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STRUCTURE, FUNCTION AND METABOLISM OF HEPATIC AND ADIPOSE TISSUE LIPID DROPLETS: IMPLICATIONS IN ALCOHOLIC LIVER DISEASE

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Abstract

For more than 30 years, lipid droplets (LDs) were considered as an inert bag of lipid for storage of energy-rich fat molecules. Following a paradigm shift almost a decade ago, LDs are presently considered an active subcellular organelle especially designed for assembling, storing and subsequently supplying lipids for generating energy and membrane synthesis (and in the case of hepatocytes for VLDL secretion). LDs also play a central role in many other cellular functions such as viral assembly and protein degradation. Here, we have explored the structural and functional changes that occur in hepatic and adipose tissue LDs following chronic ethanol consumption in relation to their role in the pathogenesis of alcoholic liver injury.

INTRODUCTION

Alcohol abuse causes more than 60% of all chronic liver diseases and accounts for 2.5 million deaths globally each year [1]. Approximately 51.5% of US citizens use alcohol regularly; nearly 18% of these individuals meet the criteria for alcohol abuse [2]. The earliest manifestation of chronic alcohol consumption is the development of alcoholic fatty liver disease (AFLD). This is characterized by the intracellular accumulation of lipids that are stored in a specialized organelle called lipid droplets (LDs). The development of hepatic steatosis (fatty liver) is considered an initial critical event that predisposes the liver to progressive inflammation, fibrosis and cirrhosis [3, 4]. Thus, the understanding of the intracellular lipid stores and LD metabolism has emerged as a cornerstone for developing therapy against hepatic steatosis.

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In this review, we will first present an overview of LD structure and function. This will be followed by examining the effects of alcohol administration on various pathways/mechanisms that are responsible for the loss of adipose tissue LDs and the concomitant increase in the accumulation of hepatic LDs. The role of these alterations in the progression of liver injury will also be discussed. We will finally present therapeutic intervention strategies that prevent changes in adipose tissue and hepatic LDs to ultimately protect against the development of AFLD.

GENERAL STRUCTURE, FUNCTION AND METABOLISM OF LDs

LDs are also referred to as adiposomes, lipid bodies, fat bodies, eicosasomes, liposomes or even great balls of fat, and in the past decade the field has settled on the preferred name, LDs [5, 6]. LDs are highly conserved organelle present from bacteria to mammals [6, 7].

In eukaryotes, LDs may arise primarily from the endoplasmic reticulum (ER) and continue to maintain tight association with the parent organelle [6, 8, 9]. Although several models for LD formation have been proposed and discussed in details [6, 8, 10–12], none have been sufficiently proven. LDs have also been shown to interact with other cell organelles including mitochondria, peroxisomes and endosomes [13–18]. Structurally, LDs are composed of a homogenous lipid core covered by a phospholipid monolayer studded with proteins as discussed below and depicted in Fig. 1.

LD STRUCTURE

LD Core

The hydrophobic core of LDs is made of neutral lipids with tri- and diacylglycerols, esterified cholesterol and retinyl esters. These core lipids are present in hepatocyte LDs whereas LD cores in adipocytes are mainly triacylglycerol (TAG) [5, 6]. LDs number and size varies significantly between different cell types [6]. White adipocytes mainly form one giant unilocular LD as large as 100 μm, while most other cell types, including hepatocytes, have multiple small LDs with diameters of 100–200 nm [19, 20]. However, the size and number of LDs can increase under pathological conditions such as in hepatocytes following ethanol administration [21, 22].

LD Surface

LDs are coated by a monolayer of phospholipids, free cholesterol and lysophospholipids. LDs have been shown to have a unique phospholipid profile in their monolayer and phosphatidylcholine (PC) is the major phospholipid (50–60%) followed by phosphatidylethanolamine (PE) and phosphatidylinositol [23]. LDs are shown to have >50 different types of PC and 45 types of PE, and 9 species of phoshatidylinositol [23]. The phospholipids in the coating have been reported to stabilize the droplets and facilitate interactions with other cellular compartments and in maintaining LD morphology [24, 25]. There are also specific proteins that localize to the surface of LDs. In 1991, Greenberg *et al* identified a novel LD bound phosphoprotein, perilipin, in adipocytes [26]. Soon after, two other structurally related LD-binding proteins (ADRP and TIP47) were also identified [5]. These three LD-binding proteins became the founding members of the PAT (perilipin,

ADRP and TIP47) family of proteins [5]. These proteins have since been renamed Perilipin1, 2 and 3 (Plin1-3) respectively. In addition, perilipin4 and perilipin5 (older name S3-1 and OXPAT respectively), CG-158, lipases, protein kinases, phosphatases and lipid transport proteins associate with LDs and play important roles in regulating LD structure and function (reviewed in [9]).

Additional Proteins Associated with LDs

Only a dozen LD-associated proteins had been identified until 2006 when a mass spectrometry approach provided an array of proteins that were associated with LDs [5]. Subsequent proteomic approaches have revealed a surprising number of proteins, many unique, to be present in LDs isolated from different cell types [27–30]. The LD proteome was shown to have histone proteins like H2A, H2Av, H2B and vesicular trafficking proteins like Rab8 and Rab11 [31]. Further studies identified a repertoire of many other Rab-family members like, Rab1, 2, 5, 10, 14, 18,19b, 21, 24, 33b, 34, 35, 39, and 41 in LDs of Chinese hamster ovary cells [32].

LDs have been documented to contain enzymes for each step of *de novo* TAG synthesis [9, 33] and reportedly have the ability to locally synthesize PC. Lysophosphatidylcholine acyltransferase-1 and -2 [34] as well as enzymes that catalyze the rate-limiting step of *de novo* PC synthesis such as CTP:phosphocholine cytidyltransferase-1 and -2 [25, 35] have all been localized to LDs.

Earlier LD proteomes were also shown to have cytoskeletal, ER, lysosomal, mitochondrial, golgi, and chaperone proteins [32] which suggested that LDs could act as a carrier of proteins [31]. However, whether these other organelle proteins in the LD proteome represent co-purified contaminating or authentic LD proteins is currently being sorted out by utilizing protein correlation profile studies [35]. The various proteins present or associated with the LDs regulate key LD functions and are linked to many physiological processes as recently reviewed [6, 9, 14, 36–41].

LD FUNCTION AND METABOLISM

LDs have been shown to perform many cellular functions (Fig. 2), including storage, transport, and metabolism of lipids, viral assembly, protein sequestration, membrane trafficking and signaling [6, 8, 10, 11, 18, 33, 35, 42, 43].

In the healthy liver, the number and size of LDs are tightly regulated and there is an orchestrated re-distribution of stored TAG following lipolysis. FAs liberated from TAGs in liver LDs have one of several fates. They can be used as building blocks for membrane lipid synthesis, used for β-oxidation to generate energy or exported to the ER for re-esterification and packaging into VLDL for secretion [44]. These latter two mechanisms are used by the liver to get rid of the fat and prevent generation of steatosis.

LD Lipolysis and Associated Lipases

LDs were initially thought to be degraded extensively by hormone sensitive lipase (HSL) in adipocytes and in the liver. However, the failure of HSL knockout mice to become obese

indicated that other lipase(s) are present that can compensate for HSL deficiency [45, 46]. Later, two independent groups identified adipose triglyceride lipase (ATGL) on LDs [47, 48] that co-localized with Plin2 and Plin3 [49]. It has been documented that ATGL and Plin2 are both delivered to LDs from ER via COPI and COPII coatomer protein-mediated pathways [50]. ATGL-deficient mice exhibit LD accumulation in many tissues examined [48, 51, 52]. Despite low ATGL expression in the liver [53], (liver-specific) deficiency of this lipase caused increased LD accumulation in hepatocytes and cholangiocytes [54, 55]. Conversely, overexpression of ATGL enhanced LD lipolysis causing a marked decrease in LD size and this appeared to occur through displacement of Plin3 from LDs [49]. HSL overexpression also resulted in the loss of LDs [56]. However, ATGL or HSL overexpression did not promote VLDL assembly; instead it improved mitochondrial fatty acid β-oxidation [56]. These results are consistent with ATGL and HSL playing a role in lipid catabolism rather than VLDL export. A lipase that functions in mobilizing liver lipids for VLDL secretion is triacylglycerol hydrolase (TGH), which primarily resides in the ER [53] and also associates with LDs [57].

Patatin-like phospholipid domain containing protein 3 (PNPLA3), a multifunctional enzyme encoded by the PNPLA3 gene, has been shown to accumulate on LDs under pathological conditions [58]. PNPLA3 and their family members have patatin-like domain at the N-terminus for the lipase activity [59] and a conserved brummer box domain that helps targeting to LDs [59]. PNPLA3 also reportedly possesses acylglycerol O-acyltransferase activity that mediates the conversion of lysophosphatidic acid into phosphatidic acid [60].

In addition to its lipase activity, PNPLA3 was also shown to interact with another family member of patatin-like domain containing lipase, PNPLA2, and functions as a co-activator for enhancing PNPLA2 lipase enzyme activity [59].

LDs and VLDL Assembly

The assembly of VLDL is regulated by the availability of TAGs stored in cytoplasmic LDs [61]. Up to 70% of TAGs in VLDLs are derived via lipolysis and re-esterification of preformed TAG stores in LDs [62–64]. While the exact mechanism that links the composition of a VLDL core to LD lipolysis is not yet known, the formation of LDs in the secretory pathway clearly plays a central role in VLDL assembly and secretion. The protein and lipid factors within LDs must be maintained in a delicate balance for effective lipolysis. Any change in the structure or interactions of these components can alter the strong relationship between the accumulation of LDs and the rate of formation of VLDL. A potentially important observation is that the relative distribution of PC or PE modifies the LD size [23]. It has been documented that changes in the phospholipid composition can promote LDs to fuse into supersized structures that appear to be resistant to normal lipolysis [65, 66]. PE is a conical lipid that can increase membrane curvature, thereby promoting membrane fusion [24, 67]. Interestingly, an increase in the LD content of PE in absolute and relative terms at the expense of PC coincides with the accumulation of LDs [67].

Phosphatidylethanolamine methyltransferase (PEMT) is an enzyme that is required for normal secretion of VLDL as it is involved in synthesis of PC moieties that are preferentially used for the VLDL synthesis [68]. We contend that this enzyme is also important for

maintaining PC on hepatic LDs, thereby, preventing the development of supersized LDs that are resistant to lipolysis. Conversely, PEMT in adipocytes has been postulated to stabilize LD structures and promote fat deposition [67, 68].

LDs and Autophagy

Autophagy is mostly a cell survival mechanism providing a source of energy during nutrient starvation by catabolizing cellular proteins. Recent studies have revealed the role of autophagy in preventing LD accumulation [69]. Autophagy occurs by forming double-membrane autophagosomes which fuses to lysosomes resulting in degradation by lysosomal proteases and lipases [70]. Inhibition of autophagy, either by using 3-methyl adenine or by knocking down Atg2, Atg6, Atg7, & Atg8 genes, resulted in an increased accumulation of LDs in hepatocytes as well as decreased VLDL secretion [69, 71]. Further, co-localization of the autophagic marker protein, LC3, with LDs suggested that these two are physiological interacting partners [69]. A recent study identified that the LD-associated proteins, Plin2 and Plin3, are degraded by chaperone-mediated autophagy [72]. Further, these Plins possess a putative pentapeptide motif that is recognized by heat shock cognate protein of 70kDa (hsc70) for their delivery to the lysosomal-associated membrane protein 2A (LAMP-2A), a lysosomal surface protein [72]. Plin2 mutants that cannot bind to hsc70 were shown to be enriched in larger sized LDs [72]. Moreover, hepatocytes and fibroblast isolated from LAMP-2A deficient mouse exhibit increases in LDs and TAG levels [72]. Taken together, accumulating evidence indicates that lipophagy (a term for autophagy related to controlling lipid levels) and chaperone-mediated autophagy of Plin2 and Plin3 may play an important role in preventing LD accumulation in the liver by promoting lipolysis and VLDL secretion [73–76].

Vesicular trafficking of LDs in Hepatocytes

Our laboratories have made valuable contributions toward understanding the trafficking network in hepatocytes [77–79]. Current work in our laboratories is examining roles of several proteins involved in vesiculation (Dynamin-2; Caveolin-1 and Src) and transport (Rab18 and Rab7) relevant to LD trafficking.

Dynamin-2 (Dyn-2), Caveolin-1 (Cav-1) and Src kinase (Src)—Dyn-2 is a large GTPase that is known to polymerize around the neck of membrane invaginations for constricting, severing (“pinch”) and releasing a newly formed, small vesicle [78]. Our recent observations implicate Dyn-2 in LD vesiculation and metabolism [80]. Specifically, we have shown that knockdown or inhibition of Dyn-2 results in increased LD accumulation in liver cells [80]. We also demonstrated that Dyn-2 plays an important role in lipophagic breakdown [80]. Dyn-2 works in concert with two other proteins, Cav-1, a coat protein, and Src, a tyrosine kinase. Cav-1 is known to bind cholesterol, is targeted to LDs by exogenous cholesterol and associates with LDs [81–83]. Importantly, Cav-1 and Dyn-2 are regulated by Src [84, 85]. However, the participation of these three proteins in LD dynamics and metabolism is currently not known.

Rab proteins—An extensive family of small GTPases named Rab proteins act as molecular switches to support the targeted transport, docking, and fusion of a donor vesicle

to a receptor compartment. Several Rab proteins have been identified on LDs [22, 31, 32], and two of these are particularly important in LD metabolism. Rab18 is a common mediator in both lipogenesis and lipolysis [86] and co-localizes with Plin1 and Plin2 in the LDs isolated from hepatic cells [87]. Rab7 is known to play an important role in lipophagy [88]. We have recently observed that starvation enhances the level of active Rab7 in the LD fraction and suggested that this increase of active Rab7 in LD favors lipolysis [89]. Additionally, activated Rab7 in LDs is critical for LD interaction with multivesicular bodies and lysosomes and in the formation of autophagolysosomes [89]. LDs associated Rab7 was also found to be decreased in LAMP-2A deficient cells suggesting the important role of Rabs and chaperone-mediated autophagy in LD metabolism [72].

LD and Hepatitis C Virus Assembly

Many viruses, such as hepatitis C virus (HCV), use host cell LDs for replication [90]. Indeed, LDs are directly involved in assembling the infectious HCV particles. It has been shown that the maturation of the core protein promotes the transport of this structural HCV protein from the ER to the surface of LDs, where the full infectious particle is assembled [91]. While the exact mechanism of recruitment of core protein to LDs is not clear, it has been shown that trafficking of core to LD involves Rab18 [92]. The localization of HCV core protein to LD depends on the host diacylglycerol acyltransferase-1, which plays a pivotal role in translocation and attachment of core protein to LD [93]. Recently, it has been reported that the presence of core at the surface of LDs also interferes with the activity of ATGL, thereby blocking lipolysis [94].

Following core protein attachment to LDs, non-structural proteins and replication complex are recruited to these LDs [95] which through multiple protein-protein interactions [96] coordinate viral assembly [97]. In addition to providing a surface for viral assembly, LD also provides a vesicular transport system for virus to be exported out of the infected cells [98].

MicroRNAs in LD Metabolism

MicroRNAs (miRs) act to fine-tune the expression of a large number of genes and can regulate cell physiology and pathology. Emerging evidence implicates miRs in LD formation, lipid catabolism and vesicular trafficking. Functionally, miRs have been shown to alter LD formation in a “spontaneous steatosis” cell model. Specifically, a panel of 327 miRs was transfected into Huh7 cells followed by automated imaging (high-content analysis) to measure LD content. Six miRs (miR-34c, miR-509, miR-29a, miR-515-5p, miR-378, miR-1) were shown to increase LD accumulation while five miRs (miR-181d, miR324, miR-451, miR-493-5p, miR-135a) decreased lipid accumulation [99]. The specific functional importance of these results remains to be defined.

CHANGES IN HEPATIC LD STRUCTURE, FUNCTION AND METABOLISM FOLLOWING ETHANOL ADMINISTRATION

The first pathological change that occurs during the early stages of AFLD is increased accumulation of LDs within hepatocytes [100, 101]. Many of the detrimental effects of alcohol on the liver, including LD accumulation, are dependent on alcohol metabolism [102–

104]. Alcohol oxidation primarily occurs via two enzymes, alcohol dehydrogenase and microsomal cytochrome p450-2E1 that generate acetaldehyde [102, 103]. This toxic metabolite is a key player in the pathogenesis of AFLD since preventing its generation significantly attenuates LD accumulation in alcohol-metabolizing hepatic cells [104] and the development of many features of alcoholic liver injury.

There are characteristic compositional changes in hepatic LDs following ethanol administration, as reviewed below, that collectively play a role in altering LD metabolism and promoting the pathogenesis of alcoholic liver injury.

Role of Plin2

An increase in Plin2 has been suggested to be a reliable diagnostic marker for excessive alcohol consumption [21, 105]. There is a direct correlation between hepatic LDs and Plin2 levels and LDs number and size increase in parallel with the induction of Plin2 expression [21, 104–106].

Increasing Plin2 expression significantly blocked TAG hydrolysis and increased the fraction of cellular TAG that was stored in LDs [21, 107]. This anti-lipolytic effect of Plin2 to slow TAG turnover is mediated via its role in impairing ATGL association with LDs [107]. Conversely, Plin2 depletion prevented the alcohol-induced LD accumulation [108] indicating an important role of Plin2 in the pathogenesis of alcoholic steatosis.

Role of PNPLA3

Genome-wide association studies have identified variants of PNPLA3 in AFLD [109–112]. A common PNPLA3 I148M variant impaired hepatic secretion of VLDL and caused accumulation of TAG and cholesterol esters in large LDs of hepatocytes [113]. This observed effect could be explained by a recent report that, unlike recombinant enzyme, mutant PNPLA3 form does not display any TAG hydrolase activity [114]. However, PNPLA3 I148M variant showed 2.1 fold higher acylglycerol O-acyltransferase activity and this biochemical gain function were suggested to promote liver steatosis in the carriers of this variant [60]. Interestingly, a search in yeast mutants which produced supersized LDs were all shown to have dysregulated phospholipid metabolism and more commonly exhibited increased phosphatidic acid levels [115].

A recent study demonstrated that PNPLA3 is highly expressed in hepatic stellate cells (HSC) and further revealed that its lipase activity was responsible for degrading the stored retinoids in LDs of these cells [116]. Activation of HSC and the loss of stored retinoids is an initial event in the fibrotic process [117, 118] and alcohol administration impaired the activities of several retinoid metabolic enzymes [119]. However, further lipidomics analyses of retinoic esters in the hepatocyte and HSC LDs are needed to understand the metabolism of retinoid and their role in the pathogenesis of alcoholic liver injury.

Role of Altered Rab Protein Content and Impaired Vesicular Trafficking

Recent studies from our laboratories have shown alterations in several small GTPases involved in both vesicle and LD trafficking after ethanol administration [22]. Isolated LDs

from ethanol-fed rat livers identified significantly decreased protein content of Rab2, 5, 7, and 18 compared to controls [22]. Of particular interest was the observed decrease in Rab7 and Rab18 GTPases as both these proteins significantly affect LD metabolism as discussed below.

Rab18—Rab18 is a well characterized trafficking protein believed to be functionally important in LD biology, especially as it relates to lipolysis and LD accumulation. After identifying the decrease in Rab18 content on LDs from ethanol-fed rats, we evaluated its precise localization and interaction(s) with other LD proteins [22]. We observed that Rab18 was present on all LDs and it co-localized with Plin2 in the livers from control-fed animals [22]. In contrast, not all the LDs were found to be coated with Rab18 in the livers from ethanol-fed animals [22]. This was especially apparent in the large LDs, where the co-localization between Rab18 and Plin2 was significantly decreased. These data are particularly intriguing since previous research showed that Rab18 was recruited to LDs upon lipolytic stimulation [87]. We believe that the impaired recruitment of Rab18 to LDs and the decreased association of Rab18 with the anti-lipolytic Plin2, [107] following ethanol administration may be directly responsible for the reduced lipolysis and consequent LD accumulation.

Rab7—We have identified decreased Rab7 on LDs isolated from ethanol-fed rats [22], and data shows that Rab7 plays an important role in lipophagy [88]. Specifically, it has been observed that starvation enhances the level of active Rab7 in the LD fraction suggesting that increased active Rab7 in LD favors lipolysis [89]. Since activated Rab7 in LDs is critical for LD interaction with multivesicular bodies and lysosomes for the formation of autophagolysosomes, it is tempting to speculate that ethanol-induced LD accumulation in the liver might be a consequence of decreased lipophagy due to the impaired association of active Rab7 with LDs.

Role of Alterations in Liver Lipids and Phospholipids

Lipid analysis with alcohol consumption reported an elevation of 22:6, 18:0 and 18:0 to 18:3 FA containing lipids in serum and liver of alcohol-fed mice [120, 121]. Lipidomic studies using pattern recognition analysis of nuclear magnetic resonance spectroscopic data revealed that PC and total fatty acyl chains were decreased in the alcohol-fed liver [122]. Recent studies employing LD lipidomics revealed increases in TAGs, cholesterol, monounsaturation of fatty acyl chains, free FAs and significant decreases in PC levels in isolated LDs from the ethanol-fed rats compared with controls [123–125]. It is yet to be demonstrated whether such decrease in PC on LD surface could potentially causes impaired lipolysis, reduced TAG turnover and impaired VLDL secretion.

Ethanol administration has been shown to decrease the activity of microsomal long chain FA elongase 5 (Elovl5) [126]. Interestingly, adenoviral induction of hepatic Elovl5 was shown to promote lipolysis by increasing ATGL mRNA and protein expression [127]. Whether such ethanol-induced decrease in Elovl5 also is affecting ATGL expression and hepatic TAG turnover is another area of research that warrants further investigation.

Role of Lipophagy

Recent studies have shown that ethanol administration in addition to causing lipid accumulation in the liver also upregulates autophagic clearance of LDs as a protective measure [17, 71, 128, 129]. Acute ethanol-induced autophagy was also shown to enhance the clearance of damaged or fragmented mitochondria, a process termed as mitophagy [71]. Mitophagy can decrease the generation of reactive oxygen species and thereby functions as a protective mechanism to reduce ethanol-induced hepatotoxicity [71]. Signaling mechanism of ethanol-induced autophagy is via the inhibition of Akt and mammalian target of Rapamycin [71, 128].

Autophagy was also shown to be directly dependent on ethanol metabolism and reactive oxygen species generation [71]. Supplementation with antioxidants decreases ethanol-induced autophagy [71]. While ethanol-induced hepatocyte apoptosis has been shown to be very minimal [130], inhibition of autophagy resulted in increased hepatocyte apoptosis. These results suggest that the ethanol-mediated induction of the autophagic process is a protective, cell survival mechanism [131]. In support of this protective role of autophagy, liver specific autophagy-related protein 7 (Atg7) knockdown mice exposed to binge alcohol exhibit more severe liver damage [132]. Similarly, silencing Atg5 resulted in an increase in hepatocyte apoptosis [131, 132]. Conversely, treatment with rapamycin, an autophagy inducer, lowers LDs accumulation and triglyceride levels [133, 134]. Further investigations are required to examine whether an autophaghic inducer can promote LD catabolism and be used for reversing alcohol-induced hepatic steatosis.

Role in HCV Assembly

While there are no published studies on the role of ethanol on LD-associated HCV assembly, we believe that ethanol-induced increased LD accumulation could be responsible for the faster progression of the infection and in the exacerbation of liver injury in HCV-infected alcoholic patients. Indeed, we have observed an increased accumulation of LDs in livers of HCV ethanol-fed core-transgenic mice (unpublished observations) and in the livers of ethanol-fed NS5A+ mice [135]. Ongoing investigations are in progress to understand the persistence of HCV infection in alcoholic patients and their poor response to anti-viral therapy.

MiRs in LD metabolism

Excellent review articles discussing ethanol-induced alterations in miRs and their role in pathogenesis of liver injury have been published recently [136, 137]. However, the mode of ethanol delivery by oral feeding or gavage appears to modulate miRs differently [138, 139]. More work is needed to dissect and understand the relevance of these observations in disease progression. Nevertheless, especially relevant to alcoholic steatosis is the observation that ethanol administration increased hepatic miR-33 expression and decreased VLDL secretion [140]. Whether therapeutic approaches to alter miRs could limit LD formation and liver injury remains to be seen.

THERAPEUTIC ROLE OF BETAINE

A variety of animal model studies [141, 142] and *in vitro* studies [143–145] by many investigators have shown that treatment with betaine can mitigate many hallmark features of alcoholic liver injury [146, 147] including alcoholic steatosis [142]. More importantly, betaine protection against the development of ALFD is mediated via its effect on LDs. Particularly, the ethanol-induced increases in the size and numbers of LDs in the centrilobular and mid zonal regions of the liver lobules are prevented by betaine administration [148].

Mechanistic studies conducted in our laboratories have implicated the alcohol-induced reduction in the hepatocellular S-adenosylmethionine (SAM) to S-adenosylhomocysteine (SAH) ratio [142, 144] impairs PEMT-catalyzed PC generation [142, 144]. This in turn, causes decreased VLDL secretion [149], contributing to increased fat accumulation in the liver [142]. We have further shown that betaine treatment normalizes PEMT-catalyzed-PC generation [142] that restores VLDL secretion rate [149] and prevent alcohol-induced LD accumulation and steatosis [142, 148]. Whether PEMT-mediated catalysis is also responsible for maintaining LD surface PC moieties and in promoting lipolysis and TAG turnover for VLDL production and secretion is currently being examined.

CHANGES IN ADIPOCYTE LDs AFTER ETHANOL ADMINISTRATION

Emerging evidence suggests that an impaired adipose-liver tissue axis can be a potential factor that affects hepatic fat overload observed in alcoholics [150–153]. White adipose tissue (WAT) is the main site to store excess nutrients in the form of LDs and recent metabolic tracing experiments have shown that ethanol enhances adipocyte lipolysis, thereby shifting the metabolic task of storing excess lipids in the liver [150, 152]. Indeed, ethanol-induced WAT loss in the three main abdominal (epididymal, perirenal and mesenteric) fat storing sites [150] occurs through activation of lipases, HSL and ATGL [150, 152]. ATGL activity is normally inhibited by insulin [154] and it has been suggested that insulin resistance that develops with chronic ethanol abuse could mechanistically be related to increased WAT lipolysis [152]. Further ethanol-induced oxidative stress [155, 156] can also induce ATGL expression in a FoxO1-dependent manner [157].

Further studies have reported that alcohol-induced decreases in the adipose SAM:SAH ratio and elevations in homocysteine levels [155, 156, 158, 159] enhances HSL activation to promote LD lipolysis [158–160]. More importantly, betaine exerts it therapeutic effect to facilitate TAG storage in adipocytes by preventing these ethanol-induced changes in adipose tissue [158, 159].

CONCLUSIONS

Here we have presented details on the general structure of LDs and the associated proteins that control many cellular functions of LDs (Fig. 3), including storage, transport, and metabolism of lipids and HCV assembly. We discussed the structural and functional alterations that occur in adipocytes and hepatocytes LDs following ethanol administration. We further presented some insights into how these compositional changes promote lipolysis

and LD loss in adipocytes (Fig. 4) and at the same time inhibit lipolysis and TAG turnover in hepatocyte LDs to impair VLDL production and secretion (Fig. 5), ultimately leading to the development of AFLD. Finally, we presented a therapeutic option of using betaine that ameliorates both ethanol-induced increases in adipose tissue lipolysis and hepatic LD accumulation and thereby prevents the development of ALFD [148, 158, 159].

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List of abbreviations

AFLD	Alcoholic fatty liver disease
ATG	Autophagy-related protein
ATGL	Adipose triglyceride lipase
Cav-1	caveolin-1
Dyn-2	dynamin-2
Elovl5	Microsomal long chain fatty acid elongase 5
ER	Endoplasmic reticulum
FA	Fatty acid
HSC70	heat shock cognate protein of 70kDa
HSL	Hormone sensitive lipase
HCV	Hepatitis C virus
HSC	Hepatic stellate cells
LAMP-2A	lysosomal-associated membrane protein 2A
LDs	lipid droplets
miRs	MicroRNAs
PNPLA3	Patatin-like phospholipid domain containing protein 3
PC	Phosphatidylcholine
PE	Phosphatidylethanolamine
Plin	Perilipin
PEMT	Phosphatidylethanolamine methyltransferase

Src	Src Kinase
SAM	S-adenosylmethionine
SAH	S-adenosylhomocysteine
TAG	Triacylglycerol
VLDL	Very-low density lipoprotein
WAT	white adipose tissue

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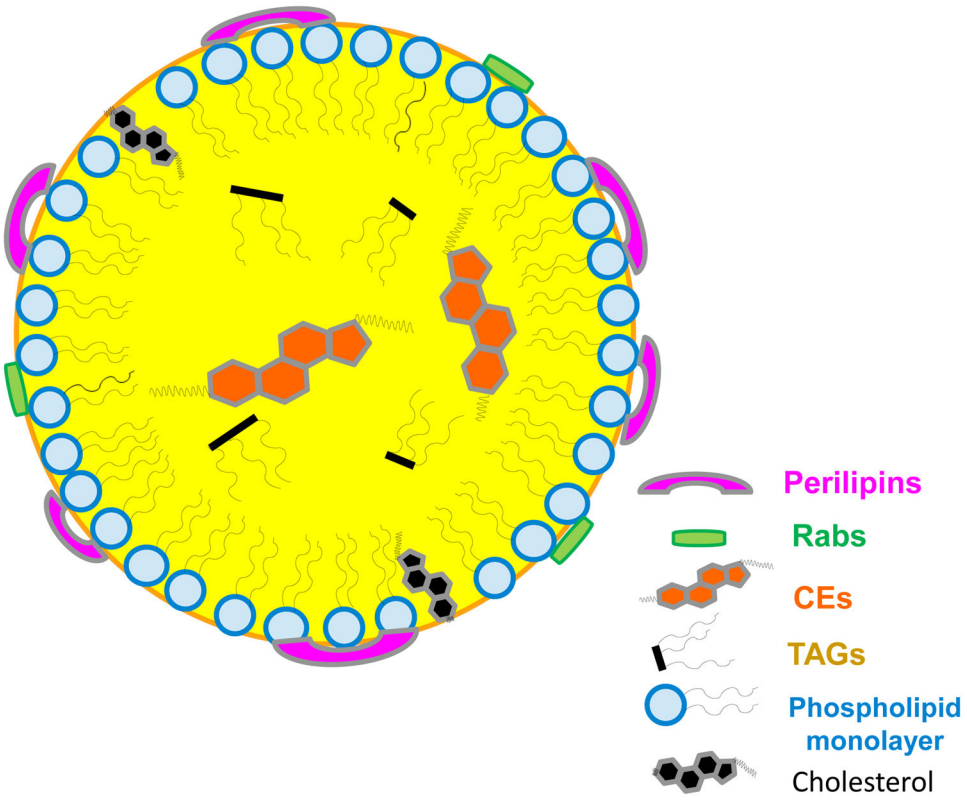

Figure 1. Diagrammatic Structure of Lipid Droplets

Lipid droplets (LDs) are composed of a homogenous lipid core covered by a monolayer coating of phospholipids, free cholesterol and lysophospholipids. The hydrophobic core of LDs is made of neutral lipids with tri- and diacylglycerols, esterified cholesterol and retinyl esters. The surface of LDs is also studded with several different proteins including perilipins and RabGTPase's.

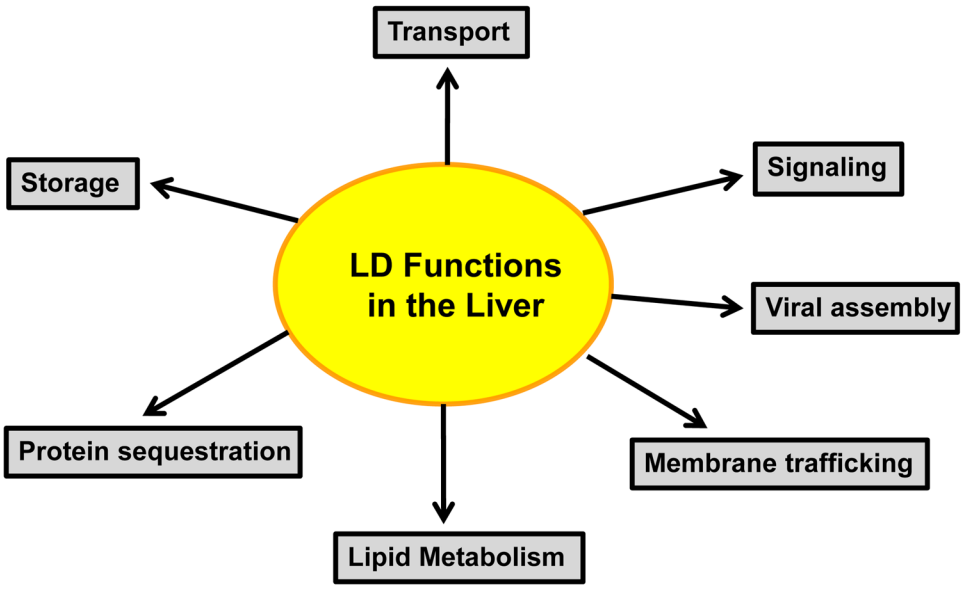

Figure 2. Diverse functions of Liver Lipid droplets

Lipid droplet (LD) is an active subcellular organelle that performs many cellular functions in the liver as shown.

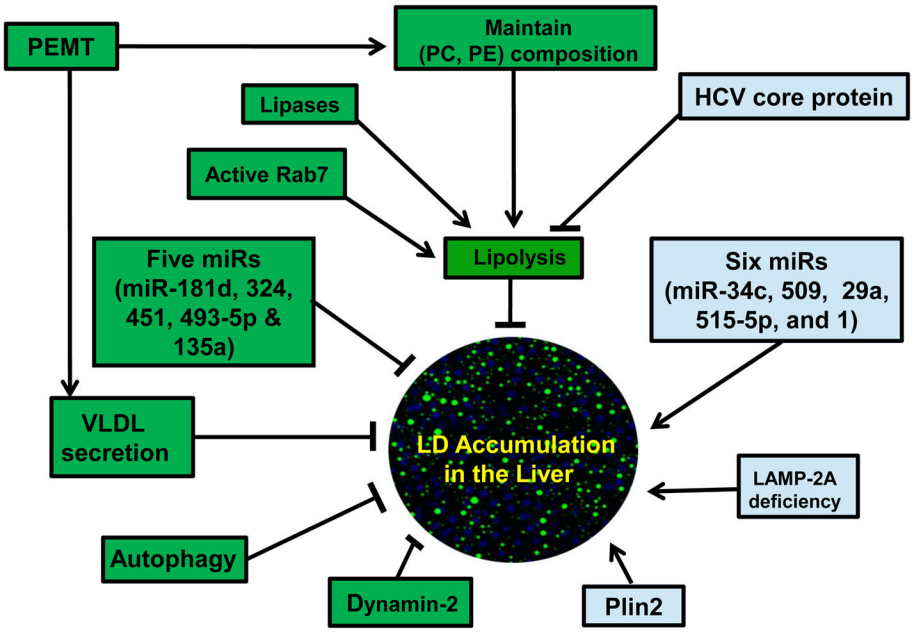

Figure 3. Schematic representation of many players that regulate lipid droplet formation and degradation

The protein and lipid factors within lipid droplets (LDs) must be maintained in a delicate balance for effective lipolysis. Any change in the structure or interactions of these components can alter lipid droplet formation and their degradation. Hepatic phosphatidylethanolamine methyltransferase (PEMT) maintains phospholipids composition of the LD for normal lipolysis to facilitate VLDL production and secretion to ultimately prevent LD accumulation. In addition, lipases, active RabGTPases such as Rab7 and dynamin 2 activate lipolysis/vesiculation and prevent LD accumulation. LD also recruits core autophagic machinery and LAMP-2A dependent chaperone-mediated autophagy of Perilipin2 (Plin2) thereby favoring lipolysis. Several microRNAs (miRs) listed reportedly prevent LD accumulation. Alternatively, several other miRs, Plin2 and HCV proteins all promote LD accumulation in the liver.

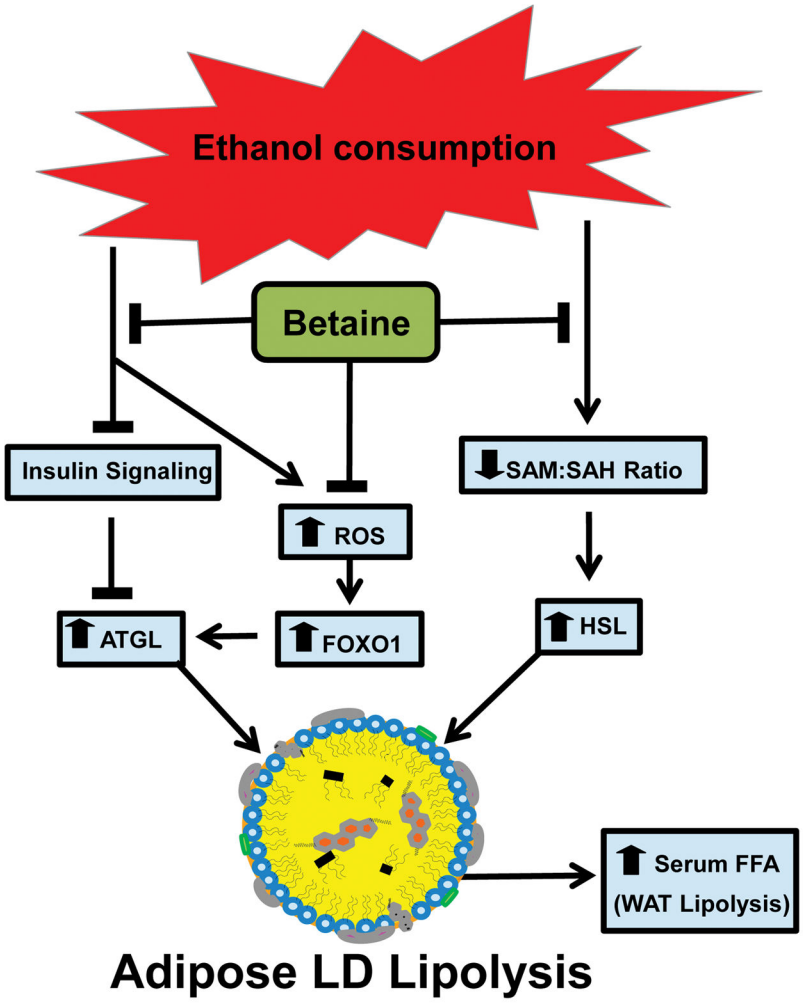

Figure 4. Major mechanisms by which ethanol promotes adipocyte LD lipolysis

Ethanol induces LD lipolysis in white adipose tissue (WAT) loss through activation of the lipases (HSL and ATGL). Insulin resistance that develops with chronic ethanol abuse promotes WAT LD lipolysis via ATGL activation. Further ethanol-induced oxidative stress also induces ATGL expression in a FoxO1-dependent manner. Ethanol-induced changes in adipose S-adenosylmethionine:S-adenosylhomocysteine (SAM:SAH) ratio enhance HSL activation to promote LD lipolysis. Betaine can prevent activation of the lipases via normalizing adipose SAM:SAH ratios and preventing homocystenemia.

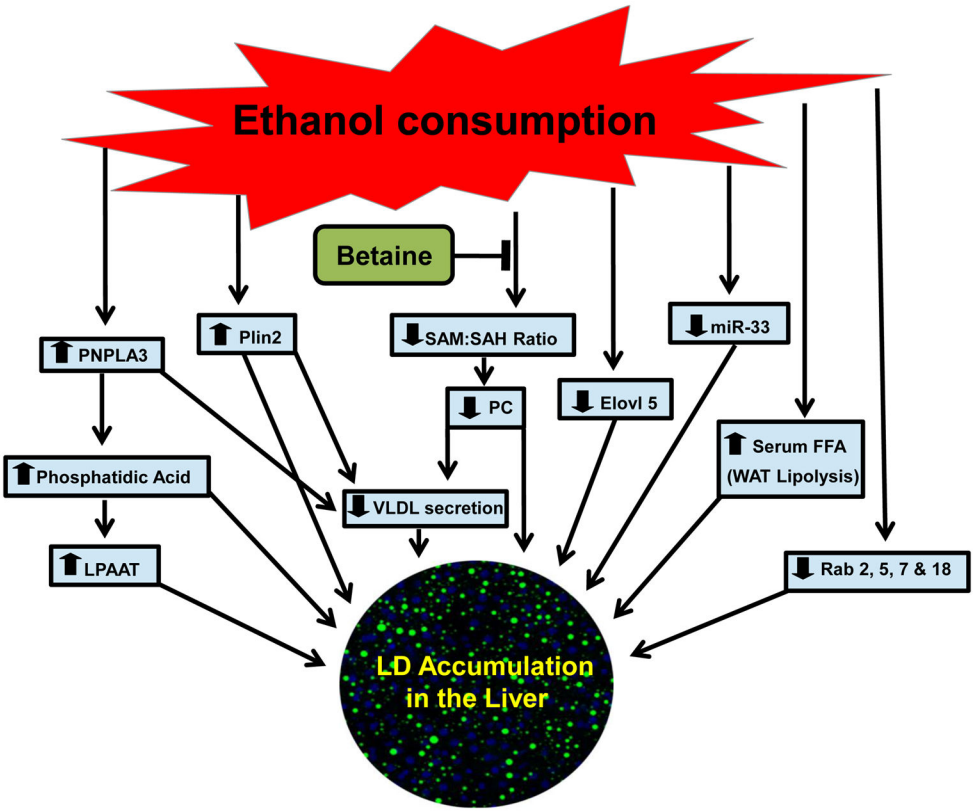

Figure 5. Multiple mechanisms by which ethanol facilitates lipid droplet accumulation in the liver

In the healthy liver, the number and size of lipid droplets (LDs) are tightly regulated and there is an orchestrated re-distribution of stored triglycerides (TAG) following lipolysis. Ethanol-induced changes in LD phospholipid and protein composition inhibits LD lipolysis, TAG turnover and VLDL secretion causing LD accumulation in the liver. Betaine via its ability to normalize hepatocellular methylation potential (S-adenosylmethionine:S-adenosylhomocysteine [SAM:SAH] ratio) could normalize LD phospholipid composition to promote lipolysis and VLDL secretion, thereby preventing LD accumulation.