



Published in final edited form as:

Exp Cell Res. 2016 January 15; 340(2): 187–192. doi:10.1016/j.yexcr.2015.10.021.

Pathophysiology of lipid droplet proteins in liver diseases

Rotonya M. Carr, M.D.¹ and Rexford S. Ahima, M.D., Ph.D.²

¹Department of Medicine, Gastroenterology and Hepatology Division, University of Pennsylvania, Perelman School of Medicine, Philadelphia, Pennsylvania, U.S.A

²Department of Medicine, Division of Endocrinology, Diabetes and Metabolism, University of Pennsylvania, Perelman School of Medicine, Philadelphia, Pennsylvania, U.S.A

Abstract

Cytosolic lipid droplets (LDs) are present in most cell types, and consist of a core comprising neutral lipids, mainly triglycerides and sterol esters, surrounded by a monolayer of phospholipids. LDs are heterogeneous in their structure, chemical composition, and tissue distribution. LDs are coated by several proteins, including perilipins and other structural proteins, lipogenic enzymes, lipases and membrane-trafficking proteins. Five proteins of the perilipin (PLIN) family (PLIN1 (perilipin), PLIN2 (adipose differentiation-related protein), PLIN3 (tail-interacting protein of 47 kDa), PLIN4 (S3-12), and PLIN5 (myocardial lipid droplet protein)), are associated with LD formation. More recently, the CIDE family of proteins, hypoxia-inducible protein 2 (HIG2), and patatin-like phospholipase domain-containing 3 (PNPLA3) have also gained attention in hepatic LD biology. Evidence suggests that LD proteins are involved in the pathophysiology of fatty liver diseases characterized by excessive lipid accumulation in hepatocytes. This review article will focus on how hepatic LDs and their associated proteins are involved in the pathogenesis of three chronic liver conditions: hepatitis C virus infection, non-alcoholic fatty liver disease, and alcoholic liver disease.

Keywords

liver; lipids; lipid droplet; perilipin; steatosis; hepatitis; obesity; diabetes; alcohol; NAFLD; HCV

Introduction

Hepatocytes are parenchymal cells of the liver responsible for mobilizing lipids for energy and storing excess lipids in the form of lipid droplets (LDs), thus making the liver the primary organ responsible for lipid homeostasis. Hepatocellular accumulation of excess LDs is called hepatic steatosis. LDs are thought to develop as either a bud, bicelle or vesicle from

Address correspondence to: Rotonya M. Carr, M.D. University of Pennsylvania Perelman School of Medicine Biomedical Research Building, Room 907 421 Curie Boulevard Philadelphia, Pennsylvania 19107, rotonya.carr@uphs.upenn.edu.

Conflict of interest: The authors have no conflicts to declare.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

the lipid bilayer of the endoplasmic reticulum¹⁻³, an organelle with which LDs share key enzymes involved in lipid synthesis and lipolysis. After a putative maturation process, the final LD structure is comprised of a core of neutral lipids (primarily triacylglycerols (TGs) and cholesterol esters) and a membrane monolayer of phospholipids and sphingomyelin. LDs ultimately distinguish themselves as bioactive organelles in their own right by associating with LD-specific proteins whose primary roles are in maintaining LD homeostasis¹ (Figure 1).

The predominant hepatocellular LD proteins are members of the perilipin family of proteins. There are five known perilipins (Perilipins 1-5 (PLIN1-5)) expressed in hepatocytes. Each perilipin is thought to have a distinct role, but few have been studied specifically in hepatocytes. More recently, the non-perilipin LD associated proteins hypoxia-inducible protein 2 (HIG2), the cell death-inducing DFFA-like effector (CIDE) family of proteins, and patatin-like phospholipase domain-containing 3 (PNPLA3/adiponutrin) have been discovered to have roles in hepatocellular lipid biology as well. HIG2 is a target of hypoxia-inducible factor 1 (HIF1), co-localizes with PLIN2 and PLIN3, and may be a marker of hepatic hypoxia⁴. The CIDE family of proteins includes CIDEA, CIDEB, and CIDEF/fat-specific protein 27 (Fsp 27)⁵. As their name implies, CIDE proteins promote cell death when upregulated, however, they associate with the LD membrane and, as a result, have roles in cellular lipid homeostasis. Finally, PNPLA3 is a protein with LD, ER and cytoplasmic distribution⁶. PNPLA3 has TG hydrolase and transacylase activity⁷ but its requirement for LD homeostasis remains controversial. A sequence variant of the PNPLA3 gene (rs738409 C>G; “G allele”) encodes an isoleucine-to-methionine substitution at position 148 and this particular single-nucleotide polymorphism (SNP) has gained significant attention since initially being associated with risk of hepatic steatosis in patients with non-alcoholic fatty liver disease (NAFLD)⁸.

The specific mechanisms that determine the fate of an individual LD remain elusive. We and others⁹ speculate that there are pools of LDs with distinct lipid and protein composition that are either targeted for lipolysis, secretion in the form of very low density lipoprotein (VLDL), or long-term lipid storage making these organelles highly regulated and integrated within the hepatocellular machinery. Hepatic steatosis, therefore, ultimately results from dysregulation of this homeostatic process. This review will summarize the evidence to date of the mechanisms of LD accumulation with specific attention to the roles of LD proteins in hepatocellular biology in three common conditions: hepatitis C, NAFLD, and alcoholic liver disease (ALD).

Hepatitis C

Chronic liver disease due to hepatitis C virus (HCV) infection affects approximately 185 million people worldwide. Infection with HCV causes chronic hepatic inflammation, fibrosis, cirrhosis, and in some cases hepatocellular carcinoma¹⁰. HCV is a single-stranded positive-sense RNA virus whose structure and life cycle have largely been unraveled. The polyprotein product of the RNA virus is comprised of structural and non-structural components. The structural components include the HCV core protein and two envelope glycoproteins (E1 and E2). The non-structural (NS) components are NS2, NS3, NS4A,

NS4B, NS5A and NS5B, most of which are involved in RNA replication through intrinsic protease or polymerase activity. Both the life cycle and infectivity of HCV are dependent on host lipid metabolism. In fact, HCV circulates as a component of host VLDL, engages the host's low density lipoprotein (LDL) receptor for hepatocellular uptake, and alters cellular lipid homeostasis to promote its own replication and release from cells¹¹. In addition, HCV has direct effects on the host's serum lipid profile and hepatocellular lipid content. The following sections summarize current understanding of the mechanisms by which HCV promotes hepatocellular LD accumulation.

Intracellular viral assembly and LD association

HCV enters hepatocytes primarily via the LDL receptor after which the virus moves to the endoplasmic reticulum (ER) and forms an ER-derived complex adjacent to LDs. The complex is comprised of HCV viral proteins enveloped by a membranous lipid bilayer, referred to as the membranous web. The HCV polyprotein is anchored to the ER by core protein and from there its components gain access to LDs¹¹ (Figure 2). These interactions with cellular LDs serve to regulate LD pool size through effects on lipid storage, oxidation, and secretion.

The effects of HCV on lipid storage are predominantly through the direct interactions of HCV proteins with the LD membrane. The interaction of core protein with the ER membrane-associated enzyme diacylglycerol acyltransferase 1 (DGAT 1) is required for the binding of HCV core protein to LDs. DGAT 1 is a rate-limiting enzyme in TG synthesis from diacylglycerol and promotes core LD-binding likely through its regulation of HCV assembly. Core protein remains associated with the ER membrane in DGAT1 inhibited cells but localizes to LD upon DGAT1 overexpression¹². Non-structural HCV proteins also play a role in LD binding. NS3 is present in the LD fractions of control-treated cells but not in cells treated with a DGAT1 inhibitor¹². The hydrophobic amino acid residues of NS4B's alpha-helices help HCV target LDs, and mutations of these residues prevent virus replication¹³. In cells with replicating virions, NS5A is found on LDs and in association with HCV replication complexes¹⁴. These data demonstrate that LDs are central to HCV's designs on the human host.

HCV and LD proteins

NS5A binds LDs through several protein-protein interactions. Namely, NS5A forms a complex with HCV core protein and DGAT-1¹⁴; associates with the Rab GTPase, Rab18, which facilitates recruitment of NS5A to LDs¹⁵; and binds PLIN3 (or tail-interacting protein of 47kD (TIP47)) via its amphipathic helix on the surface of LDs¹⁶. PLIN3 is an LD protein whose association with LDs increases in HCV-replicon cells. The interaction between NS5A and PLIN3 is required for HCV replication, as there is reduced PLIN3 binding to NS5A and a near absence of colony formation in HCV replicon cells transfected with mutant NS5A¹⁶. Although other perilipin proteins are expressed in the livers of HCV patients¹⁷, the role of these proteins in promoting HCV infection is unknown.

The roles of CIDE proteins, HIG2, and PNPLA3 in HCV infection are also not well understood. CIDEB may be important for hepatocyte differentiation and is required for HCV

infection of hepatocytes *in vitro* likely due to promotion of endosomal fusion¹⁸. HIG2 is upregulated in patients with HCV¹⁹, but the specific role of this LD protein in HCV has not been directly examined. The PNPLA3 “G allele” is associated with hepatic steatosis and risk of progression in HCV in some populations²⁰. The specific interaction between this gene product and the HCV viral machinery, however, is unknown.

Clinical implications of HCV hepatic steatosis

About 70% of HCV patients develop hepatic steatosis; and the presence of steatosis correlates with both HCV genotype and disease progression²¹. Patients with HCV genotype 3 have the highest prevalence of hepatic steatosis²¹ likely due to the direct effects of the HCV virus on host cellular lipid machinery discussed above. Patients with genotype 1 also have a high prevalence of hepatic steatosis, but in these patients, the development of hepatic steatosis correlates with BMI and insulin resistance²¹, leading many to suggest that it is the development of the metabolic syndrome rather than the virus itself that promotes hepatocellular lipid accumulation. In fact, Adinolfi et al. demonstrated that steatosis is associated with visceral obesity in 76% of patients with genotype 1 or 2 infection (or mixed); while 70% of genotype 3a patients are normal weight²¹. These differences may simply reflect an association between host genetic background and geographic distribution of viral genotypes; or may result from yet undefined viral factors that impact lipid biology.

Non-alcoholic fatty liver disease (NAFLD)

NAFLD is the hepatic manifestation of the “metabolic syndrome”. NAFLD is diagnosed by the presence of excess hepatocellular LD accumulation in the absence of other causes of chronic liver disease. NAFLD affects up to one-third of the population²², making this a significant public health issue worldwide. Despite its prevalence, there is no consensus on the primary drivers of disease progression. NAFLD can progress from hepatic steatosis to steatohepatitis, cirrhosis and hepatocellular carcinoma. Hepatic steatosis in NAFLD is at least partially due to perturbations of lipid synthetic and oxidative pathways. More recently, the role of LDs and their associated proteins has become an active area of investigation in NAFLD.

NAFLD and mechanisms of LD accumulation

Upregulation of lipogenic pathways, impairment of mitochondrial fatty acid oxidation, and inhibition of TG secretion all contribute to the development of hepatic steatosis in NAFLD. A comprehensive discussion of these pathways is beyond the scope of the current review but several key factors are thought to play a role. Increased *de novo* lipogenesis significantly contributes to the development of hepatic steatosis in NAFLD patients. The transcription factors sterol regulatory binding protein 1 (SREBP1) and carbohydrate responsive element binding protein (ChREBP) are key regulators of lipogenic genes increased in both patients²³ and rodent models of NAFLD; and deficiency of these transcription factors ameliorates hepatic steatosis in experimental models^{24, 25}. Defects of fatty acid mitochondrial oxidation are also evident in NAFLD patients and likely result from both direct and indirect effects on mitochondrial structure and function²⁶. Finally, the contribution of VLDL secretion to

hepatic steatosis in NAFLD is unclear, as it has been shown to be both reduced²⁷ and increased²⁸ in NAFLD patients.

NAFLD and LD proteins

Impairment of the pathways listed above have a net effect of hepatocellular LD accumulation, and an associated increase of both perilipin and non-perilipin proteins. Of the perilipins, PLIN2 is most upregulated in the livers of rodents²⁹ and humans with NAFLD¹⁷. PLIN2 promotes TG accumulation³⁰, inhibits fatty acid oxidation³¹, and impairs glucose tolerance^{29, 32-34}. *PLIN2* knock-out mice are protected from hepatic steatosis when fed a high fat diet^{34, 35}, a diet that induces NAFLD. When bred with obesity-prone leptin deficient mice, these mice have reduced steatosis despite some compensation of PLIN3 for PLIN2³². Our group specifically examined the role of hepatic PLIN2 with anti-sense oligonucleotide (ASO) knockdown of hepatic PLIN2 in wildtype mice fed a high-fat diet³³ and leptin-deficient mice³⁰. PLIN2-ASO reduced hepatic TG and reduced genes involved in fatty acid metabolism^{30, 33}. Notably, reduction of steatosis was also associated with an upregulation of fibrosis genes, suggesting that hepatic PLIN2 may protect the liver from lipotoxicity³³ despite its impairment of glucose homeostasis³⁰.

We next examined the role of hepatic PLIN3 in a NAFLD mouse model. PLIN3 is upregulated in the livers of mice fed a high-fat diet. We demonstrated that PLIN3 ASO reduces hepatic TG and improves insulin sensitivity³⁶. To our knowledge, this is the only study of PLIN3 hepatic reduction in experimental NAFLD.

PLIN5 is increased in the livers of fatty liver dystrophic (fld) mice³⁷. In a study by Wang, et al, *plin5* knock-out mice were protected from hepatic steatosis due to increased lipolysis and fat oxidation and like PLIN2, PLIN5 is suspected to play a protective role against lipotoxicity. To date, there have been no studies examining the liver-specific role of PLIN5 *in vivo*, but in hepatoma cells, PLIN5 binds adipose-triglyceride lipase (ATGL) and the ATGL activator, α - β -hydrolase domain-containing 5 (ABHD5)³⁸ thus providing a mechanistic link between PLIN5 and lipolysis.

Among the non-perilipin proteins, CIDEA and CIDEC appear to have a role in fatty liver. CIDEA is increased in fld mice, and adenoviral overexpression of SREBP1 in wildtype hepatocytes results in increased CIDEA expression³⁷. CIDEA is also upregulated in CIDEC-deficient mice and adenoviral delivery of short hairpin RNA CIDEA reduces hepatic steatosis in these CIDEC-deficient mice³⁹. Like CIDEA, hepatic CIDEC is increased in fld mice³⁷ and in leptin-deficient *ob/ob* mice⁴⁰. Overexpression of CIDEC promotes steatosis while CIDEC knock-down reduces steatosis in *ob/ob* mice lacking hepatic PPAR γ ⁴⁰. The contribution of CIDE proteins to the pathogenesis of NAFLD remains an active area of study.

The role of HIG2 in NAFLD is an emerging area of investigation. Inactivation of hepatic von Hippel-Lindau protein causes hepatic steatosis, inflammation and upregulation of *Hif* genes, a phenotype ameliorated by HIG2 disruption^{41, 42}. HIG2 may also regulate hepatic PLIN2 expression⁴² and prevent LD lipolysis⁴³, thus providing a link between cellular oxygenation status and LD formation.

The role of the *Pnpla3* “G allele” has been investigated in several experimental models of NAFLD, but the *in vivo* function of its protein product remains elusive. *Pnpla3* knock-out mouse models show no specific hepatic phenotype^{44, 45}, while a recent *Pnpla3* “G allele” knock-in demonstrates that this allele confers increased risk of hepatic steatosis and accumulation of larger hepatocellular LDs possibly through sequestration of comparative gene identification 58 (CGI), a co-factor required for activation of ATGL⁴⁶. However, this potential mechanism has not been supported because CGI is not increased in the LDs of *Pnpla3* transgenic mice.⁴⁶

LD proteins in human NAFLD

In humans with NAFLD, several hepatic LD proteins are upregulated. PLIN1 is upregulated in steatohepatitis due to NAFLD (non-alcoholic steatohepatitis, NASH)^{17, 47}; but PLIN1 protein is not typically expressed in normal hepatocytes¹⁷. Moreover, the distribution of hepatic PLIN1 in NAFLD patients differs from that of PLIN2 and PLIN3 in that it surrounds mostly large LDs^{17, 47}. PLIN2 is upregulated in human NAFLD^{17, 47-49} and appears to be a more reliable marker of hepatic LDs. PLIN2 is present in both hepatocytes and hepatic stellate cells (a fibrogenic cell type) and surrounds LDs of varying sizes^{47, 49}. PLIN2 LD protein expression also correlates with oxidative damage reflected in ballooned hepatocytes⁴⁷. A polymorphism of the *PLIN2* gene was recently reported⁵⁰ providing further insight into the role of this protein. A minor allele of this missense polymorphism Ser251Pro disrupts a PLIN2 alpha-helix and causes reduced plasma TG and VLDL levels⁵⁰, thus giving indirect evidence of the effects of this polymorphism on hepatic lipid homeostasis.

PLIN3 upregulation has been observed in human steatotic livers^{17, 48}. To date, there have been no human studies examining hepatic PLIN4 or PLIN5; and the role of the non-perilipin LD proteins HIG2 and CIDE family remains unclear in human NAFLD. Genome wide association studies have associated the *PNPLA3* “G allele” variant with both serum ALT and hepatic fat content⁸ in NAFLD patients. Although the biologic role of the *PNPLA3* “G allele” is debated, the independent association of this variant with NAFLD risk and progression has been established in numerous studies⁵¹. To date, however, incorporation of *PNPLA3* genetic testing is not a part of routine care of NAFLD patients.

Alcoholic Liver Disease (ALD)

ALD is the most common cause of liver failure worldwide and accounts for 44% of all liver related deaths in the United States. Similar to both NAFLD and HCV, hepatic steatosis is a key histologic feature and develops in over 90% of heavy drinkers. Only 15% of heavy drinkers with hepatic steatosis develop progressive liver disease⁵² suggesting that there are additional factors that mediate liver disease in heavy drinkers. Due to the limitations of existing ALD rodent and cellular models, the pleiotropic effects of alcohol on hepatocellular LD formation are incompletely understood. Nevertheless, steatogenic effects of chronic alcohol have been shown to result from upregulation of lipid synthetic and storage proteins, downregulation of fatty acid oxidation enzymes and impairment of LD secretory mechanisms.

The role of *de novo* lipogenesis in LD accumulation in ALD has been explored in several studies. *Srebp1c* (an isoform of *Srebp*) is activated in experimental alcohol steatosis; and its downstream fatty acid synthetic gene targets are upregulated in hepatoma cells and in mice chronically fed a 27.5% calorie content alcohol diet⁵³. Although up-regulation of *Srebp1c* appears to be involved in ethanol-induced lipid accumulation, the physiologic effects of this up-regulation may be modest at best as the contribution of *de novo* lipogenesis to hepatic lipid accumulation in response to ethanol is estimated to be less than 5%⁵⁴. These data suggest that changes in lipogenic gene and protein expression in response to ethanol may not necessarily translate into measurable physiologic endpoints and may be dependent on the duration and amount of alcohol consumption and the availability of other dietary macronutrients.

There is substantial experimental evidence of the adverse effects of alcohol on fatty acid oxidation and resultant LD accumulation, and there are likely several mechanisms involved. For example, alcohol has direct and indirect toxic effects on hepatic mitochondria and its components that ultimately impair the mitochondria's ability to oxidize fatty acids; and alcohol inhibits AMPK and PPAR α , key signaling pathways involved in fatty acid oxidation⁵⁵.

Alcohol impairs VLDL secretion. VLDL levels may be reduced by host immune response to acetaldehyde VLDL adducts, as alcoholic patients have higher serum IgG titers than non-alcoholics against VLDL; and ApoB-containing lipoproteins from these alcoholic patients are more prone to acetaldehyde adduct formation than non-alcoholic patients⁵⁶. To our knowledge, however, this study has not been replicated in patients with and without ALD.

ALD and LD proteins

PLIN2 is the most prominent LD protein seen in alcoholic fatty liver^{57, 58}, and it is overexpressed in humans with alcoholic steatohepatitis¹⁷. In experimental alcoholic steatosis, PLIN2 associated LDs temporally increase and have a zone two predominance⁵⁹. We observed this temporal correlation between hepatic PLIN2 upregulation and the development of steatosis; and additionally observed a correlation between PLIN2 upregulation and the onset of glucose intolerance in alcohol-fed mice⁶⁰. We were also the first to demonstrate that PLIN2 is required for the development of steatosis in mice chronically fed alcohol⁵⁷. However, the exact mechanism by which alcohol regulates PLIN2 requires further investigation. To our knowledge, the role of other perilipin LD proteins has not been directly examined in either human or experimental ALD. Moreover, although the *PNPLA3* I148M polymorphism is associated with ALD risk and progression^{61, 62}; the cellular role of this protein in experimental ALD has not been established.

Conclusion

Hepatocellular LDs and their associated proteins are key mediators of cellular lipid homeostasis and their biology is an emerging area of investigation in chronic liver diseases. Both perilipin and non-perilipin LD proteins can be modulated to promote lipid storage and utilization. The remarkable ability of these proteins to be regulated by both host and foreign proteins suggests that these proteins can be targeted for therapeutic purposes. However,

more research is needed to examine the optimal balance between hepatic steatosis prevention and permissive steatosis, as the latter may be required to prevent cellular exposure to toxic lipid metabolites. In addition, the mechanisms underlying differential regulation of perilipin LD proteins in the context of hepatic steatotic diseases is not well understood. Exploration of these and other areas will greatly advance our understanding of hepatic steatosis, the earliest pathologic stage of the most common liver diseases worldwide.

Acknowledgments

R.M. Carr is supported by National Institutes of Health grant K08-AA021424, P30-DK-50306 pilot and feasibility grant from the Penn Diabetes Research Center, and Robert Wood Johnson Foundation Harold Amos Medical Faculty Development Award 71586. R.S. Ahima is supported by National Institutes of Health grant P01-DK049210.

References

1. Brasaemle DL, Wolins NE. Packaging of fat: An evolving model of lipid droplet assembly and expansion. *J Biol Chem.* 2012; 287:2273–2279. [PubMed: 22090029]
2. Ploegh HL. A lipid-based model for the creation of an escape hatch from the endoplasmic reticulum. *Nature.* 2007; 448:435–438. [PubMed: 17653186]
3. Walther TC, Farese RV Jr. The life of lipid droplets. *Biochim Biophys Acta.* 2009; 1791:459–466. [PubMed: 19041421]
4. Gimm T, Wiese M, Teschemacher B, Deggerich A, Schodel J, Knaup KX, Hackenbeck T, Hellerbrand C, Amann K, Wiesener MS, Honing S, Eckardt KU, Warnecke C. Hypoxia-inducible protein 2 is a novel lipid droplet protein and a specific target gene of hypoxia-inducible factor-1. *Faseb J.* 2010; 24:4443–4458. [PubMed: 20624928]
5. Inohara N, Koseki T, Chen S, Wu X, Nunez G. Cide, a novel family of cell death activators with homology to the 45 kda subunit of the DNA fragmentation factor. *Embo J.* 1998; 17:2526–2533. [PubMed: 9564035]
6. Ruhanen H, Perttola J, Holtta-Vuori M, Zhou Y, Yki-Jarvinen H, Ikonen E, Kakela R, Olkkonen VM. Pnpla3 mediates hepatocyte triacylglycerol remodeling. *J Lipid Res.* 2014; 55:739–746. [PubMed: 24511104]
7. Jenkins CM, Mancuso DJ, Yan W, Sims HF, Gibson B, Gross RW. Identification, cloning, expression, and purification of three novel human calcium-independent phospholipase a2 family members possessing triacylglycerol lipase and acylglycerol transacylase activities. *J Biol Chem.* 2004; 279:48968–48975. [PubMed: 15364929]
8. Romeo S, Kozlitina J, Xing C, Pertsemlidis A, Cox D, Pennacchio LA, Boerwinkle E, Cohen JC, Hobbs HH. Genetic variation in pnpla3 confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet.* 2008; 40:1461–1465. [PubMed: 18820647]
9. Mashek DG, Khan SA, Sathyanarayan A, Ploeger JM, Franklin MP. Hepatic lipid droplet biology: Getting to the root of fatty liver. *Hepatology.* 2015
10. Messina JP, Humphreys I, Flaxman A, Brown A, Cooke GS, Pybus OG, Barnes E. Global distribution and prevalence of hepatitis c virus genotypes. *Hepatology.* 2015; 61:77–87. [PubMed: 25069599]
11. Scheel TK, Rice CM. Understanding the hepatitis c virus life cycle paves the way for highly effective therapies. *Nat Med.* 2013; 19:837–849. [PubMed: 23836234]
12. Herker E, Harris C, Hernandez C, Carpentier A, Kaehlcke K, Rosenberg AR, Farese RV Jr, Ott M. Efficient hepatitis c virus particle formation requires diacylglycerol acyltransferase-1. *Nat Med.* 2010; 16:1295–1298. [PubMed: 20935628]
13. Tanaka T, Kuroda K, Ikeda M, Wakita T, Kato N, Makishima M. Hepatitis c virus ns4b targets lipid droplets through hydrophobic residues in the amphipathic helices. *J Lipid Res.* 2013; 54:881–892. [PubMed: 23315449]

14. Camus G, Herker E, Modi AA, Haas JT, Ramage HR, Farese RV Jr, Ott M. Diacylglycerol acyltransferase-1 localizes hepatitis c virus ns5a protein to lipid droplets and enhances ns5a interaction with the viral capsid core. *J Biol Chem.* 2013; 288:9915–9923. [PubMed: 23420847]
15. Salloum S, Wang H, Ferguson C, Parton RG, Tai AW. Rab18 binds to hepatitis c virus ns5a and promotes interaction between sites of viral replication and lipid droplets. *PLoS Pathog.* 2013; 9:e1003513. [PubMed: 23935497]
16. Vogt DA, Camus G, Herker E, Webster BR, Tsou CL, Greene WC, Yen TS, Ott M. Lipid droplet-binding protein tip47 regulates hepatitis c virus rna replication through interaction with the viral ns5a protein. *PLoS Pathog.* 2013; 9:e1003302. [PubMed: 23593007]
17. Straub BK, Stoeffel P, Heid H, Zimbelmann R, Schirmacher P. Differential pattern of lipid droplet-associated proteins and de novo perilipin expression in hepatocyte steatogenesis. *Hepatology.* 2008; 47:1936–1946. [PubMed: 18393390]
18. Wu X, Lee EM, Hammack C, Robotham JM, Basu M, Lang J, Brinton MA, Tang H. Cell death-inducing dffa-like effector b is required for hepatitis c virus entry into hepatocytes. *J Virol.* 2014; 88:8433–8444. [PubMed: 24829338]
19. Hagist S, Sultmann H, Millonig G, Hebling U, Kieslich D, Kuner R, Balaguer S, Seitz HK, Poustka A, Mueller S. In vitro-targeted gene identification in patients with hepatitis c using a genome-wide microarray technology. *Hepatology.* 2009; 49:378–386. [PubMed: 19177570]
20. Trepo E, Pradat P, Potthoff A, Momozawa Y, Quertinmont E, Gustot T, Lemmers A, Berthillon P, Amininejad L, Chevallier M, Schlue J, Kreipe H, Deviere J, Manns M, Trepo C, Sninsky J, Wedemeyer H, Franchimont D, Moreno C. Impact of patatin-like phospholipase-3 (rs738409 c>g) polymorphism on fibrosis progression and steatosis in chronic hepatitis c. *Hepatology.* 2011; 54:60–69. [PubMed: 21488075]
21. Adinolfi LE, Gambardella M, Andreana A, Tripodi MF, Utili R, Ruggiero G. Steatosis accelerates the progression of liver damage of chronic hepatitis c patients and correlates with specific hcv genotype and visceral obesity. *Hepatology.* 2001; 33:1358–1364. [PubMed: 11391523]
22. Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, Charlton M, Sanyal AJ. The diagnosis and management of non-alcoholic fatty liver disease: Practice guideline by the american association for the study of liver diseases, american college of gastroenterology, and the american gastroenterological association. *Hepatology.* 2012; 55:2005–2023. [PubMed: 22488764]
23. Benhamed F, Denechaud PD, Lemoine M, Robichon C, Moldes M, Bertrand-Michel J, Ratziu V, Serfaty L, Housset C, Capeau J, Girard J, Guillou H, Postic C. The lipogenic transcription factor chrebp dissociates hepatic steatosis from insulin resistance in mice and humans. *J Clin Invest.* 2012; 122:2176–2194. [PubMed: 22546860]
24. Dentin R, Benhamed F, Hainault I, Fauveau V, Foufelle F, Dyck JR, Girard J, Postic C. Liver-specific inhibition of chrebp improves hepatic steatosis and insulin resistance in ob/ob mice. *Diabetes.* 2006; 55:2159–2170. [PubMed: 16873678]
25. Yahagi N, Shimano H, Hasty AH, Matsuzaka T, Ide T, Yoshikawa T, Amemiya-Kudo M, Tomita S, Okazaki H, Tamura Y, Iizuka Y, Ohashi K, Osuga J, Harada K, Gotoda T, Nagai R, Ishibashi S, Yamada N. Absence of sterol regulatory element-binding protein-1 (srebp-1) ameliorates fatty livers but not obesity or insulin resistance in lep(ob)/lep(ob) mice. *J Biol Chem.* 2002; 277:19353–19357. [PubMed: 11923308]
26. Caldwell SH, Swerdlow RH, Khan EM, Iezzoni JC, Hespeneide EE, Parks JK, Parker WD Jr. Mitochondrial abnormalities in non-alcoholic steatohepatitis. *J Hepatol.* 1999; 31:430–434. [PubMed: 10488700]
27. Vedala A, Wang W, Neese RA, Christiansen MP, Hellerstein MK. Delayed secretory pathway contributions to vldl-triglycerides from plasma nefa, diet, and de novo lipogenesis in humans. *J Lipid Res.* 2006; 47:2562–2574. [PubMed: 16929033]
28. Lambert JE, Ramos-Roman MA, Browning JD, Parks EJ. Increased de novo lipogenesis is a distinct characteristic of individuals with nonalcoholic fatty liver disease. *Gastroenterology.* 2014; 146:726–735. [PubMed: 24316260]
29. Varela GM, Antwi DA, Dhir R, Yin X, Singhal NS, Graham MJ, Crooke RM, Ahima RS. Inhibition of adrp prevents diet-induced insulin resistance. *Am J Physiol Gastrointest Liver Physiol.* 2008; 295:G621–628. [PubMed: 18669627]

30. Imai Y, Varela GM, Jackson MB, Graham MJ, Crooke RM, Ahima RS. Reduction of hepatosteatosis and lipid levels by an adipose differentiation-related protein antisense oligonucleotide. *Gastroenterology*. 2007; 132:1947–1954. [PubMed: 17484887]
31. Magnusson B, Asp L, Bostrom P, Ruiz M, Stillemark-Billton P, Linden D, Boren J, Olofsson SO. Adipocyte differentiation-related protein promotes fatty acid storage in cytosolic triglycerides and inhibits secretion of very low-density lipoproteins. *Arterioscler Thromb Vasc Biol*. 2006; 26:1566–1571. [PubMed: 16627799]
32. Chang BH, Li L, Saha P, Chan L. Absence of adipose differentiation related protein upregulates hepatic vldl secretion, relieves hepatosteatosis, and improves whole body insulin resistance in leptin-deficient mice. *J Lipid Res*. 2010; 51:2132–2142. [PubMed: 20424269]
33. Imai Y, Boyle S, Varela GM, Caron E, Yin X, Dhir R, Graham MJ, Ahima RS. Effects of perilipin 2 antisense oligonucleotide treatment on hepatic lipid metabolism and gene expression. *Physiol Genomics*. 2012; 44:1125–1131. [PubMed: 23012396]
34. McManaman JL, Bales ES, Orlicky DJ, Jackman M, MacLean PS, Cain S, Crunk AE, Mansur A, Graham CE, Bowman TA, Greenberg AS. Perilipin-2-null mice are protected against diet-induced obesity, adipose inflammation, and fatty liver disease. *J Lipid Res*. 2013; 54:1346–1359. [PubMed: 23402988]
35. Chang BH, Li L, Paul A, Taniguchi S, Nannegari V, Heird WC, Chan L. Protection against fatty liver but normal adipogenesis in mice lacking adipose differentiation-related protein. *Mol Cell Biol*. 2006; 26:1063–1076. [PubMed: 16428458]
36. Carr RM, Patel RT, Rao V, Dhir R, Graham MJ, Crooke RM, Ahima RS. Reduction of tip47 improves hepatic steatosis and glucose homeostasis in mice. *Am J Physiol Regul Integr Comp Physiol*. 2012; 302:R996–1003. [PubMed: 22378776]
37. Hall AM, Brunt EM, Chen Z, Viswakarma N, Reddy JK, Wolins NE, Finck BN. Dynamic and differential regulation of proteins that coat lipid droplets in fatty liver dystrophic mice. *J Lipid Res*. 2010; 51:554–563. [PubMed: 19749180]
38. Granneman JG, Moore HP, Mottillo EP, Zhu Z, Zhou L. Interactions of perilipin-5 (PLIN5) with adipose triglyceride lipase. *J Biol Chem*. 2011; 286:5126–5135. [PubMed: 21148142]
39. Zhou L, Park SY, Xu L, Xia X, Ye J, Su L, Jeong KH, Hur JH, Oh H, Tamori Y, Zingaretti CM, Cinti S, Argente J, Yu M, Wu L, Ju S, Guan F, Yang H, Choi CS, Savage DB, Li P. Insulin resistance and white adipose tissue inflammation are uncoupled in energetically challenged fsp27-deficient mice. *Nat Commun*. 2015; 6:5949. [PubMed: 25565658]
40. Matsusue K, Kusakabe T, Noguchi T, Takiguchi S, Suzuki T, Yamano S, Gonzalez FJ. Hepatic steatosis in leptin-deficient mice is promoted by the ppargamma target gene fsp27. *Cell Metab*. 2008; 7:302–311. [PubMed: 18396136]
41. Qu A, Taylor M, Xue X, Matsubara T, Metzger D, Chambon P, Gonzalez FJ, Shah YM. Hypoxia-inducible transcription factor 2alpha promotes steatohepatitis through augmenting lipid accumulation, inflammation, and fibrosis. *Hepatology*. 2011; 54:472–483. [PubMed: 21538443]
42. Rankin EB, Rha J, Selak MA, Unger TL, Keith B, Liu Q, Haase VH. Hypoxia-inducible factor 2 regulates hepatic lipid metabolism. *Mol Cell Biol*. 2009; 29:4527–4538. [PubMed: 19528226]
43. DiStefano MT, Danai LV, Roth Flach RJ, Chawla A, Pedersen DJ, Guilherme A, Czech MP. The lipid droplet protein hypoxia-inducible gene 2 promotes hepatic triglyceride deposition by inhibiting lipolysis. *J Biol Chem*. 2015; 290:15175–15184. [PubMed: 25922078]
44. Basantani MK, Sitnick MT, Cai L, Brenner DS, Gardner NP, Li JZ, Schoiswohl G, Yang K, Kumari M, Gross RW, Zechner R, Kershaw EE. Pnpla3/adiponutrin deficiency in mice does not contribute to fatty liver disease or metabolic syndrome. *J Lipid Res*. 2011; 52:318–329. [PubMed: 21068004]
45. Chen W, Chang B, Li L, Chan L. Patatin-like phospholipase domain-containing 3/adiponutrin deficiency in mice is not associated with fatty liver disease. *Hepatology*. 2010; 52:1134–1142. [PubMed: 20648554]
46. Smagris E, BasuRay S, Li J, Huang Y, Lai KM, Gromada J, Cohen JC, Hobbs HH. Pnpla3i148m knockin mice accumulate pnpla3 on lipid droplets and develop hepatic steatosis. *Hepatology*. 2015; 61:108–118. [PubMed: 24917523]

47. Fujii H, Ikura Y, Arimoto J, Sugioka K, Iezzoni JC, Park SH, Naruko T, Itabe H, Kawada N, Caldwell SH, Ueda M. Expression of perilipin and adipophilin in nonalcoholic fatty liver disease; relevance to oxidative injury and hepatocyte ballooning. *J Atheroscler Thromb*. 2009; 16:893–901. [PubMed: 20032580]
48. Pawella LM, Hashani M, Eiteneuer E, Renner M, Bartenschlager R, Schirmacher P, Straub BK. Perilipin discerns chronic from acute hepatocellular steatosis. *J Hepatol*. 2014; 60:633–642. [PubMed: 24269473]
49. Straub BK, Gyoengyoesi B, Koenig M, Hashani M, Pawella LM, Herpel E, Mueller W, Macher-Goepfing S, Heid H, Schirmacher P. Adipophilin/perilipin-2 as a lipid droplet-specific marker for metabolically active cells and diseases associated with metabolic dysregulation. *Histopathology*. 2013; 62:617–631. [PubMed: 23347084]
50. Magne J, Aminoff A, Perman Sundelin J, Mannila MN, Gustafsson P, Hultenby K, Wernerson A, Bauer G, Listenberger L, Neville MJ, Karpe F, Boren J, Ehrenborg E. The minor allele of the missense polymorphism ser251pro in perilipin 2 (PLIN2) disrupts an alpha-helix, affects lipolysis, and is associated with reduced plasma triglyceride concentration in humans. *Faseb J*. 2013; 27:3090–3099. [PubMed: 23603836]
51. Dongiovanni P, Donati B, Fares R, Lombardi R, Mancina RM, Romeo S, Valenti L. Pnpla3 i148m polymorphism and progressive liver disease. *World J Gastroenterol*. 2013; 19:6969–6978. [PubMed: 24222941]
52. O'Shea RS, Dasarathy S, McCullough AJ. Alcoholic liver disease. *Hepatology*. 2010; 51:307–328. [PubMed: 20034030]
53. You M, Fischer M, Deeg MA, Crabb DW. Ethanol induces fatty acid synthesis pathways by activation of sterol regulatory element-binding protein (srebp). *J Biol Chem*. 2002; 277:29342–29347. [PubMed: 12036955]
54. Siler SQ, Neese RA, Hellerstein MK. De novo lipogenesis, lipid kinetics, and whole-body lipid balances in humans after acute alcohol consumption. *Am J Clin Nutr*. 1999; 70:928–936. [PubMed: 10539756]
55. Hoek JB, Cahill A, Pastorino JG. Alcohol and mitochondria: A dysfunctional relationship. *Gastroenterology*. 2002; 122:2049–2063. [PubMed: 12055609]
56. Wehr H, Rodo M, Lieber CS, Baraona E. Acetaldehyde adducts and autoantibodies against vldl and ldl in alcoholics. *J Lipid Res*. 1993; 34:1237–1244. [PubMed: 8371070]
57. Carr RM, Peralta G, Yin X, Ahima RS. Absence of perilipin 2 prevents hepatic steatosis, glucose intolerance and ceramide accumulation in alcohol-fed mice. *PLoS One*. 2014; 9:e97118. [PubMed: 24831094]
58. Mak KM, Ren C, Ponomarenko A, Cao Q, Lieber CS. Adipose differentiation-related protein is a reliable lipid droplet marker in alcoholic fatty liver of rats. *Alcohol Clin Exp Res*. 2008; 32:683–689. [PubMed: 18341646]
59. Orlicky DJ, Roede JR, Bales E, Greenwood C, Greenberg A, Petersen D, McManaman JL. Chronic ethanol consumption in mice alters hepatocyte lipid droplet properties. *Alcohol Clin Exp Res*. 2011; 35:1020–1033. [PubMed: 21535024]
60. Carr RM, Dhir R, Yin X, Agarwal B, Ahima RS. Temporal effects of ethanol consumption on energy homeostasis, hepatic steatosis, and insulin sensitivity in mice. *Alcohol Clin Exp Res*. 2013; 37:1091–1099. [PubMed: 23398239]
61. Stickel F, Buch S, Lau K, Meyer zu Schwabedissen H, Berg T, Ridinger M, Rietschel M, Schafmayer C, Braun F, Hinrichsen H, Gunther R, Arlt A, Seeger M, Muller S, Seitz HK, Soyka M, Lerch M, Lammert F, Sarrazin C, Kubitz R, Haussinger D, Hellerbrand C, Broring D, Schreiber S, Kiefer F, Spanagel R, Mann K, Datz C, Krawczak M, Wodarz N, Volzke H, Hampe J. Genetic variation in the pnpla3 gene is associated with alcoholic liver injury in caucasians. *Hepatology*. 2011; 53:86–95. [PubMed: 21254164]
62. Tian C, Stokowski RP, Kershenovich D, Ballinger DG, Hinds DA. Variant in pnpla3 is associated with alcoholic liver disease. *Nat Genet*. 2010; 42:21–23. [PubMed: 19946271]

Highlights

- We review the major types of hepatic lipid droplet proteins, including perilipin and non-perilipin family members
- We review theoretical models of hepatic lipid droplet formation
- We discuss the evidence to date for a biologic role of lipid droplet proteins in three common hepatic steatotic diseases: Hepatitis C, non-alcoholic fatty liver disease, and alcoholic liver disease

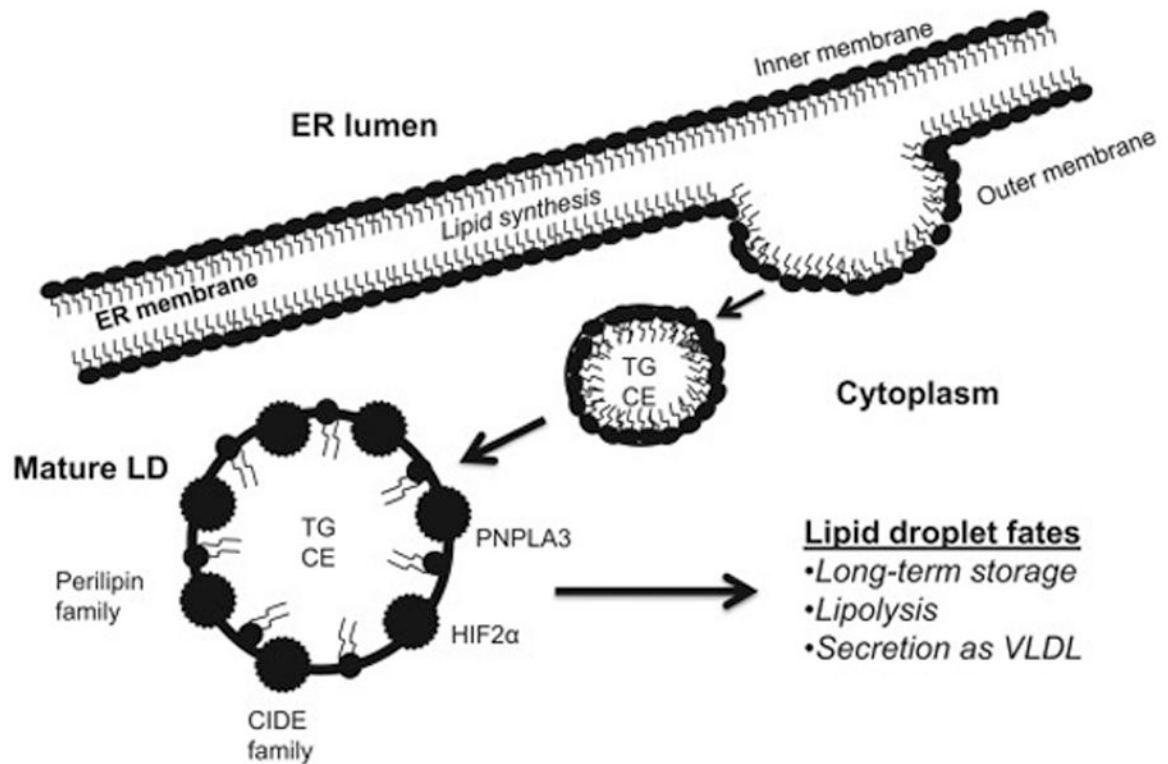


Figure 1. Biogenesis of lipid droplets (LDs)

Neutral lipids are synthesized in the ER and stored in the LDs. The canonical model of LD formation shown in the figure posits that neutral lipids form a lens within the ER bilayer which then buds from the membrane and incorporates phospholipids from the cytosolic leaflet. Alternatively, the “bicelle model” of LD formation proposes that neutral lipids accumulate between the ER membrane leaflets instead of budding. The nascent LDs are excised from the ER membrane, and incorporate phospholipids from both cytosolic and luminal leaflets of the ER². The latter model may explain how large unfolded proteins and viruses escape from the ER lumen into the cytoplasm. A “vesicular-budding model” proposes that small bilayer vesicles are tethered to the ER membrane and provide a platform for LD formation. Neutral lipids are synthesized and pumped from the ER into the vesicle bilayer, fill the intermembrane space, and the vesicular lumen eventually becomes a small inclusion inside the LDs³.

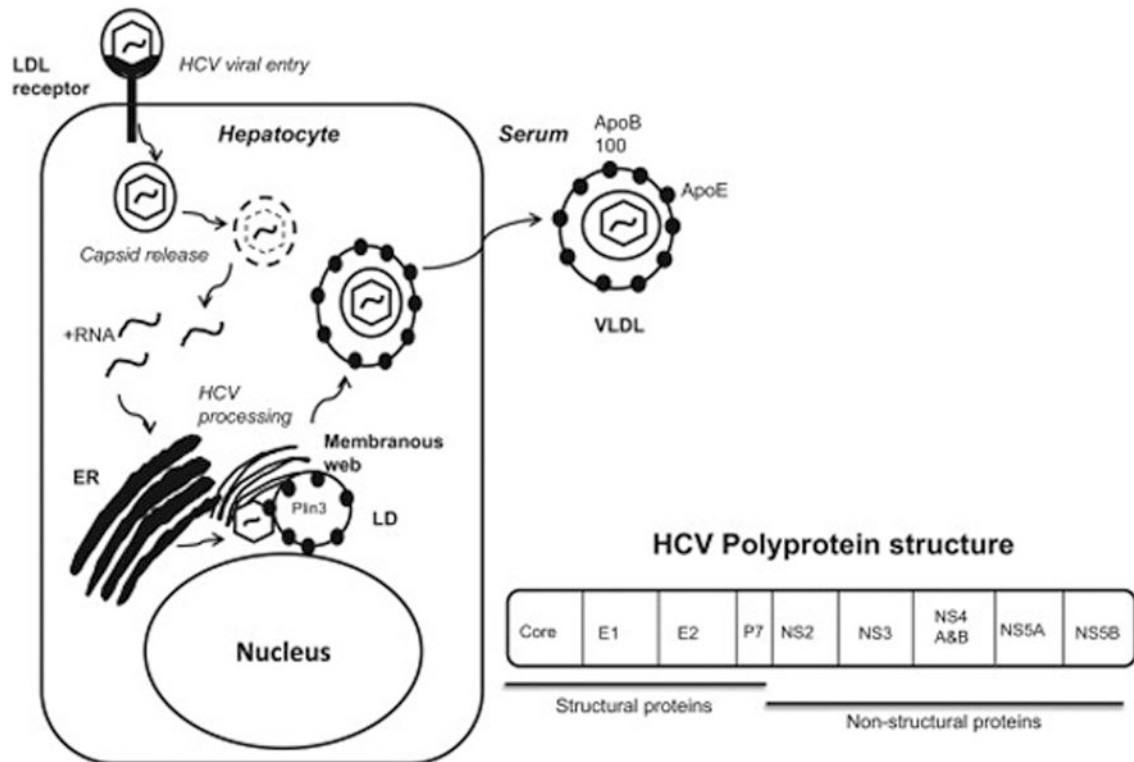


Figure 2. Interaction of HCV and LDLs

The replication of HCV RNA and viral particle production requires lipid droplets (LDs). Newly synthesized or uncoated viral genomic RNA is translated to a viral polyprotein followed by a cleaving polyprotein. Mature non-structural proteins form a complex with host factors. The positive strand of genomic RNA is enclosed with the core proteins, associated with non-structural proteins. The viral core protein is translocated to the LDs. It has been proposed that envelope proteins, e.g. NS2 and p7, determine the recruitment of viral protein from the LDs to the ER membrane. VLDL binds to viral particles before or after budding. The viral core protein increases the synthesis and storage of triglycerides and cholesterol esters in the LDs through interactions with host factors.