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Lipid droplet dynamics in alcoholic fatty liver disease

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Abstract

The rising incidence of alcohol-related liver disease (ALD) demands making urgent progress in understanding the fundamental molecular basis of alcohol-related hepatocellular damage. One of the key early events accompanying chronic alcohol usage is the accumulation of lipid droplets (LDs) in the hepatocellular cytoplasm. LDs are far from inert sites of neutral lipid storage; rather, they represent key organelles that play vital roles in the metabolic state of the cell. In this review, we will examine the biology of these structures and outline recent efforts being made to understand the effects of alcohol exposure on the biogenesis, catabolism, and motility of LDs and how their dynamic nature is perturbed in the context of ALD.

Keywords

Lipid droplet (LD); Alcohol; Autophagy; Lipolysis; Hepatocyte; Steatosis

1. Introduction

An estimated 2.4 billion individuals worldwide consume alcoholic beverages.[1] Though the exact relationship between alcohol use and overall health is complicated, it is clear that the overconsumption of alcohol is inextricably linked to liver disease, resulting in an increasingly costly socioeconomic burden. It has been estimated that between 1 and 2 million cirrhosis and chronic liver-related disease deaths occur each year, with more than half of cirrhosis deaths likely attributable to alcohol intake.[2,3] The resulting financial impact on patients and the healthcare system is unclear, but estimates have suggested that in the United States alone, the total costs of alcohol abuse surpass $166 billion.[4] Clearly, a better understanding of the biological connections between alcohol consumption and liver physiology are urgently required.

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Authors' contributions

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Conflict of interest

The authors declare that they have no conflict of interest.

Alcohol-related liver disease (ALD) encompasses a number of pathologies ranging from the benign accumulation of hepatic fat (simple steatosis) to alcoholic steatohepatitis (ASH), characterized by significant hepatic inflammation and defined histological features (*e.g.*, hepatocellular ballooning and neutrophil infiltration). Severe cases may result in alcoholic hepatitis (AH), an acute form of ASH resulting in high short-term mortality with limited treatment options. In a certain percentage of patients, ALD may further progress to include the development of cirrhosis or even hepatocellular carcinoma (HCC). Liver transplantation is a viable intervention for these advanced stages of ALD; however, due to requirements related to sobriety, continued adherence to a plan of alcohol abstinence remains a concern for these patients.[5]

The consumption of alcohol results in myriad mechanisms of damage to hepatocytes. Many of the effects of alcohol result from secondary metabolites (*i.e.*, acetaldehyde and acetate) rather than alcohol itself. Initially, exposure of the hepatocyte to alcohol results in the rapid oxidation of alcohol to acetaldehyde by alcohol dehydrogenase (ADH) and cytochrome P450 2E1 (CYP2E1). Acetaldehyde can then be subsequently converted to acetate via the enzymatic activity of various aldehyde dehydrogenase (ALDH) isoforms located within the mitochondria, endoplasmic reticulum (ER), and cytosol. The combined consequences of alcohol, acetaldehyde, and acetate exposure are numerous. For example, the ratio of nicotinamide adenine dinucleotide (reduced/oxidized) ($NADH:NAD^+$) is significantly increased as a result of alcohol metabolism by both ADH and ALDH. One net effect of this disrupted balance is a rise in the activity of key lipogenic pathways (*i.e.*, sterol regulatory element binding protein 1c (SREBP1c) activation) that are coupled to concomitant decreases in mitochondrial β-oxidation, a key catabolic pathway for the degradation of stored fatty acids.[6,7] Another key consequence resulting from the production of these metabolites is the formation of adducts (*i.e.*, aldehyde adducts and protein hyperacetylation) that can have profound effects on lipid, protein, and nucleic acid biochemistry. In addition to lipid and protein modification, the generation of reactive oxygen species (ROS) due to CYP2E1 activity has considerable effects on the cell and can lead to not only apoptosis and ER stress, but also upregulation of lipogenic pathways as well. Finally, remnant acetate generated by ALDH activity that is not secreted into the bloodstream is likely to be converted to acetyl-CoA, at least a portion of which can serve as an important substrate used to generate malonyl-CoA in the rate-limiting initial step of fatty acid biosynthesis.

A current focus with potential therapeutic implications has thus centered on the hepatic fat accumulation that almost universally accompanies chronic alcohol consumption and often precedes the development of more severe sequelae. Greater than 90% of individuals who chronically consume large amounts of alcohol will develop fatty liver.[8] In all cells, this fat has been shown to accumulate in the form of lipid droplets (LDs), unique organelles that serve as repositories for neutral lipid. Under normal physiological conditions, LDs are constantly being synthesized and catabolized in a tightly regulated fashion that is largely dictated by the nutritional status of the cell. In this review, we will detail current research that has begun to address how alcohol exposure can impact the dynamic nature of hepatic LDs and result in their potentially detrimental accumulation with liver disease progression.

2. LDs

The major storage form of neutral lipid within the hepatocyte (and indeed, all mammalian cells) is the cytosolic LD. These organelles are formed subsequent to the synthesis of neutral lipids (predominantly triacylglycerols (TAG) and cholesteryl esters (CE)) by numerous enzymes located within the bilayer of the ER. TAG synthesis primarily occurs through the activity of diacylglycerol:acyltransferases (DGATs) and CE biosynthesis occurs via the action of acyl-coenzyme A:cholesterol acyltransferases (ACATs). Following the formation of a lipid 'lens' within this membrane, the eventual distension of the cytoplasmic leaflet results in the sequestration of a hydrophobic mixture of TAG and CE within the ER-derived delimiting phospholipid monolayer. These structures bud directionally outward toward the cytoplasm in a biophysical process dependent on membrane phospholipid composition and asymmetry.[9–11]

Studding the surface of LDs are a plethora of proteins, many of which are thought to be specific to the LD surface. A subset of these proteins are thought to possess a function analogous to a structural barrier, preventing unwarranted access of cytosolic lipases to the neutral lipids stored within the LD. Amongst these coat proteins are the perilipins, all of which contain N-terminal amphipathic helices critical to binding the LD surface phospholipid monolayer.[12,13] Aside from the perilipins, a number of additional proteins appear to be only transiently associated with the LD surface; the identity of and roles for these proteins in LD biology are only now beginning to come into focus.[14–16] In the past two decades, it has become increasingly appreciated that the LD represents a *bona fide* organelle that undergoes constant remodeling in response to the nutritional status of the cell.[17] As such, insights into the effects of perturbations to the composition of lipids and proteins comprising these organelles may be critical to better understanding the natural history of ALD.

3. Effects of alcohol on LD biosynthesis

One of the first, nearly immediate hallmarks of alcohol-induced liver damage is the accumulation of LDs within hepatocytes. This rapid increase in steatosis is ordinarily a trivial matter for the liver—the transient appearance of significant quantities of hepatic LDs occurs on a regular basis with normal physiological feeding/fasting cycles. However, continued chronic alcohol insult results in a persistent steatotic phenotype that appears to be a prerequisite for the development of advanced ALD. In response to chronic alcohol consumption, the hepatocyte retains progressively increased amounts of lipid within LDs. Upon histological examination, this increased lipid content can be in the form of relatively large LDs (macrovesicular) or take the form of an accumulation of small LDs (microvesicular steatosis). The underlying biological differences between the large and small LDs observed in these two phenotypes remain unclear. As stated above, there are a number of effects that alcohol has on lipogenic programming within the cell, all of which serve to increase the intracellular availability of free fatty acids (FFAs) that can be used for incorporation into the neutral lipids comprising nascent LDs.

Largely under the control of transcriptional factors such as SREBP1c, critical lipogenic enzymes such as stearoyl CoA desaturase-1 (SCD1), acetyl-coA carboxylase (ACC), and fatty acid synthase (FASN) are all upregulated at the genetic level in response to alcohol consumption.[7,18–22] Upregulation of SREBP1c at the transcriptional level appears to be mediated by one of the metabolites of alcohol metabolism, acetaldehyde.[7,23] Furthermore, the NAD^+-dependent deacetylase sirtuin1 (SIRT1), a suppressor of SREBP1c, is downregulated transcriptionally (via miR-217) and has reduced signaling (via lipin-1) following alcohol consumption.[24–27]

Carbohydrate-responsive element binding protein (ChREBP) represents a second important regulator of hepatic lipid synthesis that is also negatively affected by alcohol intake.[28] In a murine model of acute alcohol treatment, significant LD accumulation was observed in the liver alongside enhanced ChREBP activity, likely attributable to alcohol-induced acetylation of ChREBP.[28] Also upregulated is the pregnane X nuclear receptor (PXR), a key regulator of the expression of numerous metabolic enzymes and of transport of numerous small molecule compounds into the hepatocyte.[29] Indeed, *Pxr*-null mice appear to have reduced lipogenic gene expression and reduced hepatic steatosis in response to chronic alcohol feeding.[30]

The species of fatty acids available for incorporation into the LD may also be altered by ethanol consumption. Nuclear magnetic resonance (NMR) analysis of the hepatic lipidome following administration of a Lieber-DeCarli diet to rats revealed significant alterations to the metabolism of cholesterol, triglyceride and phospholipids within the liver.[31] These results showed substantial decreases in phosphatidylcholine levels, an important component of the LD monolayer and regulator of LD size, in agreement with previous observations.[32–34] Also affected by ethanol exposure are the levels of long-chain ceramides, both of which are increased in the livers of alcohol-fed mice and in patients with chronic ALD.[35,36]

Concomitant with the numerous alterations listed above that lead to increased substrate biosynthesis is a reduction in hepatic mitochondrial β-oxidation of these same FFAs.[37,38] This decrease in levels of β-oxidation is likely to be a direct result of downregulated components (such as carnitine palmitoyltransferase 1 (CPT1) α, acyl-coenzyme A oxidase (ACOX) 1, and peroxisome proliferator-activated receptor (PPAR) α). Experiments performed in an alcohol-metabolizing human hepatoma cell line (VL-17A) demonstrated that ethanol exposure can drive the accumulation of LDs, as determined by a substantial reduction in mitochondrial β-oxidation as well as increases in both triglyceride as well as the LD-specific surface protein perilipin 2 (PLIN2).[39] Isolation of LDs from livers of a rat model of ALD showed similar increases in triglyceride and PLIN2, along with altered surface phosphatidylcholine:phosphatidylethanolamine ratios, suggesting a linkage between phospholipid identity on the LD surface monolayer and the nature of proteins able to be recruited to the LD.[34] Interestingly, this effect could be ameliorated by exogenous addition of a short-chain ceramide (C2 ceramide) to cells, possibly via a PPARα-mediated mechanism. Consistent with the detrimental accumulation of PLIN2-coated LDs following alcohol exposure, use of a *Plin2* knockout mouse resulted in evidence showing that elimination of this perilipin protein afforded protection against alcoholic steatosis as well as improved insulin resistance and glucose tolerance.[40,41]

Finally, alcohol exposure also results in enhanced uptake of circulating FFAs directly into the hepatocyte.[42,43] This increased transport of FFAs appears to be partially due to an upregulation in the expression of the cell surface FFA transporter CD36.[44] Together, the net result is that the alcohol-damaged hepatocyte is primed for lipid storage. The numerous ways described above in which alcohol exposure results in increased FFA availability for LD production represent only one side of the equation, however. As will be discussed in the next section, alcohol exposure can also directly interfere in the normal ability of the hepatocyte to recoup these FFAs from storage within the LD in times of energetic deficit.

4. Effects of alcohol on LD catabolism by autophagy and lipolysis

Following packaging into the LD, the neutral lipid largely remains stably sequestered from the cytosol in an ‘oil-in-water immersion’ that shields the hepatocyte from the many cytotoxic consequences associated with cellular FFA overload.[45,46] During times of increased metabolic demand, however, the LD itself can be readily catabolized (in a highly regulated manner) to release these energy-rich FFAs as required to fuel various biochemical processes. As might be expected, alcohol exposure can interfere in this aspect of LD dynamics as well.

4.1. Effects of alcohol on autophagy of LDs

One of the mechanisms used for the catabolism of hepatocellular LDs is a selective form of autophagy referred to as lipophagy.[47] During lipophagy, a dedicated membrane (phagophore) encapsulates the LD, ultimately sequestering it within a double-membrane structure referred to as an ‘autophagosome’. These autophagosomes are recognized by the lysosomal compartment, which subsequently fuses with autophagosomes to form degradative organelles called ‘autolysosomes’. The acid lipases found within the lysosomal lumen are deposited into LD-containing autolysosomes, and by this mechanism, the neutral lipids at the core of LDs can be catabolized. Because of its potential in mediating the selective degradation of LDs, modulation of the autophagic pathway represents an attractive pathway that might be exploited for the resolution of the steatosis observed in early stages of ALD.[48]

The overall effects of alcohol exposure on autophagy of LDs appear to vary with the length of exposure to alcohol. In this sense, autophagy can thus be considered to be a hepatoprotective mechanism that can ultimately be undermined with continuous alcohol insult. For example, acute ethanol exposure seems to promote a large increase in autophagosomal biogenesis to promote the selective targeting of both mitochondria as well as LDs for autophagic degradation.[49,50] In contrast, chronic alcohol consumption appears to have the opposite effect, preventing autophagic progression and eventually resulting in hepatic LD accumulation.[51] This differential autophagic response appears to be related to alterations in the regulation of a key transcription factor, transcription factor EB (TFEB), that serves as a master regulator of lysosomal biogenesis and autophagy.[51–53] Indeed, levels of TFEB were found to be increased in the nuclear fractions of livers from mice that were acutely exposed to ethanol but not in those chronically administered with ethanol.[51]

Another group of proteins with potentially important roles in autophagy and ALD are members of a small guanosine triphosphatase (GTPase) family of Ras-related proteins—the Rab GTPases, many of which have well-established roles in membrane trafficking pathways as well in autophagy.[54,55] Through various proteomic screens, numerous Rab GTPases (*e.g.*, Rab7, Rab 10, and Rab18) have been localized to the surface of LDs, where they appear to moonlight on this compartment together with roles elsewhere in the cell.[14,16,56–58] Rats fed an alcohol-containing Lieber-DeCarli diet for an extended period of time were found to have defects in the ability of one of these Rabs (Rab7) to respond as expected to starvation-induced cues.[59] As with other Rab GTPases, Rab7 is found in either a guanosine triphosphate (GTP)-bound (active) state or a guanosine diphosphate (GDP)-bound (inactive) state.[54] Rab7 was previously shown to be abundant on the LD surface and to play critical roles in the autophagy-mediated catabolism of LDs under starvation conditions.[60] The finding that alcohol exposure can interfere with the activation of this Rab warrants the future examination of GTP cycling amongst the other Rabs found on the LD surface. In addition to defective enzymatic function, the ability of these Rabs to associate with and populate the LD surface in the first place may also be compromised in ALD. For example, the levels of LD-localized Rab18 appear to be significantly reduced with chronic alcohol feeding of rats.[61] The consequences of this finding remain unclear.

The lysosome itself may also be a direct target that is negatively affected by chronic alcohol intake. A recent study demonstrated that in mice, chronic ethanol feeding plus an acute binge resulted in significant decreases in hepatic lysosomal biogenesis, through interference with the TFEB transcriptional regulatory system.[62,63] A consequence of reduced lysosomal biogenesis is an attenuation in hepatocellular autophagic flux (insufficient autophagy). This is consistent with findings showing that chronic alcohol hinders the proteolytic function of hepatic lysosomes.[64] Further evidence for a role of TFEB was the finding that its overexpression led to an improvement in the expression of key fatty acid oxidation genes that are normally decreased in response to alcohol exposure (*i.e.*, *CPT1a*, *ACOX1*, and *PPARα*).[63] Moreover, ethanol also appears to impair dynamin-2, a mechanochemical GTPase involved in endocytosis, but also playing roles in recycling of autolysosomal membranes following the conclusion of lipophagy in a process termed ‘autophagic lysosomal reformation’.[65–68] Importantly, recent data in rats show that these effects may be mitigated by withdrawal of alcohol insult.[69]

Impaired autophagic removal of mitochondria also affects the ability of LDs to be catabolized. Knockout of *Parkin*, an E3 ubiquitin ligase that decorates the surface of damaged mitochondria during their selective autophagic turnover (in a process referred to as ‘mitophagy’), resulted in enhanced LD content compared to wild-type mice in an acute-binge model of acute alcohol exposure.[70] Promotion of autophagy by treatment with mammalian target of rapamycin complex 1 (mTORC1)-inhibitory compounds (*i.e.*, rapamycin) as well as carbamazepine improves hepatic steatosis as well as further liver injury in mice.[71] These results suggest that the continued exploration of the connection between autophagy and LDs may yield important insights into alcohol-induced steatosis.

4.2. Effects of alcohol on lipolysis

Another mechanism involved in the catabolism of LDs is that of lipolysis. Much of our understanding of the principles of cytosolic lipolysis stems from studies performed in adipocytes, which contain a single large unilocular LD (compared to the smaller multilocular phenotype of numerous LDs observed in hepatocytes). During hormone-stimulated lipolysis, soluble lipases are recruited to the surface of the LD to catalyze the removal of FFAs from the neutral lipid stored within LDs. Adipose triglyceride lipase (ATGL), hormone-sensitive lipase (HSL), and monoglyceride lipase (MGL) sequentially remove individual fatty acid moieties, releasing FFAs that can be immediately utilized in the mitochondria (for β-oxidation) or released into the bloodstream by adipocytes and later taken up in the liver by hepatocytes.

Exposure of mice to chronic alcohol feeding results in clear increases in lipolysis in the adipose tissue. As a consequence, the significant release of FFAs into the bloodstream and uptake in the liver results in substantial hepatic steatosis following conversion to triglyceride.[43] During lipolysis, AMP-activated protein kinase (AMPK) can be inactivated by chronic alcohol consumption.[72] Lipolysis within hepatocytes can also be inhibited in response to alcohol exposure.[73] This effect appears to arise due to inhibition of β-adrenergic receptor stimulation of hepatocellular lipases (and inhibition of the cyclic AMP/protein kinase A (cAMP/PKA) pathway), the net result being decreased LD turnover. Acetaldehyde, the metabolite resulting from ethanol metabolism also appears to play a role in modulation of cytosolic lipolysis. Whereas acetaldehyde appears to suppress the PPARα transcriptional network (resulting in decreased β-oxidation), it also suppresses PPARγ, resulting in enhanced lipolysis and biasing the adipose tissue-liver axis towards hepatic fat accumulation (Fig. 1).[74]

5. Effects of alcohol on LD motility

As reported above, the chronic administration of ethanol to rats resulted in decreased activation of the small GTPase Rab7.[59] This appears to be a partial explanation for the accumulation of hepatic LDs. Hepatocytes derived from these same animals also exhibited reduced lysosomal motility, implying that ethanol likely has broad effects on cytoskeletal trafficking networks. This is consistent with the findings of others that alcohol administration impairs microtubules; a clear consequence of an impaired microtubule network is that LD mobilization can be significantly attenuated. As intracellular organelles, LDs rely on an intact cytoskeletal network of microtubules to be transported from sites of synthesis to locations in the cell where they can be metabolized or interact with other compartments of the cell.[75,76] Linkages of LDs to the microtubule network is well established, with motor proteins such as kinesin-1 recruited to the LD surface to facilitate transport of LDs to the smooth ER for secretion as very low-density lipoprotein (VLDL) particles.[77] As stated above, acetaldehyde and acetate, byproducts of alcohol metabolism can form adducts to numerous proteins; among these proteins are soluble and polymeric tubulin.[78,79] Alcohol exposure results in enhanced microtubule acetylation of α-tubulin (on lysine 40) in polarized WIF-B cells as well as the ethanol-metabolizing VL-17A cell line.[80,81] These alterations of the central network used by LDs for intracellular motility represent

a third factor (along with enhanced lipogenesis and decreased LD breakdown) that together contributes to the steatosis that almost universally appears following alcohol consumption.

6. Future perspectives

How LDs respond to perturbations of the cellular environment is becoming an area of intense research interest. Because these organelles play central roles as sites of readily available energy storage, an understanding of their metabolic regulation is essential to understanding diseases such as obesity, diabetes, and fatty liver. We are still learning a great deal about the molecular machinery that controls many aspects of LD biology. For example, a number of recent studies have identified important roles for machinery that regulates the influx of triglyceride from the ER bilayer and into nascently forming LDs. One protein that appears to be involved in this process, Seipin, appears to stabilize ER-LD contact sites to allow for the unidirectional flow of neutral lipid into the LD during biogenesis.[82–85] Might alcohol exposure be involved in promoting these early stages of Seipin-driven LD synthesis?

Additionally, recent genetic data suggests that advanced liver disease may involve a number of genes that are intimately tied to LD dynamics. For example, the genetic mutation patatin-like phospholipase domain containing 3 (*PNPLA3*; I148M) has been recently identified to play a role in hepatic lipid accumulation. This variant (rs738409) was found to be strongly associated with both ALD as well as alcohol-related cirrhosis in various populations.[86,87] The mechanism whereby this mutant appears to influence LD biology is via defective ubiquitination of the mutant. Normally, PNPLA3 can be extracted from the LD surface and turned over by the proteasome. The I148 M mutation, however, renders the protein unable to be ubiquitinated, and as a consequence, it accumulates on the surface of the LD.[88] A side effect of this accumulation appears to be the inability of LDs to undergo turnover by cytosolic lipases. Intriguingly, a truncation variant in the gene encoding another LD-associated protein, *HSD17B13*, is thought to confer protection against the development of advanced ALD.[89,90] Future experiments addressing the mechanisms whereby this occurs are paramount.

Clearly, there is a complex interplay between alcohol and fat accumulation in ALD. As such, it is more urgent than ever to tease apart the molecular basis for the effects of alcohol consumption on the dynamics of LDs within the hepatocyte. Aside from diet/lifestyle modifications or bariatric surgery, which can reduce steatosis and associated inflammation/fibrosis, no approved pharmacological treatments are available to reduce LD content directly. A number of compounds that target lipid biosynthetic pathways and steatosis are currently in various stages of development, however, highlighting the ongoing interest in developing mechanisms of therapy related to LD biology for the treatment of ALD.[91]

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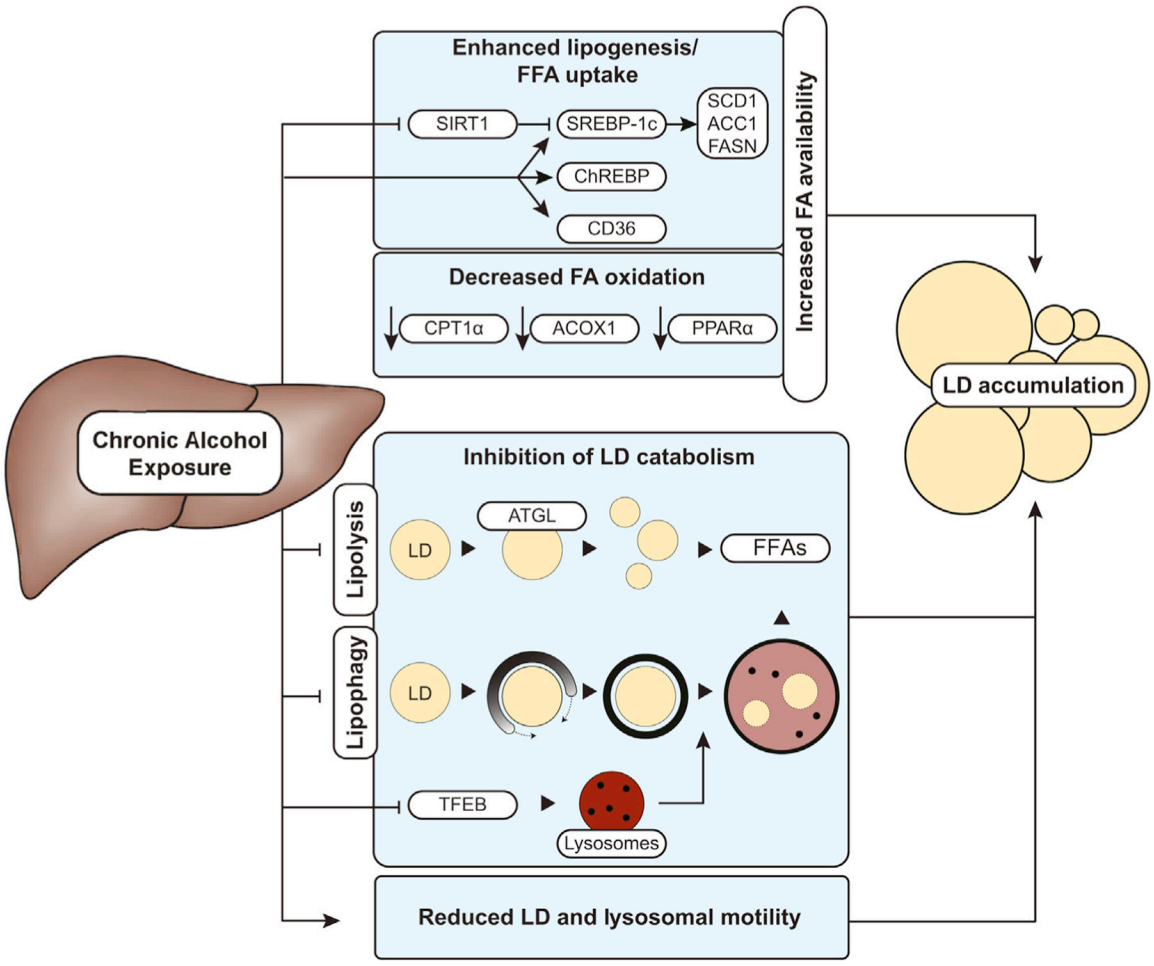

Fig. 1. Effects of chronic alcohol exposure on LD dynamics.

The effects of chronic alcohol consumption have numerous effects on hepatic LD dynamics including: (i) elevated FA substrate availability due to increased lipogenesis and decreased mitochondrial β-oxidation; (ii) inhibition of canonical LD catabolic pathways such as lipolysis and lipophagy; and (iii) reduced LD and lysosomal motility due to alterations of the microtubule network on which these organelles are trafficked throughout the cell. Abbreviations: FFA, free fatty acid; FA, fatty acid; SIRT1, sirtuin1; SREBP1c, sterol regulatory element binding protein 1c; ChREBP, carbohydrate-responsive element binding protein; SCD1, stearoyl CoA desaturase-1; ACC, acetyl-coA carboxylase; FASN, fatty acid synthase; LD, lipid droplet; CPT1α, carnitine palmitoyltransferase 1α; ACOX1, acyl-coenzyme A oxidase 1; PPARα, peroxisome proliferator-activated receptor α; TFEB, transcription factor EB; ATGL, adipose triglyceride lipase.