complex 1 to prevent transcription factor EB from entering the nucleus and activating its coordinated lysosomal expression and regulation (CLEAR) gene network of lysosomal genes in ethanol-fed hepatocytes. This prevents macrolipophagic degradation of LDs as well as autophagy of mitochondria and other cargoes also contributing to LD accumulation in hepatocytes.

Microlipophagy occurs independent of macroautophagy machinery as LDs can be directly engulfed by lysosomes, multivesicular bodies, and late endosomes.[77] This process is complex and requires LD targeting, trafficking, and engulfment by a variety of intracellular vesicles. It is likely that the vesicle trafficking family of Ras-associated binding (RAB) GTPases is at the forefront of this process, although RAB GTPases can also facilitate macrolipophagy. For example, RAB10 can direct light chain 3−positive autophagosomes to the LD surface via its interaction with endocytic machinery.[78,79] It is likely that microlipophagy is mediated by endocytic vesicle trafficking, especially the late endosomal RAB7, which marks endocytic maturation and fusion with acidic lysosomes.[80] Recent work has also demonstrated that RAB5 operates upstream of RAB7, possibly serving in the initial targeting of LDs during the early stages of microlipophagy.[81] Mechanistically, ethanol's action on lipophagy is thought to occur primarily through the inhibition of RAB7 GTPase activity to prevent lysosomal degradation LDs.[82] Ethanol did not impact RAB5 GTPase activity, but the current model suggests that ethanol inhibition of RAB7 leads to a dramatic

accumulation of RAB5-positive early endosomes on LDs, suggesting an RAB5-to-RAB7 conversion process is needed for hepatocellular microlipophagy[81] (Figure 1). In support of this model, a recent study showed that active RAB7 may serve as potential therapeutic to restore lipophagy in response to ethanol insult.[83] However, another compelling model for microlipophagy is the direct injection of lipids from LDs into lysosomes, demonstrated by both live-cell microscopy and electron microscopy, but the impact of ethanol on this process is unclear.[84] Future studies are needed to further define the interactions between LDs and autophagosomes, endosomes, and lysosomes, and to test the relative prevalence of macrolipophagy versus microlipophagy following ethanol insult *in vivo*. Beyond impairing lipid degradation pathways like lipophagy, chronic alcohol exposure also disrupts the structural and functional integrity of LDs themselves through extensive remodeling of their protein and lipid composition.

Alcohol Remodeling of Lipid Droplets: Proteins, Lipids, and Post-Translational Modifications

Altered Lipid Droplet Proteome in Alcoholic Steatohepatitis

Lipid droplets have a unique and diverse proteome that facilitates LD growth, fusion, trafficking, and catabolism. Recent studies demonstrate that chronic ethanol consumption can alter this proteome, leading to LD dysfunction. For example, proteomic analysis of liver LDs from rats fed a 6-week chronic ethanol diet versus isocaloric control diet (as well as postethanol recovery with or without fasting)[85] showed that chronic ethanol consumption modified 338 distinct LD membrane-associated proteins and caused significant alterations in the LD proteome network. Proteomic analysis and Western blot analysis reconfirmation revealed up-regulation of genes involved in cholesterol and steroid biosynthetic pathway, such as hydroxysteroid 17-β dehydrogenase (*HSD17B7/11/13*), squalene monooxygenase (*SQLE*), and 7-dehydrocholesterol reductase (*DHCR7*), resulting in fatty liver in ethanol-fed animals, whereas the levels of proteins associated with the LD breakdown were significantly reduced, including the cytoplasmic lipase ATGL and the endosome/lipophagy protein RAB5. Contrastingly, refeeding or fasting ethanol fed reversed some of these effects. Fasted animals showed a twofold decrease in lipogenesis-related proteins, with a concomitant increase in proteins promoting β-oxidation and lipophagy.[85]

As LD size is implicated in LD dynamics and degradation, another interesting study analyzed the proteome of large-, medium-, and small-sized LDs isolated from the livers of ethanol-fed rats.[86] Large-sized LDs displayed higher amounts of proteins associated with fatty acid synthesis, such as ATP citrate lyase, fatty acid synthase, acyl-CoA synthetase long-chain family member 4, and 1-acylglycerol-3-phosphate O-acyltransferase 2; fatty acid transport, such as fatty acid translocase (CD36), solute carrier family 27, and sterol carrier protein (SCP2); and lipolysis inhibitors compared with smaller-sized LDs.[86]

Altered Lipid Droplet Lipidome in Alcoholic Steatohepatitis

A study performed by Arumugam et al[87] precisely analyzed the LD lipidome and quantified alterations in these organelles along the progression of ethanol-induced steatosis. Chronic ethanol diet markedly modified the lipid composition of LDs, including phospholipids and glycerophospholipids in LDs of differing sizes. Furthermore, elevated concentrations of sphingolipids were observed, including a 2.7-fold increase in ceramide (Cer) C18:1 (24:0), an 18.8-fold increase in hexosylceramide (HexCer) C18:1 (20:0), and a 15-fold increase in HexCer C18:1 (22:0) across all fractions of LDs from the livers of ethanol-fed rats. Also, the increment was observed, particularly in 16- and 18-carbon fatty acids, including C (16:1), C (18:0), and C (18:3), as well as eicosatrienoic acid C (20:3) and docosahexaenoic acid C (22:6), in the LD fractions of ethanol-fed rats in comparison to control subjects. Few studies have examined phospholipid architecture and noted a decrease in phosphatidylcholine levels in the livers of ethanol-fed rats. This study showed that larger LDs exhibited a significantly lower phosphatidylcholine/phosphatidylethanolamine ratio compared with the smaller LDs. Moreover, this ratio was markedly reduced in LD fractions from ethanol-fed rats in comparison to their pair-fed counterparts.[87] Prior research demonstrated that the phosphatidylcholine/phosphatidylethanolamine ratio governs LD size, which subsequently affects the accessibility of lipases to the TAG reserves inside the LDs.[88,89] Together with proteomic and lipidomic remodeling, post-translational modifications (PTMs) of LD-associated proteins represent an additional layer by which alcohol disrupts LD function and turnover in ASH.

Post-Translational Modification of Lipid Droplet Proteins in Alcoholic Steatohepatitis

Protein modifications by alcohol have long been attributed to liver injury during ALD. For example, reactive oxygen species—generated malondialdehyde reacts with the ethanol metabolite acetaldehyde to form numerous protein adducts in hepatocytes.[90] Ethanol can also alter protein acetylation, especially tubulin, which can impact several pathways related to LD trafficking.[91] On the LD surface, there is a specific proteome that can be heavily influenced by PTMs, including phosphorylation, ubiquitylation, acetylation, and lipidation. Given the impact of alcohol on PTMs in other hepatocellular compartments, an area of growing interest is the impact of alcohol on PTMs of the LD proteome.

Lipid droplet proteins are highly sensitive to ubiquitylation, which drives selective degradation of LD proteins.[92] Polyubiquitin chains are added to specific LD resident proteins, which are then retrotranslocated to the proteasome for degradation via the protein segregase p97/valosin-containing protein (VCP). Ubiquitin PTMs can also signal protein catabolism by lysosomes. A recent study linked ALD to decreased proteasome and lysosome activity in hepatocytes, leading to the accumulation and stabilization of many ubiquitylated proteins on the LD surface identified by mass spectrometry.[93] One of these heavily ubiquitylated proteins was the steroidogenic enzyme hydroxysteroid 17-beta dehydrogenase 11 (HSD17B11), which is stabilized to the LD surface and displaces ATGL, the major triacylglycerol lipase in hepatocytes, resulting in diminished lipolysis and LD accumulation in ALD.[93] This supports previous work demonstrating a reduction in ATGL recruitment to LDs during ALD.[72] ATGL itself can be modified post-translationally. Although various phosphorylation sites have been identified previously, a recent study in nonalcoholic fatty liver in mice discovered a novel PTM site on ATGL, whereby Cys15 is palmitoylated by the acyltransferase enzyme zinc finger DHHC-type containing 11 (zDHHC11).[94] Interestingly, Cys15 palmitoylation was not necessary for ATGL localization to the LD, but ATGL (C15S) mutants were catalytically inactive, similar to enzymatically dead ATGL (S47A).

Phosphorylation of LD proteins is a critical regulator of LD biology. Canonically, PKA phosphorylation is central cAMP-stimulated lipolysis. In ALD, cAMP activity is severely down-regulated, with reduced PKA phosphorylation of substrates as well as reduced cAMP levels by up-regulation of phosphodiesterase 4.[71,72,95–97] This limits lipolytic activity in ALD and leads to steatosis. It is likely that several LD-resident proteins undergo phosphorylation on the LD surface, although the complete phosphoproteome of LDs has not been defined to date. However, a recent phosphoproteomics study profiled liver biopsies from a cohort of patients with alcoholic hepatitis (AH) and alcoholic cirrhosis.[98] Although this study focused on top hits related to protein secretion and fibrosis in ALD, several known LD-associated proteins were also identified in their study. Cross-referencing their phosphoproteomics data set with a modified list of high-confidence LD-associated proteins[99] identified several phosphopeptides of LD-associated proteins that were significantly altered in ASH or alcoholic cirrhosis compared with normal liver biopsies. A summary table for significantly altered phosphorylation sites is provided in Table 1[82,98–114] (AH versus normal) and Table 2[80,82,98–106,111–113,115–117] (alcoholic cirrhosis versus normal). Two caveats must be considered in these results. First, although these proteins are found on LDs based on proteomic studies, many of them have other functions in cells that may not be directly related to LD biology. The second caveat is that changes in phosphorylated peptide abundance may be a function of similar changes in total protein levels. Thus, of special interest are significantly altered phosphopeptides with no change, or opposite change, in total protein levels (Tables 1 and 2). For example, the phosphorylation of the LD biogenesis protein glycerol-3-phosphate acyltransferase 3 on serine 66 was greatly reduced in AH versus normal liver, with no change in total protein abundance in these samples. Glycerol-3-phosphate acyltransferase 3 Ser66 has been implicated as a putative phosphotarget in insulin signaling,[110] but its exact role in ALD is yet to be defined. The autophagy receptor SQSTM1/p62 also showed a modest but significant reduction in phosphorylation of serine 403 in alcoholic cirrhosis versus normal liver, with no change in total protein levels. As Ser403 phosphorylation enhances SQSTM1/p62 ubiquitin binding, this may provide a potential mechanistic insight into reduced autophagy in ALD. Conversely, SQSTM1/p62 has many phosphopeptides in AH, which could suggest a hyperphosphorylated state in AH versus cirrhosis. It is noteworthy that the function of many of these phosphosites is unknown, and future studies will be necessary to validate these hits and identify any putative roles for these phosphorylated LD proteins along the lifecycle of LD biogenesis, fusion, trafficking, and catabolism during ALD. In addition to hepatocytes, LD also plays a critical role in other liver cell types, particularly hepatic stellate cells (HSCs), as discussed below.

Lipid Droplets as Distinct Morphologic Marker of Hepatic Stellate Cells

HSCs are nonparenchymal cells of the liver, originally named fat-storing cells, which regulate inflammation, fibrosis, and tissue repair. HSCs have unique cytoplasmic LDs serving as the storage site for retinoids (vitamin A).[118,119] Retinyl esters and TAGs are the most abundant lipids found at similar concentrations within HSC LDs, surrounded by a phospholipid layer with an embedded surface LD-associated protein called PLIN, which helps control LD lipolysis.[120] In response to ASH, quiescent HSCs undergo activation, which regulate hepatocyte injury and inflammation that precedes liver fibrosis.[121] Chronic alcohol exposure induces oxidative stress and inflammation, which contributes to LD loss and depletion of hepatic retinoid in HSCs through induction of cytochrome P450 family 2 subfamily E member 1, which accelerates HSC activation and transformation to fibrogenic myofibroblast-like cells, leading to fibrosis, thereby exacerbating liver damage.[122,123]

According to lipidomic analysis, HSCs have two distinct metabolic LD pools: an original pool within quiescent HSCs and a new generated pool of LDs emerging on HSC activation.[118–120] In the quiescent HSCs, the LD pool consists of large LDs containing mainly retinyl esters and TAGs, which are localized mainly to the perinuclear area where LDs are degraded by lysosomes with a half-life of a

few days.[124] In the activated HSCs, the LD quantity is increased but is significantly smaller in size. This pool of small LDs is enriched mainly with TAGs, containing polyunsaturated fatty acids with less retinyl ester content. Furthermore, these LDs are mainly localized to the cell periphery and are less resistant to lysosomal degradation, with a turnover rate of <8 hours.[125] As a therapeutic implication, targeting lipid metabolism by restoring LD function and preventing their depletion in HSCs could offer new treatment strategies for ASH, which may modulate HSC activation and mitigate liver fibrosis. This insight opens new avenues for developing antifibrotic therapies for ASH.

Future Directions: Cellular Mechanisms and Therapeutics

Lipid droplets will continue to be an exciting area for mechanistic research into the cell biology of ALD. One interesting area that has recently been clarified is the role of LD-mitochondria contacts, which are highly prevalent in hepatocytes and best appreciated by high-resolution electron microscopy. Until recently, it was assumed that LD-mitochondrial contacts facilitated lipid transport into mitochondria following lipolysis to complete the next step of β-oxidation. However, recent studies have emerged in liver and adipose showing that LD-mitochondria contacts play a larger role in lipid biosynthesis, as opposed to lipid catabolism.[126–128] In fact, it is the cytoplasmic mitochondria, detached from LDs, that exhibit higher levels of lipid oxidation. Future studies will be needed to understand this dynamic in the context of ALD, and whether the hyperfused megamitochondria characteristic of ALD aid in LD biogenesis.[129] In addition, the activity of lipophagy *in vivo* remains unclear, particularly the balance between lipolysis, microlipophagy, and macrolipohagy. These studies would be particularly interesting in the context of microsteatosis and macrosteatosis, as LD catabolism is heavily influence by LD size.[70] Third, greater clarity is needed regarding the mechanisms and contribution of LDs in nonparenchymal liver cell types, especially resident and nonresident immune cells, in the context of ALD. Finally, given the obesity epidemic worldwide, expanding the use of mouse models combining alcohol with high-fat diet (MetALD) has the potential to enhance the ability to define effective therapeutic targets.[130]

Alcoholic steatohepatitis remains associated with high mortality, yet treatment options remain limited. Notwithstanding variations in origins, ASH and metabolic dysfunction–associated steatohepatitis (MASH) exhibit several parallels that could open the possibility for MASH drug repurposing in the treatment of ASH. For example, resmetirom is a liver-specific thyroid hormone receptor β agonist that has recently obtained US Food and Drug Administration clearance as the first pharmaceutical intervention for MASH.[131] Resmetirom's liver specificity prevents deleterious thyromimetic consequences on other thyroid hormone–responsive organs, including the cardiovascular system, bone, and pituitary gland. Resmetirom's mechanism of action in MASH could have a parallel effect in ASH by reversing liver steatosis and fibrosis.[132] Other promising candidates, including the diacylglycerol O-acyltransferase 2 (DGAT2) target (ervogastat), which inhibits LD biogenesis, as well as the acetyl-CoA carboxylase (ACC) target (clesacostat), which inhibits DNL, can prove to be an effective intervention to resolve MASH with fibrosis.[133] More recent studies indicate that ATGL inhibition could also serve as a MASH therapeutic, likely through protection against lipotoxicity.[134] These therapeutic strategies can be successfully repurposed to treat ASH.

Pharmacotherapy for ASH has largely remained unchanged since corticosteroids were introduced in the 1970s.[135] However, emerging therapeutic strategies are being explored, including autophagy inducers, such as rapamycin analogs, which may help counteract ethanol-induced lipid accumulation by enhancing autophagic flux. Additionally, antioxidants that target oxidative stress could mitigate mitochondrial dysfunction and support lipophagy, whereas mTOR inhibitors aimed at restoring autophagic activity by targeting mTOR complex 1 may also offer therapeutic benefits.

Currently, several clinical trials are investigating novel targeted therapies for ASH, with multiple approaches, including the following: i) Targeting hepatic injury—chronic ethanol exposure depletes glutathione, increasing susceptibility to oxidative stress.[136] Although classic antioxidants, like N-acetylcysteine, have shown limited efficacy in severe ASH because of their lack of mitochondrial specificity, S-adenosylmethionine, which restores mitochondrial glutathione, has demonstrated promise in preclinical studies.[137,138] Also, liver regeneration strategies, including granulocyte colony-stimulating factors and IL-22 agonists, have shown promise in early studies.[139,140] ii) Reducing inflammatory responses—chronic inflammation plays a pivotal role in ASH progression, making immune modulation a compelling therapeutic avenue. Anti–tumor necrosis factor agents, including infliximab and etanercept, have failed in clinical trials.[141,142] Ongoing trials assess IL-1 inhibitors, such as canakinumab and anakinra, whereas toll-like receptor 4 antagonists, like HA35 (NCT05018481, *https://clinicaltrials.gov*, last accessed May 26, 2025), have shown promise in preclinical models but remain in early-stage human trials. The farnesoid X receptor agonists (NCT02039219, *https://clinicaltrials.gov*, last accessed May 26, 2025), which exhibit hepatoprotective effects, were explored in a clinical trial involving obeticholic acid, but the trial was halted because of hepatotoxicity. iii) Microbiome-based therapies—emerging evidence suggests that gut microbiota dysfunction contributes to ASH progression, positioning microbiome-targeted therapies as a

potential treatment avenue. Probiotic trials, including *Lactobacillus* rhamnosus GG, have shown promise in reducing inflammation.[143] However, antibiotic therapies have not demonstrated significant benefits in mitigating hepatitis and systemic inflammation. Fecal microbiota transplantation has yielded encouraging results in severe ASH, with studies indicating improved survival rates and beneficial microbiota changes. Additionally, cytolysin-secreting *Enterococcus faecalis* strains have been implicated in worsening ALD severity, and bacteriophage therapy targeting these strains may provide a novel therapeutic approach.[144,145]

Overall, ASH is a perilous condition with a restricted outlook and continues to be a predominant cause of liver pathology globally, with an increasing disease burden. Approaching ASH with multitargeted therapeutic strategies is of great importance to achieve the required clinical outcomes, before liver transplant is needed. Ongoing research is primarily focused on evaluating combination therapies for MASH. Notably, the combination of farnesoid X receptor agonist (cilofexor) and acetyl-CoA carboxylase inhibitor (firsocostat) has demonstrated synergistic effects in preclinical studies.[146] The dual approach targets key disease pathways—bile acid metabolism via farnesoid X receptor activation and DNL via acetyl-CoA carboxylase inhibition. By simultaneously addressing both metabolic dysregulation and fibrotic progression, this strategy holds promise for broader therapeutic benefits, including in the context of ASH.[147] A triplicate therapy consisting of semaglutide, cilofexor, and firsocostat, under investigation for MASH, may have potential implications for ASH as well.[148]

In the past 10 years, several new therapeutic targets have been identified, with clinical studies either finished or currently underway in individuals with ALD. Despite the availability of excellent medicinal therapy for ALD, long-term outcomes are contingent on abstaining from alcohol use across all spectrums of ALD.

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References

1. Osna NA, Rasineni K, Ganesan M, Donohue TM Jr: Kharbanda KK: Pathogenesis of alcohol-associated liver disease. J Clin Exp Hepatol 2022, 12:1492−1513
2. Hyun J, Han J, Lee C, Yoon M, Jung Y: Pathophysiological aspects of alcohol metabolism in the liver. Int J Mol Sci 2021, 22:5717
3. Chaudhry H, Sohal A, Iqbal H, Roytman M: Alcohol-related hepatitis: a review article. World J Gastroenterol 2023, 29:2551−2570
4. Bou Saleh M, Louvet A, Ntandja-Wandji LC, Boleslawski E, Gnemmi V, Lassailly G, Truant S, Maggiotto F, Ningarhari M, Artru F, Anglo E, Sancho-Bru P, Corlu A, Argemi J, Dubois-Chevalier J, Dharancy S, Eeckhoute J, Bataller R, Mathurin P, Dubuquoy L: Loss of hepatocyte identity following aberrant YAP activation: a key mechanism in alcoholic hepatitis. J Hepatol 2021, 75:912−923
5. Rachakonda V, Bataller R, Duarte-Rojo A: Recent advances in alcoholic hepatitis. F1000Res 2020, 9. F1000 Faculty Rev-97
6. Hernández-Évole H, Jiménez-Esquivel N, Pose E, Bataller R: Alcohol-associated liver disease: epidemiology and management. Ann Hepatol 2024, 29:101162
7. Schulze RJ, Ding WX: Lipid droplet dynamics in alcoholic fatty liver disease. Liver Res 2019, 3:185−190
8. Seitz HK, Bataller R, Cortez-Pinto H, Gao B, Gual A, Lackner C, Mathurin P, Mueller S, Szabo G, Tsukamoto H: Alcoholic liver disease. Nat Rev Dis Primers 2018, 4:16
9. Walther TC, Chung J, Farese RV: Lipid droplet biogenesis. Annu Rev Cell Dev Biol 2017, 33:491−510
10. Dempsey JL, Ioannou GN, Carr RM: Mechanisms of lipid droplet accumulation in steatotic liver diseases. Semin Liver Dis 2023, 43: 367−382
11. Ma J, Guillot A, Yang Z, Mackowiak B, Hwang S, Park O, Peiffer BJ, Ahmadi AR, Melo L, Kusumanchi P, Huda N, Saxena R, He Y, Guan Y, Feng D, Sancho-Bru P, Zang M, Cameron AM, Bataller R, Tacke F, Sun Z, Liangpunsakul S, Gao B: Distinct histopathological phenotypes of severe alcoholic hepatitis suggest different mechanisms driving liver injury and failure. J Clin Invest 2022, 132:e157780
12. Allameh A, Niayesh-Mehr R, Aliarab A, Sebastiani G, Pantopoulos K: Oxidative stress in liver pathophysiology and disease. Antioxidants 2023, 12:1653
13. Basiglio CL, Crocenzi FA, Sánchez Pozzi EJ, Roma MG: Oxidative stress and localization status of hepatocellular transporters: impact on bile secretion and role of signaling pathways. Antioxid Redox Signal 2021, 35:808−831
14. Ferdouse A, Clugston RD: Pathogenesis of alcohol-associated fatty liver: lessons from transgenic mice. Front Physiol 2022, 13:940974
15. Jeon S, Carr R: Alcohol effects on hepatic lipid metabolism. J Lipid Res 2020, 61:470−479
16. Berk PD, Zhou S, Bradbury MW: Increased hepatocellular uptake of long chain fatty acids occurs by different mechanisms in fatty livers due to obesity or excess ethanol use, contributing to development of steatohepatitis in both settings. Trans Am Clin Climatol Assoc 2005, 116:335−344
17. Ciardullo S, Mantovani A, Morieri ML, Muraca E, Invernizzi P, Perseghin G: Impact of MASLD and MetALD on clinical outcomes: a meta-analysis of preliminary evidence. Liver Int 2024, 44: 1762−1767
18. Choudhary V, Ojha N, Golden A, Prinz WA: A conserved family of proteins facilitates nascent lipid droplet budding from the ER. J Cell Biol 2015, 211:261−271
19. Klug YA, Deme JC, Corey RA, Renne MF, Stansfeld PJ, Lea SM, Carvalho P: Mechanism of lipid droplet formation by the yeast Sei1/Ldb16 Seipin complex. Nat Commun 2021, 12:5892
20. Green CJ, Pramfalk C, Charlton CA, Gunn PJ, Cornfield T, Pavlides M, Karpe F, Hodson L: Hepatic de novo lipogenesis is suppressed and fat oxidation is increased by omega-3 fatty acids at the expense of glucose metabolism. BMJ Open Diabetes Res Care 2020, 8:e000871
21. Chung J, Wu X, Lambert TJ, Lai ZW, Walther TC, Farese RV Jr: LDAF1 and seipin form a lipid droplet assembly complex. Dev Cell 2019, 51:551−563.e7
22. Ferreira JV, Carvalho P: Pex30-like proteins function as adaptors at distinct ER membrane contact sites. J Cell Biol 2021, 220: e202103176
23. Klasson TD, LaGory EL, Zhao H, Huynh SK, Papandreou I, Moon EJ, Giaccia AJ: ACSL3 regulates lipid droplet biogenesis and

ferroptosis sensitivity in clear cell renal cell carcinoma. Cancer Metab 2022, 10:14
24. Zadoorian A, Du X, Yang H: Lipid droplet biogenesis and functions in health and disease. Nat Rev Endocrinol 2023, 19:443−459
25. Chorlay A, Thiam AR: An asymmetry in monolayer tension regulates lipid droplet budding direction. Biophys J 2018, 114:631−640
26. Wang S, Idrissi FZ, Hermansson M, Grippa A, Ejsing CS, Carvalho P: Seipin and the membrane-shaping protein Pex30 cooperate in organelle budding from the endoplasmic reticulum. Nat Commun 2018, 9:2939
27. Hegaard FV, Klenow MB, Simonsen AC: Lens nucleation and droplet budding in a membrane model for lipid droplet biogenesis. Langmuir 2022, 38:9247−9256
28. Becuwe M, Bond LM, Pinto AFM, Boland S, Mejhert N, Elliott SD, Cicconet M, Graham MM, Liu XN, Ilkayeva O, Saghatelian A, Walther TC, Farese RV Jr: FIT2 is an acyl-coenzyme A diphosphatase crucial for endoplasmic reticulum homeostasis. J Cell Biol 2020, 219:e202006111
29. Wilfling F, Thiam AR, Olarte M-J, Wang J, Beck R, Gould TJ, Allgeyer ES, Pincet F, Bewersdorf J, Farese RV Jr: Walther TC: Arf1/COPI machinery acts directly on lipid droplets and enables their connection to the ER for protein targeting. Elife 2014, 3:e01607
30. Deslandes F, Thiam AR, Forêt L: Lipid droplets can spontaneously bud off from a symmetric bilayer. Biophys J 2017, 113:15−18
31. Thiam AR, Ikonen E: Lipid droplet nucleation. Trends Cell Biol 2021, 31:108−118
32. Xu L, Li L, Wu L, Li P, Chen F-J: CIDE proteins and their regulatory mechanisms in lipid droplet fusion and growth. FEBS Lett 2024, 598:1154−1169
33. Santinho A, Salo VT, Chorlay A, Li S, Zhou X, Omrane M, Ikonen E, Thiam AR: Membrane curvature catalyzes lipid droplet assembly. Curr Biol 2020, 30:2481−2494.e6
34. Charidemou E, Ashmore T, Li X, McNally BD, West JA, Liggi S, Harvey M, Orford E, Griffin JL: A randomized 3-way crossover study indicates that high-protein feeding induces de novo lipogenesis in healthy humans. JCI Insight 2019, 4:e124819
35. Belew GD, Silva J, Rito J, Tavares L, Viegas I, Teixeira J, Oliveira PJ, Macedo MP, Jones JG: Transfer of glucose hydrogens via acetyl-CoA, malonyl-CoA, and NADPH to fatty acids during de novo lipogenesis. J Lipid Res 2019, 60:2050−2056
36. Silva JCP, Marques C, Martins FO, Viegas I, Tavares L, Macedo MP, Jones JG: Determining contributions of exogenous glucose and fructose to de novo fatty acid and glycerol synthesis in liver and adipose tissue. Metab Eng 2019, 56:69−76
37. Lee DS, An TH, Kim H, Jung E, Kim G, Oh SY, Kim JS, Chun HJ, Jung J, Lee E-W, Han B-S, Han DH, Lee Y-H, Han T-S, Hur K, Lee C-H, Kim D-S, Kim WK, Park JW, Koo S-H, Seong JK, Lee SC, Kim H, Bae K-H, Oh K-J: Tcf7l2 in hepatocytes regulates de novo lipogenesis in diet-induced non-alcoholic fatty liver disease in mice. Diabetologia 2023, 66:931−954
38. Daswani R, Kumar A, Anikhindi SA, Sharma P, Singla V, Bansal N, Arora A: Predictors of 90-day mortality in patients with severe alcoholic hepatitis: experience with 183 patients at a tertiary care center from India. Indian J Gastroenterol 2018, 37:141−152
39. Klepp TD, Sloan ME, Soundararajan S, Ramsden CE, Cinar R, Schwandt ML, Diazgranados N, Vatsalya V, Ramchandani VA: Elevated stearoyl-CoA desaturase 1 activity is associated with alcohol-associated liver disease. Alcohol 2022, 102:51−57
40. Marmier S, Dentin R, Daujat-Chavanieu M, Guillou H, Bertrand-Michel J, Gerbal-Chaloin S, Girard J, Lotersztajn S, Postic C: Novel role for carbohydrate responsive element binding protein in the control of ethanol metabolism and susceptibility to binge drinking. Hepatology 2015, 62:1086−1100
41. You M, Matsumoto M, Pacold CM, Cho WK, Crabb DW: The role of AMP-activated protein kinase in the action of ethanol in the liver. Gastroenterology 2004, 127:1798−1808
42. Goikoetxea-Usandizaga N, Bravo M, Egia-Mendikute L, Abecia L, Serrano-Maciá M, Urdinguio RG, et al: The outcome of boosting mitochondrial activity in alcohol-associated liver disease is organ-dependent. Hepatology 2023, 78:878−895
43. Lu Y, Chen Y, Hu W, Wang M, Wen X, Yang J: Inhibition of ACSS2 attenuates alcoholic liver steatosis via epigenetically regulating de novo lipogenesis. Liver Int 2023, 43:1729−1740
44. Syed-Abdul MM, Jacome-Sosa M, Hu Q, Gaballah AH, Winn NC, Lee NT, Mucinski JM, Manrique-Acevedo C, Lastra G, Anderson JM, Al Juboori A, Bartholow BD, Parks EJ: The Tailgate Study: differing metabolic effects of a bout of excessive eating and drinking. Alcohol 2021, 90:45−55
45. Yin F, Wu MM, Wei XL, Ren RX, Liu MH, Chen CQ, Yang L, Xie RQ, Jiang SY, Wang XF, Wang H: Hepatic NCoR1 deletion exacerbates alcohol-induced liver injury in mice by promoting CCL2-mediated monocyte-derived macrophage infiltration. Acta Pharmacol Sin 2022, 43:2351−2361
46. Chen H, Shen F, Sherban A, Nocon A, Li Y, Wang H, Xu MJ, Rui X, Han J, Jiang B, Lee D, Li N, Keyhani-Nejad F, Fan JG, Liu F, Kamat A, Musi N, Guarente L, Pacher P, Gao B, Zang M: DEP domain-containing mTOR-interacting protein suppresses lipogenesis and ameliorates hepatic steatosis and acute-on-chronic liver injury in alcoholic liver disease. Hepatology 2018, 68:496−514
47. Correnti J, Lin C, Brettschneider J, Kuriakose A, Jeon S, Scorletti E, Oranu A, McIver-Jenkins D, Kaneza I, Buyco D, Saiman Y, Furth EE, Argemi J, Bataller R, Holland WL, Carr RM: Liver-specific ceramide reduction alleviates steatosis and insulin resistance in alcohol-fed mice. J Lipid Res 2020, 61:983−994
48. Rodgers JT, Lerin C, Haas W, Gygi SP, Spiegelman BM, Puigserver P: Nutrient control of glucose homeostasis through a complex of PGC-1[alpha] and SIRT1. Nature 2005, 434:113−118
49. Yin H, Hu M, Liang X, Ajmo JM, Li X, Bataller R, Odena G, Stevens SM Jr, You M: Deletion of SIRT1 from hepatocytes in mice disrupts lipin-1 signaling and aggravates alcoholic fatty liver. Gastroenterology 2014, 146:801−811
50. Zhong W, Zhao Y, Tang Y, Wei X, Shi X, Sun W, Sun X, Yin X, Sun X, Kim S, McClain CJ, Zhang X, Zhou Z: Chronic alcohol exposure stimulates adipose tissue lipolysis in mice: role of reverse triglyceride transport in the pathogenesis of alcoholic steatosis. Am J Pathol 2012, 180:998−1007
51. Donnelly KL, Smith CI, Schwarzenberg SJ, Jessurun J, Boldt MD, Parks EJ: Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. J Clin Invest 2005, 115:1343−1351
52. Zhao C, Liu Y, Xiao J, Liu L, Chen S, Mohammadi M, McClain CJ, Li X, Feng W: FGF21 mediates alcohol-induced adipose tissue lipolysis by activation of systemic release of catecholamine in mice. J Lipid Res 2015, 56:1481−1491
53. Granneman JG, Moore H-PH, Granneman RL, Greenberg AS, Obin MS, Zhu Z: Analysis of lipolytic protein trafficking and interactions in adipocytes. J Biol Chem 2007, 282:5726−5735
54. Granneman JG, Moore H-PH, Krishnamoorthy R, Rathod M: Perilipin controls lipolysis by regulating the interactions of AB-hydrolase containing 5 (Abhd5) and adipose triglyceride lipase (Atgl). J Biol Chem 2009, 284:34538−34544
55. Desai BN, Singhal G, Watanabe M, Stevanovic D, Lundasen T, Fisher fM, Mather ML, Vardeh HG, Douris N, Adams AC, Nasser IA, FitzGerald GA, Flier JS, Skarke C, Maratos-Flier E: Fibroblast growth factor 21 (FGF21) is robustly induced by ethanol and has a protective role in ethanol associated liver injury. Mol Metab 2017, 6:1395−1406
56. Krycer JR, Quek L-E, Francis D, Zadoorian A, Weiss FC, Cooke KC, Nelson ME, Diaz-Vegas A, Humphrey SJ, Scalzo R, Hirayama A, Ikeda S, Shoji F, Suzuki K, Huynh K, Giles C, Varney B, Nagarajan SR, Hoy AJ, Soga T, Meikle PJ, Cooney GJ, Fazakerley DJ, James DE: Insulin signaling requires glucose to

promote lipid anabolism in adipocytes. J Biol Chem 2020, 295: 13250−13266
57. Mahalingam S, Bellamkonda R, Kharbanda KK, Arumugam MK, Kumar V, Casey CA, Leggio L, Rasineni K: Role of ghrelin hormone in the development of alcohol-associated liver disease. Biomed Pharmacother 2024, 174:116595
58. Dou X, Xia Y, Chen J, Qian Y, Li S, Zhang X, Song Z: Rectification of impaired adipose tissue methylation status and lipolytic response contributes to hepatoprotective effect of betaine in a mouse model of alcoholic liver disease. Br J Pharmacol 2014, 171:4073−4086
59. Song Z, Zhou Z, Deaciuc I, Chen T, McClain CJ: Inhibition of adiponectin production by homocysteine: a potential mechanism for alcoholic liver disease. Hepatology 2008, 47:867−879
60. Wang Z, Dou X, Yao T, Song Z: Homocysteine inhibits adipogenesis in 3T3-L1 preadipocytes. Exp Biol Med 2011, 236: 1379−1388
61. Arumugam MK, Talawar S, Listenberger L, Donohue TM, Osna NA, Kharbanda KK: Role of elevated intracellular S-adenosylhomocysteine in the pathogenesis of alcohol-related liver disease. Cells 2020, 9:1526
62. Sugimoto T, Yamashita S, Ishigami M, Sakai N, Hirano K, Tahara M, Matsumoto K, Nakamura T, Matsuzawa Y: Decreased microsomal triglyceride transfer protein activity contributes to initiation of alcoholic liver steatosis in rats. J Hepatol 2002, 36: 157−162
63. Mathur K, Vilar-Gomez E, Connelly MA, He H, Sanyal AJ, Chalasani N, Jiang ZG: Circulating high density lipoprotein distinguishes alcoholic hepatitis from heavy drinkers and predicts 90-day outcome: lipoproteins in alcoholic hepatitis. J Clin Lipidol 2021, 15: 805−813
64. Jiang ZG, Tapper EB, Connelly MA, Pimentel CF, Feldbrügge L, Kim M, Krawczyk S, Afdhal N, Robson SC, Herman MA, Otvos JD, Mukamal KJ, Lai M: Steatohepatitis and liver fibrosis are predicted by the characteristics of very low density lipoprotein in nonalcoholic fatty liver disease. Liver Int 2016, 36:1213−1220
65. Vedhachalam C, Chetty PS, Nickel M, Dhanasekaran P, Lund-Katz S, Rothblat GH, Phillips MC: Influence of apolipoprotein (Apo) A-I structure on nascent high density lipoprotein (HDL) particle size distribution. J Biol Chem 2010, 285:31965−31973
66. Hu K, Perez-Matos MC, Argemi J, Vilar-Gomez E, Shalaurova I, Bullitt E, , et alLipoprotein Z: a hepatotoxic lipoprotein, predicts outcome in alcohol-associated hepatitis. Hepatology 2022, 75: 968−982
67. Cui W, Sathyanarayan A, Lopresti M, Aghajan M, Chen C, Mashek DG: Lipophagy-derived fatty acids undergo extracellular efflux via lysosomal exocytosis. Autophagy 2021, 17:690−705
68. Yang L, Yang C, Thomes PG, Kharbanda KK, Casey CA, McNiven MA, Donohue TM Jr: Lipophagy and alcohol-induced fatty liver. Front Pharmacol 2019, 10:495
69. Corbo JH, Chung J: Mechanisms of lipid droplet degradation. Curr Opin Cell Biol 2024, 90:102402
70. Schott MB, Weller SG, Schulze RJ, Krueger EW, Drizyte-Miller K, Casey CA, McNiven MA: Lipid droplet size directs lipolysis and lipophagy catabolism in hepatocytes. J Cell Biol 2019, 218: 3320−3335
71. Avila DV, Barker DF, Zhang J, McClain CJ, Barve S, Gobejishvili L: Dysregulation of hepatic cAMP levels via altered Pde4b expression plays a critical role in alcohol-induced steatosis. J Pathol 2016, 240:96−107
72. Schott MB, Rasineni K, Weller SG, Schulze RJ, Sletten AC, Casey CA, McNiven MA: beta-Adrenergic induction of lipolysis in hepatocytes is inhibited by ethanol exposure. J Biol Chem 2017, 292: 11815−11828
73. Wyatt TA, Sisson JH: Chronic ethanol downregulates PKA activation and ciliary beating in bovine bronchial epithelial cells. Am J Physiol Lung Cell Mol Physiol 2001, 281:L575−L581
74. Thomes PG, Trambly CS, Fox HS, Tuma DJ, Donohue TM Jr: Acute and chronic ethanol administration differentially modulate hepatic autophagy and transcription factor EB. Alcohol Clin Exp Res 2015, 39:2354−2363
75. Chao X, Wang S, Zhao K, Li Y, Williams JA, Li T, Chavan H, Krishnamurthy P, He XC, Li L, Ballabio A, Ni HM, Ding WX: Impaired TFEB-mediated lysosome biogenesis and autophagy promote chronic ethanol-induced liver injury and steatosis in mice. Gastroenterology 2018, 155:865−879.e12
76. Chao X, Ni HM, Ding WX: Insufficient autophagy: a novel autophagic flux scenario uncovered by impaired liver TFEB-mediated lysosomal biogenesis from chronic alcohol-drinking mice. Autophagy 2018, 14:1646−1648
77. Schott MB, Rozeveld CN, Weller SG, McNiven MA: Lipophagy at a glance. J Cell Sci 2022, 135:jcs259402
78. Li Z, Schulze RJ, Weller SG, Krueger EW, Schott MB, Zhang X, Casey CA, Liu J, Stockli J, James DE, McNiven MA: A novel Rab10-EHBP1-EHD2 complex essential for the autophagic engulfment of lipid droplets. Sci Adv 2016, 2:e1601470
79. Li Z, Weller SG, Drizyte-Miller K, Chen J, Krueger EW, Mehall B, Casey CA, Cao H, McNiven MA: Maturation of lipophagic organelles in hepatocytes is dependent upon a Rab10/Dynamin-2 complex. Hepatology 2020, 72:486−502
80. Schroeder B, Schulze RJ, Weller SG, Sletten AC, Casey CA, McNiven MA: The small GTPase Rab7 as a central regulator of hepatocellular lipophagy. Hepatology 2015, 61:1896−1907
81. Schott MB, Rozeveld CN, Bhatt S, Crossman B, Krueger EW, Weller SG, Rasineni K, Casey CA, McNiven MA: Ethanol disrupts hepatocellular lipophagy by altering Rab5-centric LD-lysosome trafficking. Hepatol Commun 2024, 8:e0446
82. Schulze RJ, Rasineni K, Weller SG, Schott MB, Schroeder B, Casey CA, McNiven MA: Ethanol exposure inhibits hepatocyte lipophagy by inactivating the small guanosine triphosphatase Rab7. Hepatol Commun 2017, 1:140−152
83. Salete-Granado D, Carbonell C, Puertas-Miranda D, Vega-Rodríguez VJ, García-Macia M, Herrero AB, Marcos M: Autophagy, oxidative stress, and alcoholic liver disease: a systematic review and potential clinical applications. Antioxidants (Basel) 2023, 12:1425
84. Schulze RJ, Krueger EW, Weller SG, Johnson KM, Casey CA, Schott MB, McNiven MA: Direct lysosome-based autophagy of lipid droplets in hepatocytes. Proc Natl Acad Sci U S A 2020, 117: 32443−32452
85. Casey CA, Donohue TM Jr, Kubik JL, Kumar V, Naldrett MJ, Woods NT, Frisbie CP, McNiven MA, Thomes PG: Lipid droplet membrane proteome remodeling parallels ethanol-induced hepatic steatosis and its resolution. J Lipid Res 2021, 62:100049
86. Perumal SK, Day LZ, Arumugam MK, Chava S, Kumar V, Osna NA, Jacobs J, Rasineni K, Kharbanda KK: Lipid droplet-associated proteins in alcohol-associated fatty liver disease: a proteomic approach. Alcohol Clin Exp Res (Hoboken) 2024, 48:2010−2021
87. Arumugam MK, Perumal SK, Rasineni K, Donohue TM Jr, Osna NA, Kharbanda KK: Lipidomic analysis of liver lipid droplets after chronic alcohol consumption with and without betaine supplementation. Biology (Basel) 2023, 12:462
88. Fei W, Du X, Yang H: Seipin, adipogenesis and lipid droplets. Trends Endocrinol Metab 2011, 22:204−210
89. van der Veen JN, Kennelly JP, Wan S, Vance JE, Vance DE, Jacobs RL: The critical role of phosphatidylcholine and phosphatidylethanolamine metabolism in health and disease. Biochim Biophys Acta Biomembr 2017, 1859:1558−1572
90. Tuma DJ, Thiele GM, Xu D, Klassen LW, Sorrell MF: Acetaldehyde and malondialdehyde react together to generate distinct protein adducts in the liver during long-term ethanol administration. Hepatology 1996, 23:872−880
91. Groebner JL, Tuma PL: The altered hepatic tubulin code in alcoholic liver disease. Biomolecules 2015, 5:2140−2159

92. Roberts MA, Olzmann JA: Protein quality control and lipid droplet metabolism. Annu Rev Cell Dev Biol 2020, 36:115−139
93. Thomes PG, Strupp MS, Donohue TM, Kubik JL, Sweeney S, Mahmud R, Schott MB, Schulze RJ, McNiven MA, Casey CA: Hydroxysteroid 17β-dehydrogenase 11 accumulation on lipid droplets promotes ethanol-induced cellular steatosis. J Biol Chem 2023, 299:103071
94. Zheng Y, Chen J, Macwan V, Dixon CL, Li X, Liu S, Yu Y, Xu P, Sun Q, Hu Q, Liu W, Raught B, Fairn GD, Neculai D: S-acylation of ATGL is required for lipid droplet homoeostasis in hepatocytes. Nat Metab 2024, 6:1549−1565
95. Montoya-Durango D, Walter MN, Rodriguez W, Wang Y, Chariker JH, Rouchka EC, Maldonado C, Barve S, McClain CJ, Gobejishvili L: Dysregulated cyclic nucleotide metabolism in alcohol-associated steatohepatitis: implications for novel targeted therapies. Biology (Basel) 2023, 12:1321
96. Elnagdy M, Barve S, McClain C, Gobejishvili L: cAMP signaling in pathobiology of alcohol associated liver disease. Biomolecules 2020, 10:1433
97. Wahlang B, McClain C, Barve S, Gobejishvili L: Role of cAMP and phosphodiesterase signaling in liver health and disease. Cell Signal 2018, 49:105−115
98. Hardesty J, Day L, Warner J, Warner D, Gritsenko M, Asghar A, Stolz A, Morgan T, McClain C, Jacobs J, Kirpich I: Hepatic protein and phosphoprotein signatures of alcohol-associated cirrhosis and hepatitis. Am J Pathol 2022, 192:1066−1082
99. Bersuker K, Peterson CWH, To M, Sahl SJ, Savikhin V, Grossman EA, Nomura DK, Olzmann JA: A proximity labeling strategy provides insights into the composition and dynamics of lipid droplet proteomes. Dev Cell 2018, 44:97−112.e7
100. Kassan A, Herms A, Fernández-Vidal A, Bosch M, Schieber NL, Reddy BJ, Fajardo A, Gelabert-Baldrich M, Tebar F, Enrich C, Gross SP, Parton RG, Pol A: Acyl-CoA synthetase 3 promotes lipid droplet biogenesis in ER microdomains. J Cell Biol 2013, 203:985−1001
101. Salameh A, Daquinag AC, Staquicini DI, An Z, Hajjar KA, Pasqualini R, Arap W, Kolonin MG: Prohibitin/annexin 2 interaction regulates fatty acid transport in adipose tissue. JCI Insight 2016, 1:e86351
102. Velikkakath AK, Nishimura T, Oita E, Ishihara N, Mizushima N: Mammalian Atg2 proteins are essential for autophagosome formation and important for regulation of size and distribution of lipid droplets. Mol Biol Cell 2012, 23:896−909
103. Pezeshkian W, Chevrot G, Khandelia H: The role of caveolin-1 in lipid droplets and their biogenesis. Chem Phys Lipids 2018, 211:93−99
104. Reddy BJ, Mattson M, Wynne CL, Vadpey O, Durra A, Chapman D, Vallee RB, Gross SP: Load-induced enhancement of Dynein force production by LIS1-NudE in vivo and in vitro. Nat Commun 2016, 7:12259
105. Kageyama S, Gudmundsson SR, Sou YS, Ichimura Y, Tamura N, Kazuno S, Ueno T, Miura Y, Noshiro D, Abe M, Mizushima T, Miura N, Okuda S, Motohashi H, Lee JA, Sakimura K, Ohe T, Noda NN, Waguri S, Eskelinen EL, Komatsu M: p62/SQSTM1-droplet serves as a platform for autophagosome formation and anti-oxidative stress response. Nat Commun 2021, 12:16
106. Kumar AV, Mills J, Lapierre LR: Selective autophagy receptor p62/SQSTM1, a pivotal player in stress and aging. Front Cell Dev Biol 2022, 10:793328
107. Davies SP, Sim AT, Hardie DG: Location and function of three sites phosphorylated on rat acetyl-CoA carboxylase by the AMP-activated protein kinase. Eur J Biochem 1990, 187:183−190
108. Kim CG, Chang SN, Park SM, Hwang BS, Kang SA, Kim KS, Park JG: Moringa oleifera mitigates ethanol-induced oxidative stress, fatty degeneration and hepatic steatosis by promoting Nrf2 in mice. Phytomedicine 2022, 100:154037
109. Liu G, Wang N, Zhang C, Li M, He X, Yin C, Tu Q, Shen X, Zhang L, Lv J, Wang Y, Jiang H, Chen S, Li N, Tao Y, Yin H: Fructose-1,6-bisphosphate aldolase B depletion promotes hepatocellular carcinogenesis through activating insulin receptor signaling and lipogenesis. Hepatology 2021, 74:3037−3055
110. Yu J, Loh K, Song ZY, Yang HQ, Zhang Y, Lin S: Update on glycerol-3-phosphate acyltransferases: the roles in the development of insulin resistance. Nutr Diabetes 2018, 8:34
111. Butt E, Günder T, Stürzebecher P, Kowalski I, Schneider P, Buschmann N, Schäfer S, Bender A, Hermanns HM, Zernecke A: Cholesterol uptake in the intestine is regulated by the LASP1-AKT-NPC1L1 signaling pathway. Am J Physiol Gastrointest Liver Physiol 2024, 327:G25−G35
112. Okumura T: Role of lipid droplet proteins in liver steatosis. J Physiol Biochem 2011, 67:629−636
113. Kimmel AR, Sztalryd C: The perilipins: major cytosolic lipid droplet−associated proteins and their roles in cellular lipid storage, mobilization, and systemic homeostasis. Annu Rev Nutr 2016, 36:471−509
114. Ge Y, Cao Y, Li F, Wang J, Liu Y, Guo W, Liu J, Fu S: Growth, fusion and degradation of lipid droplets: advances in lipid droplet regulatory protein. Arch Physiol Biochem 2025, 131:109−118
115. Wu L, Xu D, Zhou L, Xie B, Yu L, Yang H, Huang L, Ye J, Deng H, Yuan YA: Rab8a-AS160-MSS4 regulatory circuit controls lipid droplet fusion and growth. Dev Cell 2014, 30:378−393
116. Ouyang Q, Chen Q, Ke S, Ding L, Yang X, Rong P, Feng W, Cao Y, Wang Q, Li M: Rab8a as a mitochondrial receptor for lipid droplets in skeletal muscle. Dev Cell 2023, 58:289−305.e6
117. Kim KH: Regulation of mammalian acetyl-coenzyme a carboxylase. Annu Rev Nutr 1997, 17:77−99
118. Molenaar MR, Vaandrager AB, Helms JB: Some lipid droplets are more equal than others: different metabolic lipid droplet pools in hepatic stellate cells. Lipid Insights 2017, 10:1178635317747281
119. Molenaar MR, Penning LC, Helms JB: Playing Jekyll and Hyde-the dual role of lipids in fatty liver disease. Cells 2020, 9:2244
120. Mak KM, Wu C, Cheng CP: Lipid droplets, the Holy Grail of hepatic stellate cells: in health and hepatic fibrosis. Anat Rec (Hoboken) 2023, 306:983−1010
121. Arab JP, Cabrera D, Sehrawat TS, Jalan-Sakrikar N, Verma VK, Simonetto D, Cao S, Yaqoob U, Leon J, Freire M, Vargas JI, De Assuncao TM, Kwon JH, Guo Y, Kostallari E, Cai Q, Kisseleva T, Oh Y, Arrese M, Huebert RC, Shah VH: Hepatic stellate cell activation promotes alcohol-induced steatohepatitis through Igfbp3 and SerpinA12. J Hepatol 2020, 73:149−160
122. Liu C, Russell RM, Seitz HK, Wang XD: Ethanol enhances retinoic acid metabolism into polar metabolites in rat liver via induction of cytochrome P4502E1. Gastroenterology 2001, 120:179−189
123. Ferdouse A, Agrawal RR, Gao MA, Jiang H, Blaner WS, Clugston RD: Alcohol induced hepatic retinoid depletion is associated with the induction of multiple retinoid catabolizing cytochrome P450 enzymes. PLoS One 2022, 17:e0261675
124. Tuohetahuntila M, Molenaar MR, Spee B, Brouwers JF, Wubbolts R, Houweling M, Yan C, Du H, VanderVen BC, Vaandrager AB, Helms JB: Lysosome-mediated degradation of a distinct pool of lipid droplets during hepatic stellate cell activation. J Biol Chem 2017, 292:12436−12448
125. Tuohetahuntila M, Molenaar MR, Spee B, Brouwers JF, Houweling M, Vaandrager AB, Helms JB: ATGL and DGAT1 are involved in the turnover of newly synthesized triacylglycerols in hepatic stellate cells. J Lipid Res 2016, 57:1162−1174
126. Najt CP, Adhikari S, Heden TD, Cui W, Gansemer ER, Rauckhorst AJ, Markowski TW, Higgins L, Kerr EW, Boyum MD, Alvarez J, Brunko S, Mehra D, Puchner EM, Taylor EB, Mashek DG: Organelle interactions compartmentalize hepatic fatty acid trafficking and metabolism. Cell Rep 2023, 42:112435

127. Veliova M, Petcherski A, Liesa M, Shirihai OS: The biology of lipid droplet-bound mitochondria. Semin Cell Dev Biol 2020, 108:55−64
128. Freyre CAC, Rauher PC, Ejsing CS, Klemm RW: MIGA2 links mitochondria, the ER, and lipid droplets and promotes de novo lipogenesis in adipocytes. Mol Cell 2019, 76:811−825.e14
129. Ma X, Chen A, Melo L, Clemente-Sanchez A, Chao X, Ahmadi AR, Peiffer B, Sun Z, Sesaki H, Li T, Wang X, Liu W, Bataller R, Ni HM, Ding WX: Loss of hepatic DRP1 exacerbates alcoholic hepatitis by inducing megamitochondria and mitochondrial maladaptation. Hepatology 2023, 77:159−175
130. Schonfeld M, O'Neil M, Villar MT, Artigues A, Averilla J, Gunewardena S, Weinman SA, Tikhanovich I: A Western diet with alcohol in drinking water recapitulates features of alcohol-associated liver disease in mice. Alcohol Clin Exp Res 2021, 45: 1980−1993
131. Harrison SA, Bedossa P, Guy CD, Schattenberg JM, Loomba R, Taub R, Labriola D, Moussa SE, Neff GW, Rinella ME, Anstee QM, Abdelmalek MF, Younossi Z, Baum SJ, Francque S, Charlton MR, Newsome PN, Lanthier N, Schiefke I, Mangia A, Pericàs JM, Patil R, Sanyal AJ, Noureddin M, Bansal MB, Alkhouri N, Castera L, Rudraraju M, Ratziu V: A phase 3, randomized, controlled trial of resmetirom in NASH with liver fibrosis. N Engl J Med 2024, 390:497−509
132. Shahi A, Yadav A, Rajak S, Raza S, Tewari A, Gupta P, Sinha RA: Liver-specific thyroid hormone receptor-beta agonism alleviates alcoholic steatohepatitis (ASH) in mice. Biochem Biophys Res Commun 2024, 734:150742
133. Amin NB, Darekar A, Anstee QM, Wong VW, Tacke F, Vourvahis M, Lee DS, Charlton M, Alkhouri N, Nakajima A, Yunis C: Efficacy and safety of an orally administered DGAT2 inhibitor alone or coadministered with a liver-targeted ACC inhibitor in adults with non-alcoholic steatohepatitis (NASH): rationale and design of the phase II, dose-ranging, dose-finding, randomised, placebo-controlled MIRNA (Metabolic Interventions to Resolve NASH with fibrosis) study. BMJ Open 2022, 12:e056159
134. Dixon ED, Claudel T, Nardo AD, Riva A, Fuchs CD, Mlitz V, Busslinger G, Scharnagl H, Stojakovic T, Seneca J, Hinteregger H, Grabner GF, Kratky D, Verkade H, Zimmermann R, Haemmerle G, Trauner M: Inhibition of ATGL alleviates MASH via impaired PPARalpha signalling that favours hydrophilic bile acid composition in mice. J Hepatol 2025, 82:658−675
135. Porter HP, Simon FR, Pope CE 2nd, Volwiler W, Fenster LF: Corticosteroid therapy in severe alcoholic hepatitis: a double-blind drug trial. N Engl J Med 1971, 284:1350−1355
136. Lauterburg BH, Velez ME: Glutathione deficiency in alcoholics: risk factor for paracetamol hepatotoxicity. Gut 1988, 29:1153−1157
137. Moreno C, Langlet P, Hittelet A, Lasser L, Degré D, Evrard S, Colle I, Lemmers A, Devière J, Le Moine O: Enteral nutrition with or without N-acetylcysteine in the treatment of severe acute alcoholic hepatitis: a randomized multicenter controlled trial. J Hepatol 2010, 53:1117−1122
138. Colell A, García-Ruiz C, Morales A, Ballesta A, Ookhtens M, Rodés J, Kaplowitz N, Fernández-Checa JC: Transport of reduced glutathione in hepatic mitochondria and mitoplasts from ethanol-treated rats: effect of membrane physical properties and S-adenosyl-L-methionine. Hepatology 1997, 26:699−708
139. Garg V, Garg H, Khan A, Trehanpati N, Kumar A, Sharma BC, Sakhuja P, Sarin SK: Granulocyte colony-stimulating factor mobilizes CD34(+) cells and improves survival of patients with acute-on-chronic liver failure. Gastroenterology 2012, 142:505−512.e1
140. Arab JP, Sehrawat TS, Simonetto DA, Verma VK, Feng D, Tang T, Dreyer K, Yan X, Daley WL, Sanyal A, Chalasani N, Radaeva S, Yang L, Vargas H, Ibacache M, Gao B, Gores GJ, Malhi H, Kamath PS, Shah VH: An open-label, dose-escalation study to assess the safety and efficacy of IL-22 agonist F-652 in patients with alcohol-associated hepatitis. Hepatology 2020, 72:441−453
141. Naveau S, Chollet-Martin S, Dharancy S, Mathurin P, Jouet P, Piquet MA, Davion T, Oberti F, Broët P, Emilie D: A double-blind randomized controlled trial of infliximab associated with prednisolone in acute alcoholic hepatitis. Hepatology 2004, 39:1390−1397
142. Boetticher NC, Peine CJ, Kwo P, Abrams GA, Patel T, Aqel B, Boardman L, Gores GJ, Harmsen WS, McClain CJ, Kamath PS, Shah VH: A randomized, double-blinded, placebo-controlled multicenter trial of etanercept in the treatment of alcoholic hepatitis. Gastroenterology 2008, 135:1953−1960
143. Bull-Otterson L, Feng W, Kirpich I, Wang Y, Qin X, Liu Y, Gobejishvili L, Joshi-Barve S, Ayvaz T, Petrosino J, Kong M, Barker D, McClain C, Barve S: Metagenomic analyses of alcohol induced pathogenic alterations in the intestinal microbiome and the effect of Lactobacillus rhamnosus GG treatment. PLoS One 2013, 8: e53028
144. Philips CA, Phadke N, Ganesan K, Ranade S, Augustine P: Corticosteroids, nutrition, pentoxifylline, or fecal microbiota transplantation for severe alcoholic hepatitis. Indian J Gastroenterol 2018, 37:215−225
145. Philips CA, Pande A, Shasthry SM, Jamwal KD, Khillan V, Chandel SS, Kumar G, Sharma MK, Maiwall R, Jindal A, Choudhary A, Hussain MS, Sharma S, Sarin SK: Healthy donor fecal microbiota transplantation in steroid-ineligible severe alcoholic hepatitis: a pilot study. Clin Gastroenterol Hepatol 2017, 15: 600−602
146. Schwabl P, Hambruch E, Budas GR, Supper P, Burnet M, Liles JT, Birkel M, Brusilovskaya K, Königshofer P, Peck-Radosavljevic M, Watkins WJ, Trauner M, Breckenridge DG, Kremoser C, Reiberger T: The non-steroidal FXR agonist cilofexor improves portal hypertension and reduces hepatic fibrosis in a rat NASH model. Biomedicines 2021, 9:60
147. Dufour JF, Caussy C, Loomba R: Combination therapy for non-alcoholic steatohepatitis: rationale, opportunities and challenges. Gut 2020, 69:1877−1884
148. Nathani RR, Bansal MB: Update on clinical trials for nonalcoholic steatohepatitis. Gastroenterol Hepatol (N Y) 2023, 19:371−381