Triglyceride Metabolism in the Liver

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ABSTRACT

Triglyceride molecules represent the major form of storage and transport of fatty acids within cells and in the plasma. The liver is the central organ for fatty acid metabolism. Fatty acids accrue in liver by hepatocellular uptake from the plasma and by *de novo* biosynthesis. Fatty acids are eliminated by oxidation within the cell or by secretion into the plasma within triglyceride-rich very low-density lipoproteins. Notwithstanding high fluxes through these pathways, under normal circumstances the liver stores only small amounts of fatty acids as triglycerides. In the setting of overnutrition and obesity, hepatic fatty acid metabolism is altered, commonly leading to the accumulation of triglycerides within hepatocytes, and to a clinical condition known as nonalcoholic fatty liver disease (NAFLD). In this review, we describe the current understanding of fatty acid and triglyceride metabolism in the liver and its regulation in health and disease, identifying potential directions for future research. Advances in understanding the molecular mechanisms underlying the hepatic fat accumulation are critical to the development of targeted therapies for NAFLD.
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Didactic Synopsis

Major teaching points

- A variety of endogenous and exogenous sources provide fatty acids that can be assembled into triglycerides within the liver in health and disease;
- Multiple hepatocellular mechanisms regulate fatty acid uptake, synthesis, transport, and oxidation;
- Triglycerides can be stored within hepatocytes, undergo lipolysis, or be exported into the bloodstream depending upon physiological and pathological conditions;
- Mitochondria, endoplasmic reticulum, Golgi apparatus, peroxisomes, and lipid droplets are examples of organelles that participate in fatty acid and triglyceride metabolism;
- The metabolism of fatty acids and triglyceride is regulated by transcriptional and posttranscriptional mechanisms; and
- Alterations in fatty acid and triglyceride metabolism lead to non-alcoholic fatty liver disease (NAFLD), which is a common consequence of overnutrition.

Introduction

The liver is the central organ that controls lipid homeostasis by means of complex, but precisely regulated biochemical, signaling, and cellular pathways. Hepatocytes are the main liver parenchymal cells, which control hepatic biochemical and metabolic functions in the liver, including triglyceride metabolism. Additional cell types within the liver include cholangiocytes, Kupffer cells, stellate cells, and endothelial cells, each of which has specialized functions in hepatic pathobiology (261).

This review will focus on the hepatic metabolism of fatty acids (FAs) and their neutral storage form, triglycerides (TG), which occurs primarily in hepatocytes. Under normal circumstances on a daily basis, the liver processes large quantities of FA, but stores only small amounts in the form of TG, with steady state TG contents of less than 5% (32). This is because the rates of acquisition of FA by uptake from the plasma and from *de novo* synthesis within the liver are balanced by rates of FA oxidation and secretion into plasma as TG-enriched very low-density lipoprotein (VLDL-TG). The relatively small quantities of TG stored within the liver are localized in cytoplasmic lipid droplets (LDs).

NAFLD is the most common chronic liver disease and is characterized by excess TG accumulation within the liver. It is associated with obesity, type 2 diabetes and dyslipidemia, and commonly occurs in the setting of insulin resistance (22). NAFLD encompasses a spectrum of liver histopathologies from simple hepatic steatosis, often referred to as nonalcoholic fatty liver (NAFL), to nonalcoholic steatohepatitis (NASH) in which hepatic steatosis is accompanied by inflammation, cell death and fibrosis (5). Hepatic complications of NASH include cirrhosis and hepatocellular carcinoma. Currently, there are no effective therapies for NAFLD apart from changes in lifestyle that lead to improved physical fitness and weight loss. To develop effective pharmacologic therapies,

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detailed knowledge of the cellular and molecular mechanisms leading to altered hepatic lipid metabolism is essential. In addition to describing the major pathways of hepatic FA and TG metabolism and their regulation, we will discuss recent advances in our understanding of how they are altered in the setting of NAFLD.

Hepatic Fatty Acid Metabolism

FA within the liver originate from either dietary or endogenous sources. Dietary TG are emulsified by bile acids within the intestinal lumen following their hydrolysis primarily by pancreatic lipase (166), which yields *sn*-2-monoacylglycerols and free FA as products. Following emulsification, these lipid molecules are taken up by enterocytes and resynthesized into TG. TGs are packaged into chylomicrons, secreted into the lymphatic system and ultimately reach the plasma (112, 122). Much of the chylomicron TG are taken up by muscle and adipose tissue due to the activity of lipoprotein lipase, which is expressed on the luminal surfaces of capillary endothelial cells of these tissues (Fig. 1). TG remaining within the chylomicron remnants are delivered to the liver when these particles are taken up by receptor mediated endocytosis, and FA are released during lysosomal processing of the particles (54).

When carbohydrates are abundant, the liver converts glucose into FA, a process referred to as *de novo* lipogenesis (DNL) (120) (Fig. 1). The control of DNL is primarily transcriptional. Plasma insulin activates the endoplasmic reticulum membrane-bound transcription factor sterol regulatory element binding protein 1C (SREBP1c), the N-terminus of which translocates to the nucleus and upregulates all genes in the FA biosynthetic pathway (116). The hepatic uptake of excess plasma glucose promotes the nuclear translocation of carbohydrate response element binding protein (ChREBP), a transcription factor that also upregulates transcription of the majority of FA biosynthetic genes plus pyruvate kinase (89, 205), which increases the availability of citrate for FA synthesis.

Another important source of FA is direct uptake from the plasma. In human subjects, FA from plasma constitute the main source of hepatic TGs during fasting (72). Under fasting conditions when plasma insulin concentrations are low, a lipolytic program is initiated in white adipose tissue, which increases the plasma FA pool that is available for uptake by the liver (12, 214) (Fig. 1). FA are largely albumin-bound within the circulation. Several steps are involved in hepatic FA uptake. These include dissociation of FAs from albumin, transport across the hepatocyte plasma membrane, binding to intracellular proteins, and esterification to coenzyme A (CoA). Plasma FA are also the principal source of VLDL-TG in both fasting and fed states (72).

Within the hepatocyte, FA are esterified to glycerol-3-phosphate (G3P) and to cholesterol to generate TG or cholesteryl esters, respectively. These neutral lipids can be either stored in cytoplasmic LDs, or secreted into bloodstream as VLDL particles (135) (Fig. 1). FA within the liver may also be used for the synthesis of other complex lipids, including phospholipids (PL). During fasting, FA are used both as local energy supply as well as substrate for ketone bodies production. Overall, hepatic FA metabolism is tightly regulated by multiple interrelated transcriptional and signaling pathways that remain the subject of intensive investigation and discovery.

Fatty acid uptake

Notwithstanding their ability to diffuse across a lipid bilayer (232), FA are taken up by hepatocytes via plasma membrane-associated proteins. A variety of proteins have been associated with long-chain FA transport, including plasma membrane FA-binding protein (FABPpm), FA translocase (FAT)/CD36, caveolin-1, and very long-chain acyl-CoA synthetases (ACSVL/FA transport proteins, also named FATP/solute carrier family 27A1-6, SLC27A1-6) (27, 253) (Fig. 2).

The roles of CD36 and FABPpm proteins in FA uptake are established for heart and skeletal muscle, but their function in hepatocytes remains unresolved. Although expression levels are low in the liver, CD36 mRNA levels are positively correlated with hepatic TG contents in a rat model of hepatic steatosis (35), and its protein expression is increased in patients with NAFLD (181). However, the pathophysiological relevance of this protein to the liver is unclear. Whereas CD36 liver-specific deletion in mice leads to reduced hepatic lipid contents and decreased FA uptake in the setting of high fat feeding, suggesting the direct contribution of CD36 to hepatic FA metabolism under these conditions (279), whole body knockout of CD36 in mice significantly impairs FA uptake by heart, adipose tissue, and muscle, but normal uptake is

Figure 1 Major sources for hepatic fatty acids. The three major sources for hepatic fatty acids (FAs) are dietary lipids, adipose tissue derived-FA and *de novo*-synthesized FA. After a meal, dietary lipids are hydrolyzed within the intestinal lumen. Upon intestinal uptake, FA are reesterified to form TG molecules, which are packaged into chylomicrons and delivered primarily to muscle and adipose tissue. The remaining TG present in chylomicron remnants is then transported to the liver and processed intracellularly, leading to FA release within hepatocytes. Carbohydrates, particularly glucose, are utilized in hepatic *de novo* lipogenesis (DNL) for the production of FA. To be metabolized, FAs are activated to form acyl-CoA molecules, which can undergo oxidation or be incorporated into complex lipids. Locally synthesized TG can be stored in intracellular lipid droplets (LDs) or packed into VLDL and secreted into the plasma. Upon fasting, intracellular TG stores are mobilized from adipocytes and hepatocytes to release FA products. Hepatic DNL may also contribute to form an acyl-CoA pool available for energy production, undergoing oxidation by mitochondria, or for VLDL-TG production. In the setting of overnutrition and insulin resistance, hepatic FA levels are increased due to enhanced lipolysis within adipocytes, which leads to increased circulating levels of FA in the bloodstream, and increased hepatic DNL. Excess FA cannot be consumed by oxidative pathways and FA are instead directed toward the synthesis of TG, leading to increased hepatic TG storage and VLDL overproduction. Arrow thickness denotes rates of metabolic activities.

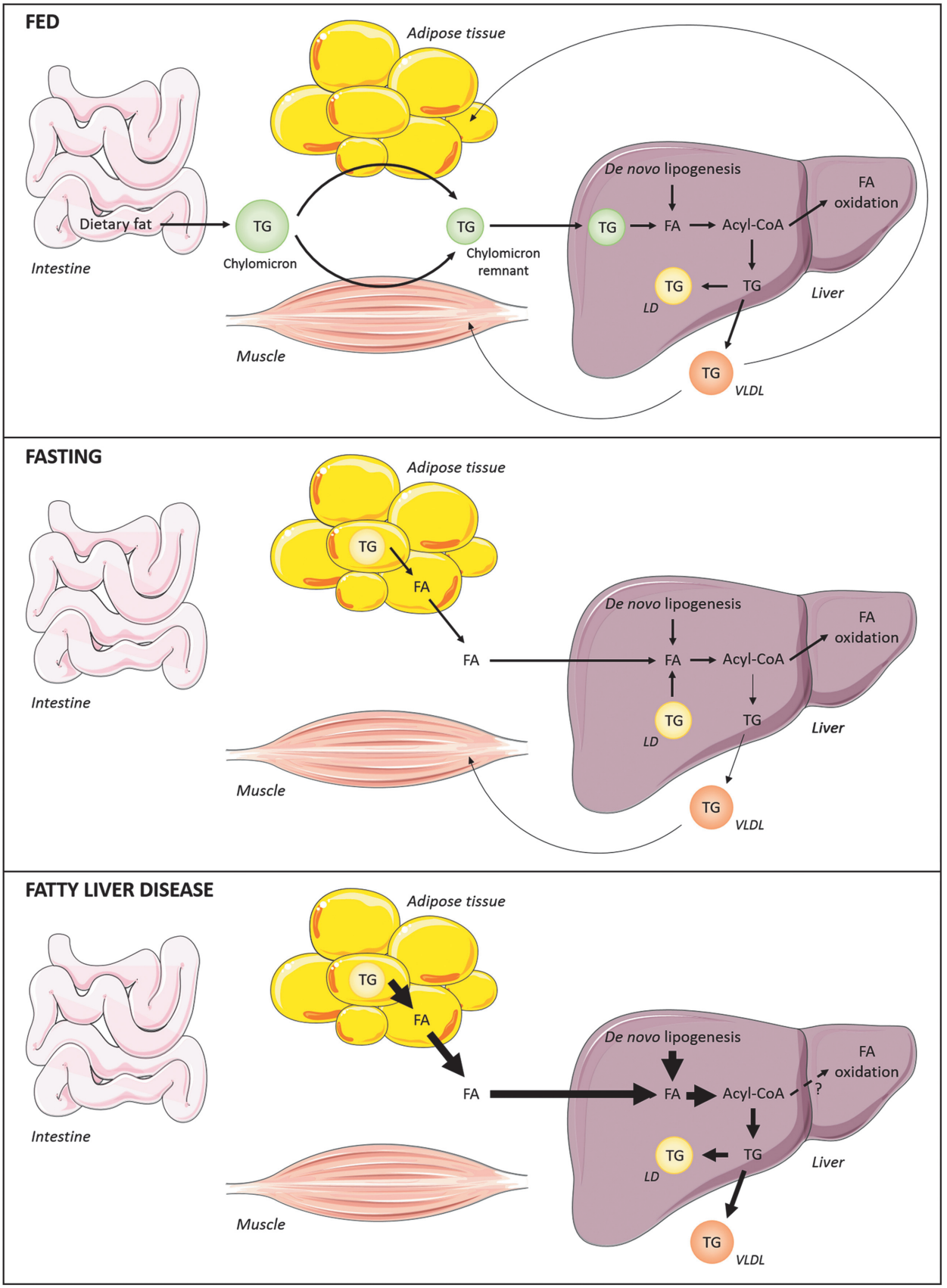

Figure 1

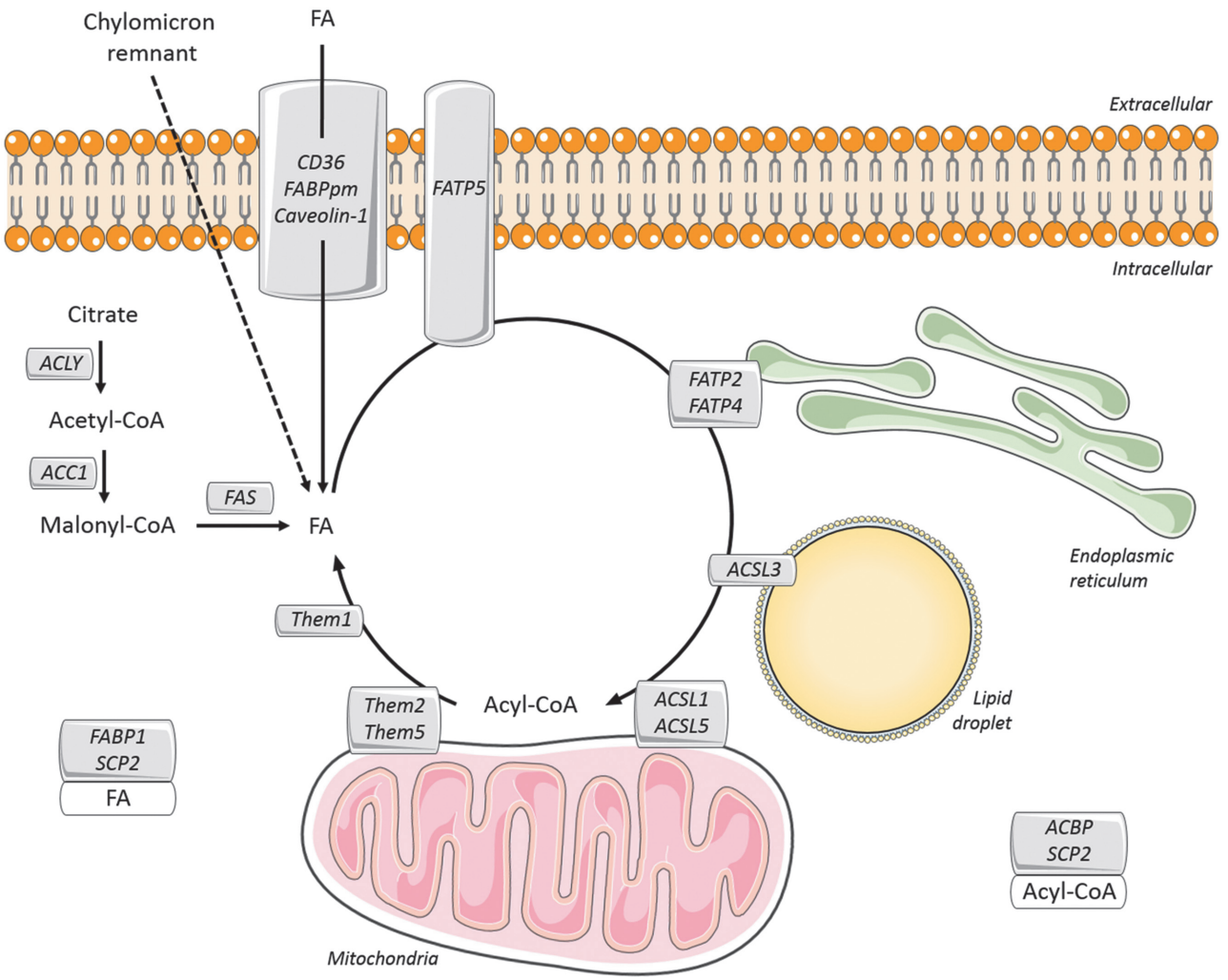

Figure 2 Hepatic fatty acid transport and metabolism. Within the plasma membrane, FA translocase (FAT)/CD36, plasma membrane FA-binding protein (FABPpm), and Caveolin-1 mediate the uptake of fatty acids (FAs) that is bound to circulating albumin. Alternatively, hepatic FA can be obtained by the internalization of chylomicron remnants or by *de novo* lipogenesis. The latter occurs through the activity of three key enzymes: ATP-citrate lyase (ACLY), acetyl-CoA carboxylase (ACC), and fatty acid synthase (FAS). In the cytosol, FAs are bound to the fatty acid-binding protein-1 (FABP1) and sterol carrier protein-2 (SCP2), which may control their cellular distribution. FA transport proteins (FATP2, 4, and 5) and long-chain acyl-CoA synthetases (ACSL1, 3 and 5) mediate the activation of long-chain FA to acyl-CoA molecules and their channeling to metabolic pathways. Although associated with mitochondria, ACSL5 may function to promote triglyceride biosynthesis. In the cytosol, acyl-CoAs are bound to acyl-CoA-binding protein (ACBP) or SCP2. Acyl-CoA thioesterases (ACOT)/thioesterase superfamily members (Them1, 2, and 5) appear to counteract ACSL activity by catalyzing the hydrolysis of acyl-CoA molecules into FA and CoA. This may provide additional means of controlling the balance between FA and acyl-CoA within hepatocytes.

observed in the liver (52). There also seems to be a role for CD36 in hepatic lipogenesis. CD36 is a target gene for the aryl hydrocarbon receptor (AhR), and is required for the TG accumulation observed in the liver in response to its activation (154). In addition, the pregnane X receptor (PXR) induces hepatic CD36 mRNA expression, along with increased FA uptake and TG accumulation in the liver (294). Finally, CD36 is a transcriptional target of peroxisome proliferator activated receptor (PPAR) γ (259), which is upregulated by PXR (294).

Functional evidence for protein-mediated FA transport in the rat hepatocyte plasma membrane led to the identification of FABPpm as a protein that mediates this activity (247, 249). FABPpm was subsequently identified as mitochondrial aspartate aminotransferase (20,28). The mRNA encoding this protein is increased in hepatocytes of genetically obese mouse models (180), and the binding of oleate to rat hepatocyte plasma membranes is reduced after treatment with specific antiserum (246). In cultured hepatocytes, ethanol treatment increases mRNA and plasma membrane protein contents, and these changes correlate with increased FA uptake (295). However, the relative contribution of FABPpm to FA uptake by the liver remains unknown.

FATP proteins (1-6) are FA-transporting proteins that are situated in the plasma membrane, as well as intracellular membranes. Because of their capacity to also activate long- or very-long-chain FA (69), these proteins have been re-classified in the very-long-chain acyl-CoA synthetase

(ACSVL) family as ACSVL1-6 (106). FATPs couple FA uptake with intracellular FA esterification into acyl-CoAs, a metabolic trapping mechanism that has been referred to as vectorial acylation (77, 258). Although well characterized, neither FATP1 nor FATP4 are normally expressed at substantial levels in the liver (239). Expression of FATP4, an ER membrane-associated isoform (226, 273), is increased in the setting of hepatic steatosis and may play a role in palmitate-mediated lipoapoptosis (226). FATP2 is highly expressed in liver and kidney (85). In HepG2 cells, FATP2 localizes to the ER, but its overexpression increases uptake of long-chain FA in cell culture (148). FATP5 is expressed in the liver, and studies in knockout mice and cell culture systems support a role in FA uptake (70).

Caveolins (types 1-3) are integral membrane proteins that are essential components of caveolae, vesicular membrane microdomains that are rich in cholesterol and sphingolipids (6). Caveolins were initially characterized with respect to cholesterol transport (204). However, their roles in FA transport have been appreciated in experiments using HepG2 hepatoma cells (203), wherein Caveolin-1, CD36, FABPpm, and calcium-independent membrane phospholipase A2 (iPLA2) form a heterotetrameric protein complex within plasma membrane microdomains (248). These complexes promote FA accumulation within caveolae-derived vesicular structures for transport into the cell (247). In support of this mechanism, mice deficient in caveolin-1 show reduced hepatic TG content and impaired LD accumulation (87).

Although multiple factors lead to hepatic steatosis, circulating FA are the main source of hepatic lipids in NAFLD (72). The overflow of FA derived from excessive lipolysis in the adipose tissue contributes to the pathogenesis of insulin resistance in mice and humans (159), leading to both type 2 diabetes (213) and NAFLD (27, 171). Although FA transporters may exert a central role in the control of FA flux into the liver, additional studies are needed to clarify specific functions and physiological contributions of each FA transporter, along with their differential regulation in the context of NAFLD.

De novo lipogenesis

Under normal circumstances in humans, pathways of hepatic DNL appear to be utilized sparingly (114). However, in addition to the enhanced FA uptake, increased DNL can contribute substantially to hepatic steatosis (72). The first step in DNL pathway is catalyzed by ATP-citrate lyase (ACLY), which converts citrate to acetyl-CoA that is then carboxylated to malonyl-CoA by acetyl-CoA carboxylase (ACC). ACC is followed by a series of reactions that convert malonyl-CoA into palmitate. Fatty acid synthase (FAS) is the key rate-limiting enzyme in palmitate synthesis, and its activity is regulated by multiple mechanisms (125, 143, 218, 228) (Fig. 2). Palmitate can be modified by elongases and desaturases to generate a variety of FA species.

There are two ACC isoforms, cytosolic ACC1 and mitochondrial ACC2, which are encoded by separate genes. The presence of distinct ACC enzymes enables the formation of two distinct pools of malonyl-CoA. ACC1-generated malonyl-CoA is utilized as a substrate by FAS, whereas the malonyl-CoA produced by ACC2 plays a key role in the negative regulation of β-oxidation by inhibiting carnitine palmitoyltransferase 1 (CPT1) (266). ACC1 is highly expressed in lipogenic organs, including the liver, wherein the gene expression and enzyme activity are induced by carbohydrate-rich, low-fat diet, and are suppressed in the setting of starvation or diabetes (139, 149). Whole body disruption of the ACC1 gene results in embryonic lethality, but liver-specific knockout mice are viable and exhibit 70–75% reductions in malonyl-CoA levels and 40% to 70% reductions in hepatic TG contents (170). Despite similar malonyl-CoA levels as wild type animals, livers of ACC2 knockout mice are characterized by reduced rates of β-oxidation and TG contents. These mice resist diet-induced obesity and diabetes (2, 3). In keeping with these observations, antisense-mediated knockdown of both ACC1 and ACC2 led to improved hepatic insulin responsiveness in rats with experimental NAFLD (217).

FAS expression is ubiquitous, but evidence of elevated transcription and enzymatic activity are observed in the liver of murine models of obesity, such as Zucker (*fa/fa*) rats (227). The transcription of both ACC and FAS genes is enhanced in livers of NAFLD subjects (146). Similar to ACC knockout mice, whole body disruption of FAS leads to embryonic lethality (51). Surprisingly, liver-specific knockout of FAS results in hypoglycemia and hepatic steatosis in mice fed a zero-fat diet (45). Because this phenotype is reversed by treatment with a PPARα agonist, it has been suggested that newly synthesized FA and their derivatives, including phospholipids (44), constitute a distinct pool that provides endogenous ligands for PPARα. This in turn stimulates hepatic gluconeogenesis and FA oxidation (45).

In leptin-deficient obese (*ob/ob*) mice, increased transcription of *Acc1* and *Fas* is linked to the increased activation of ChREBP (64). Additionally, SREBP-1c and ChREBP can synergistically induce the mRNA expression of *Acc* and *Fas* in the liver (65). Expression of both transcriptional factors are upregulated in livers of mouse models of NAFLD (19), however only SREBP-1c is upregulated in patients with NAFLD (115).

Among TG found in VLDL in normal subjects, less than 5% contain FA derived from DNL (68). However, inhibition of DNL impairs production and secretion of VLDL-TG in rats (95). The rate of incorporation of DNL-synthesized FA into VLDL-TG is increased in normal subjects in the setting of high-carbohydrate diet (223, 224), alcohol consumption, and infectious states (88), and is positively correlated with increased plasma VLDL-TG content (223). DNL is also increased in obese (67, 244) and NAFLD subjects (68, 88). These observations suggest that hypertriglyceridemia is at least in part attributable to increased availability of DNL-synthesized FA for TG synthesis and VLDL production (223).

Ongoing research is needed to clarify mechanisms of altered DNL in a variety of pathogenic conditions, including NAFLD.

Fatty acid activation

Activation by thioesterification to CoA to form fatty acyl-CoA molecules is an obligatory step in the metabolism of long-chain FA. This reaction is catalyzed by members of the long-chain acyl-CoA synthetase (ACSL) family of enzymes (Fig. 2). The five ACSL isoforms found in mammalians have specific tissue distributions, subcellular locations, and substrate preferences (106). Additionally, distinct ACSL isoforms play key roles in partitioning fatty acyl-CoAs into different metabolic pathways (57, 106).

ACSL1, the best-characterized isoform, is abundantly expressed in a variety of tissues. Because *Acsl1* is a target gene of PPARα in rat liver, this enzyme has been associated with FA catabolism (220). ACSL1 interacts physically with CPT1 in the outer mitochondrial membrane of rat hepatocytes, possibly functioning to channel fatty acyl-CoA products into mitochondria for β-oxidation (155). However, this process is not fully understood because liver-specific ACSL1 knockout mice exhibit only modestly decreased rates of β-oxidation and reduced fatty acyl-chain incorporation into newly-synthesized TG, although the total hepatic TG contents remain unchanged (161).

ACSL3 is expressed on lipid droplets in HuH7 hepatoma cells (94), and its expression is upregulated in livers of *ob/ob* mice and in mice fed a high-carbohydrate diet (34). Knockdown of ACSL3 in rat hepatocytes reduces DNL through diminished activation of lipogenic transcriptional factors (i.e., PPARγ, ChREBP, SREBP-1c, and LXRα) possibly due to the reduced availability of FA species, acyl-CoA, or their lipid intermediates (34).

Suggestive of a role for ACSL5 in TG synthesis, mRNA levels are increased by insulin and by SREBP-1c activation (4). In support of this lipogenic role, overexpression of ACSL5 in rat hepatoma cells increases uptake of exogenous FA, as well as its partitioning into TG, but not into PL (174). Knockdown of ACSL5 in rat primary hepatocytes strongly influences the partitioning of fatty acyl-CoAs between lipogenic and lipolytic pathways. In the absence of this enzyme, the incorporation of exogenous and endogenous FA into complex lipids is reduced, the formation of LD is decreased and the oxidation of FA is stimulated (33).

The reaction catalyzed by ACSL isoforms is reversed by the activities of acyl-CoA thioesterases (ACOTs) (Fig. 2). These enzymes catalyze the hydrolysis of acyl-CoA molecules into FA and CoA. Despite their relatively well-investigated enzymatic activities, the biological functions of the fifteen ACOT family members remain less well understood (53, 142, 256). *Acots 1-6*, which constitute the type I enzymes, are upregulated in the livers of fasted and high fat-fed mice under the transcriptional control of PPARα (79). These observations suggest a role of type I ACOTs in lipid catabolism. Indeed, overexpression of ACOT2 in the liver leads to increased FA oxidation and ketogenesis, although the mechanism underlying this effect remains unclear (182).

ACOTS 7-15 comprise the type II enzymes, and some of these contribute to hepatic FA metabolism. ACOT11 (synonym, Thioesterase superfamily member 1, Them1) is highly expressed in brown adipose tissue, where it functions to suppress energy expenditure (292) by decreasing rates of fat oxidation (196). *Acot11* gene expression is also strongly induced in the liver of obese mice (79). Whereas deficiency of this enzyme results in decreased hepatic steatosis, improved glucose homeostasis, and resistance to diet-induced diabetes in high fat fed mice (292), it is unclear whether these phenotypes are indirect effects of increased energy expenditure.

ACOT13 (a.k.a. Them2) is a mitochondria-associated enzyme that is highly expressed in the liver and other oxidative tissues. Them2 knockout mice are resistant to diet-induced hepatic steatosis and exhibit improved glucose metabolism (130). Moreover, Them2-deficiency results in diminished expression of PPARα and HNF4α, possibly due to the role of this enzyme in the control of FA ligands of these nuclear receptors (53) and the reduced FA oxidation (135). Them2 also controls lipid and glucose metabolism by interacting with phosphatidylcholine transfer protein (PC-TP) to suppress hepatic insulin signaling by reducing the activation of both the insulin receptor substrate 2 (IRS2) and the mammalian target of rapamycin (mTOR) (80).

ACOT15 (a.k.a. Them5) appears to play a key role in maintaining the integrity of mitochondrial membranes. Mice lacking ACOT15 experience deficiencies in cardiolipin remodeling in the inner mitochondrial membrane of hepatocytes. This phenotype leads to mitochondrial dysfunction, including altered morphology, decreased mitochondrial FA contents, reduced FA oxidation, and the development of liver steatosis (296).

It is plausible that ACSL and ACOT enzymes coordinate the control of the intracellular balance of fatty acyl-CoAs and FA, intracellular and intra-organelle concentrations of CoA, and the availability of lipid substrates for a variety of metabolic pathways. Both types of enzymes exhibit specific transcriptional patterns of expression that vary depending on cell type and physiological state, and the coordinated regulation of ACSL and ACOT enzymes has been recently described (79). Although elevated hepatic concentrations of long-chain acyl-CoAs have been associated with increased plasma insulin levels and hepatic insulin resistance (48), decreased hepatic fatty acyl-CoA concentrations in the setting of liver-specific deletion of ACSL1 do not prevent the development of insulin resistance in high-fat fed mice (161). As illustrated by these apparently contradictory observations, mechanisms whereby ACSL and ACOT enzymes control FA metabolism within hepatocytes are incompletely understood, and it remains uncertain whether their altered regulation contributes to insulin resistance and related metabolic abnormalities.

Intracellular fatty acid transport

Long-chain FAs and their acyl-CoA derivatives act not only as substrates for lipid synthesis and oxidation, but are also involved in a range of diverse intracellular processes, such as protein palmitoylation, intracellular signaling, and activation of transcription factors (83, 106, 222). Considering this multiplicity of cellular functions, their relative insolubility and potential cytotoxicity (102, 103), it is logical that the intracellular concentrations and localization of free FA and fatty acyl-CoAs are tightly regulated. This is accomplished at least in part due to the activities of lipid binding proteins, several of which have been implicated in the control of the intracellular concentration and the partitioning of long-chain FAs and acyl-CoAs within hepatocytes. These include the liver FA-binding protein (L-FABP, synonym FABP1), acyl-CoA binding protein (ACBP; also named as acyl-CoA-binding domain containing protein-1, ACBD1, or diazepam binding inhibitor, DBI), and sterol carrier protein-2 (SCP2) (8, 103) (Fig. 2).

The FABP protein family comprises 10 members (237). FABP1 is abundant in the cytosol of hepatocytes, accounting for 2–5% of the cytosolic protein content (177) and reversibly binds long-chain FA, fatty acyl-CoAs, lysophospholipids, cholesterol, and other lipids (14, 36, 254, 255). *Fabp1*$^{-/-}$ male mice fed standard chow diet exhibit normal body and liver weights (172, 192). However, these animals have reduced total hepatic TG contents as well as decreased rates of FA esterification into TG, with preserved or increased esterification into PL (172, 192). Because the overexpression of FABP1 in L-cell fibroblasts enhances the intracellular traffic of short-, medium-, and long-chain FA from the cytosol to the nucleus (119), it has been conjectured that FABP1 must enter nuclei to promote the entry of FA (222). Indeed, FABP1 directly interacts with PPARα within nuclei of primary hepatocytes (117). This interaction may represent a mechanism for the delivery of FA ligands that activate this key transcription factor (222). FABP1 ablation is protective against diet-induced obesity and hepatic steatosis and improves glucose metabolism in mice fed a Western (high saturated fat, high cholesterol) diet (193). Taken together, these findings suggest a role for FABP1 in FA partitioning into specific metabolic pathways.

ACBP binds medium- and long-chain acyl-CoA with high affinity (84, 207, 211). Levels of ACBP mRNA and protein are highest in the liver (26). In the mouse, ACBP expression is modulated by nutritional status. Fasting results in decreased hepatic ACBP expression levels, whereas high fat feeding increases it (21). Overexpression of ACBP in rat hepatoma cells increases TG content (283). In addition, hepatic TG and PL concentrations are increased in transgenic mice that overexpress ACBP in the liver (118). Under these conditions, microsome-associated glycerol-3-phosphate acyltransferase (GPAT) activity is induced, suggesting that ACBP has a role in the formation and partitioning of an acyl-CoA pool toward glycerolipid synthesis (118).

Expression of SCP-2 is high in liver and intestine (15). This protein has high affinity for binding both long-chain FA and acyl-CoAs, with K_d values in the same range as reported for FABP and ACBP (103, 144, 177). Knockout mice exhibit reduced plasma FA concentrations, decreased hepatic cholesteryl ester and TG contents as well as increased rates of β-oxidation (225). SCP-2 ablation also results in alterations in the composition and physical properties of lipid rafts from mice primary hepatocytes, including the enrichment in PL contents (9). Moreover, hepatic SCP-2 and FABP1 act to facilitate hydrolysis of cholesteryl ester molecules associated with high-density lipoprotein (HDL) particles and increasing the biliary secretion of bile acids (270) and cholesterol (92).

Because the metabolic fates of FA and acyl-CoA molecules are linked to these intracellular lipid-binding proteins, it is tempting to speculate that they could be leveraged to manipulate the flux of lipids between anabolic and catabolic pathways to defend against the FA-mediated lipotoxicity that contributes to the pathogenesis of NAFLD.

Triglyceride Metabolism

The assembly of TG molecules constitutes the principal means by which the liver stores and exports FA. Under normal conditions, the liver stores little TG, but exports considerable amounts in the form of VLDL particles that deliver FA to muscle and fat tissue, depending on nutritional status. Whereas previously it was considered that excess TG stores in the setting of NAFLD contributed to lipotoxicity (61), emerging concepts suggest that increased TG storage and VLDL secretion are instead protective against FA-mediated hepatotoxicity.

Triglyceride synthesis

In most mammalian cell types, the G3P pathway is the principal route for the synthesis of TG, contributing over 90% of total TG synthesis (56, 62). The first and rate-limiting step of this pathway is the esterification of long-chain acyl-CoA to G3P, which is catalyzed by mitochondrial and microsomal G3P acyltransferase (GPAT) enzymes. Lysophosphatidic acid (LPA) molecules produced in this reaction are then acylated to form phosphatidic acid (PA) by the acylglycerol-3-phosphate acyltransferases (AGPAT) present in the ER membrane. PA can be converted into cytidine diphosphate-diacylglycerol (CDP-DG), which is a substrate for the synthesis of certain glycerolphospholipids and cardiolipins (113, 231) or can be dephosphorylated by phosphatidate phosphohydrolase (PAP, synonym Lipin) to form DGs, which serve as precursor molecules for the synthesis of TG, as well as phosphatidylcholine (PC) and phosphatidylethanolamine (PE) (56, 73). DG acyltransferase (DGAT) catalyzes the acylation of DG, constituting the final step of TG synthesis (55). Newly synthesized TG molecules can then be directed from ER lipid bilayer to form cytosolic LDs (99, 278) (Fig. 3).

Four isoforms of GPAT are encoded by distinct genes (276). In the liver, the mitochondria-associated GPAT

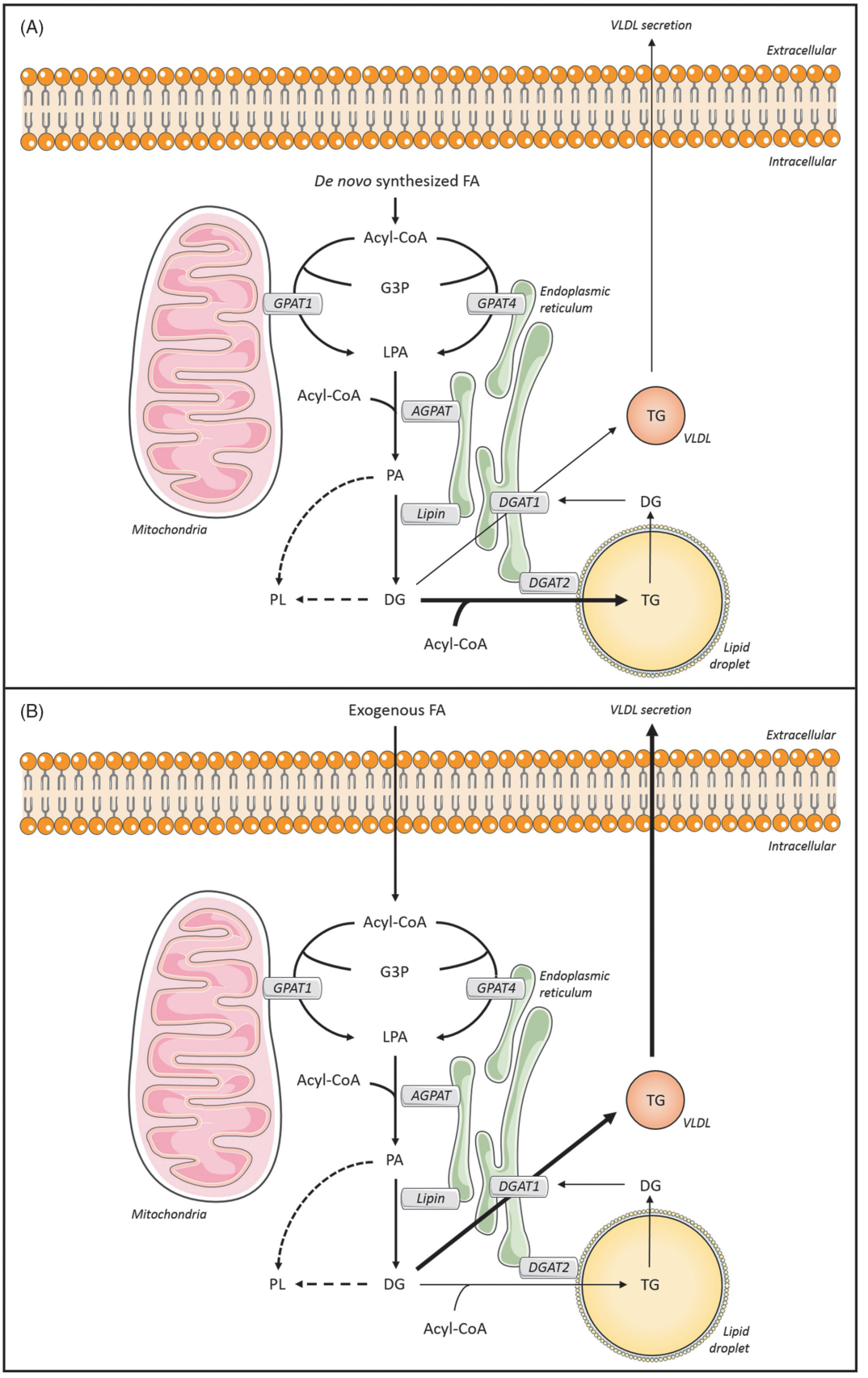

Figure 3

isoforms (GPAT1 and 2) contribute 30% to 50% of total GPAT activity (56). GPAT1 is highly expressed in the liver (157). It is induced after fasting/refeeding and in response to insulin, and downregulated via the cAMP-dependent signaling pathway during nutrient deprivation (76, 186, 230). GPAT1-deficient mice exhibit increased rates of FA oxidation and concentrations of plasma β-hydroxybutyrate. Moreover, hepatic TG contents are decreased by 60%, suggesting that GPAT1 functions in the partitioning of FA toward TG synthesis and away from oxidation in the liver (110). GPAT1 deficiency also leads to reduced plasma TG levels, reduced VLDL secretion rates and decreased total body weights (110,191). Accordingly, the overexpression of GPAT1 in rat hepatocytes leads to increased esterification of oleate into LPA, DG, and TG, and to increased intracellular TG contents (158, 165, 188). Although highly expressed in the liver, the metabolic role(s) of GPAT2 in the lipid metabolism remain unclear. This isoform is not regulated by either insulin or fasting/refeeding (271), and mouse models with GPAT2 loss or gain of function have yet to be reported.

GPAT3 and GPAT4 account for microsomal GPAT activity. These isoforms are modulated by nutritional status and hormonal variations. Although initially considered to mainly mediate PL synthesis (55), more recent studies have revealed their contributions to TG metabolism in adipose tissue. Specific roles for GPAT3 and GPAT4 in hepatic lipid metabolism remain largely undefined. The overexpression of GPAT4 in HepG2 cells leads to 20% increase in intracellular TG content (189), and its knockout in mice results in 40-50% reduction in hepatic TG (37, 262). It is noteworthy that hepatocytes from mice lacking GPAT4 are able to incorporate *de novo* synthesized FA into TG (275), suggesting that this isoform could be relevant for the metabolism of exogenous FA.

The second step in TG synthesis is mediated by AGPAT enzymes. Although ten AGPAT enzymes have been identified on the basis of sequence homology, the enzymatic activity for LPA acylation has been confirmed for only a few (252). Certain isoforms initially described as AGPAT were later reclassified into different acyltransferase groups. For example, AGPAT6 and AGPAT8 are currently designated as GPAT4 and GPAT3, respectively (38, 49, 189). AGPAT1 and AGPAT2 are localized in the ER (74, 97, 150, 277) and are highly expressed in the liver (75, 240, 252, 277). However, the contribution of these enzymes to the hepatic metabolism remains unknown.

Lipins-1-3 are PAP enzymes. Under lipogenic stimuli, these proteins translocate from the cytosol to ER membrane to access their substrate PA and contribute the TG biosynthetic pathway (31, 173). Additionally, lipin-1 can localize in the nucleus of hepatocytes, where it acts as a transcriptional coactivator (234). Fasting and glucocorticoid administration result in increased lipin-1 expression in mouse livers (90, 169, 195). This effect is mediated by PPARγ coactivator 1α (PGC-1α) and is required to sustain the fasting-induced expression of PPARα and its downstream targets. Lipin-1 physically interacts with PGC-1α and PPARα in the nucleus, leading to upregulation of oxidative FA metabolism by mitochondria (90). Accordingly, mice lacking lipin-1 exhibit hepatic steatosis (90), enhanced stearoyl-CoA desaturase-1 (Scd1) gene expression, increased rates of VLDL secretion, and increased plasma concentrations of TG without changes in TG synthetic rates (50). Reduced lipin-1 expression is observed in livers of obese diabetic mice, and the overexpression of lipin-1 in this animal inhibits VLDL secretion and improves hepatic insulin responsiveness (50). The effects of lipin-1 on VLDL secretion are independent of PAP enzymatic activity and instead linked to its nuclear receptor interactions (50). In addition to lipin-1, high levels of lipin-2 expression are also observed in the liver (71) and hepatic lipin-3 transcription is increased in lipin-1-deficient mice (71). Moreover, lipin-2 mRNA levels are induced in lipin-1-deficient mice, supporting the hypothesis that lipin isoforms could compensate to preserve PAP activity and restore levels of TG synthesis (107).

In mouse liver, DGAT activity is detected in two microsomal membrane locations: luminal activity (DGAT1, measured as latent-luminal-DGAT activity) and cytosol-accessible activity (predominantly DGAT2, measured as overt DGAT activity) (200). The former contributes to the synthesis of TG that are packaged into VLDL. The latter contributes to the synthesis of a TG pool to be stored in the cytoplasmic LDs (1, 200, 272). Interestingly, the DGAT proteins are genetically unrelated. DGAT1 is part of the membrane bound O-acyl transferase (MBOAT) protein family, which comprises a diverse group of acyltransferases, including acyl-CoA:cholesterol acyltransferase (ACAT) and protein-cysteine *N*-palmitoyltransferase (286). DGAT2 is a member of diacylglycerol acyltransferase (DAGAT) family that also includes the monoacylglycerol acyltransferase (MGAT) and acyl-CoA wax alcohol acyltransferases (AWAT) (286).

Although DGAT1 and DGAT2 are able to catalyze the same reaction, these enzymes do not appear to be redundant in their respective functions. DGAT1 mRNA is modestly expressed in the liver in comparison to other organs, such as skeletal muscle and small intestine (41). This enzyme is able to catalyze acyltransferase reactions using substrates other than DG. For example, DGAT1 acylates MG, wax esters and retinol *in vitro* (285). DGAT1 predicted topology combined with results obtained from loss of function studies support

Figure 3 Hepatic triglyceride metabolism. Acyl-CoA molecules can be esterified to glycerol-3-phosphate (G3P) by isoforms of glycerol-3-phosphate acyltransferase (GPAT). In hepatocytes, the isoforms are predominantly GPATs 1 and 4. The resulting lysophosphatidic acid (LPA) is acylated by acylglycerol-3-phosphate acyltransferases (AGPATs) to form phosphatidic acid (PA), which can be dephosphorylated by phosphatidic acid phosphatase (PAP) to form diacylglycerol (DG). Both PA and DG can be directed toward phospholipid (PL) synthesis. Additionally, diacylglycerol acyltransferases (DGATs 1 and 2) synthesize triglyceride (TG) by acylation of DG. (A) Fatty acids (FAs) synthesized *de novo* are likely to be channeled to VLDL-TG production through DGAT1 activity. (B) Exogenous FA appear to be directed toward TG synthesis for storage in lipid droplets by the activity of DGAT2. In addition, lipid droplet-TG can undergo hydrolysis, with FA rerouted into VLDL by DGAT1.

the involvement of this enzyme in both the overt and latent DGAT activities (286, 289). Accordingly, overexpression of DGAT1 in rat hepatoma cells leads to increased intracellular TG contents and also increased rates of VLDL-TG secretion (164). *Dgat1* knockout mice have slightly reductions in hepatic TG concentrations when fed a low fat diet, with more prominent decreases being observed in response to high fat feeding (47, 238). High fat-fed *Dgat1* knockout mice are also resistant to obesity and have improved insulin responsiveness (238). Moreover, DGAT1 expression is increased in NAFLD subjects and may contribute to hypertriglyceridemia (146).

By contrast, *Dgat2* transcripts are abundant in the liver, and are decreased upon fasting and increased after refeeding (179). DGAT2 exhibits *in vitro* specificity for the use of DG molecules as substrates (42). The predicted topology of this protein suggests its contribution to overt activity, and this was confirmed in HepG2 cells by studies using DGAT2 inhibitors (98). Accordingly, DGAT2 is found in close association to cytosolic LDs and mitochondria-associated membranes, where enzymes involved in TG synthesis and storage are also localized (178, 242). *Dgat2* knockout mice exhibit severe lipopenia and die in the first day after birth (243). The liver-specific overexpression of DGAT2 in chow-fed mouse leads to the development of hepatic steatosis but no abnormalities in plasma glucose and insulin signaling were observed, suggesting that hepatic TG accumulation is not a cause of insulin resistance (183). In agreement with this observation, human DGAT2 genetic variants reduce hepatic steatosis. However, the decrease in fat content does not lead to improved insulin sensitivity (131).

In keeping with specialized cellular functions of the enzymes, gain and loss of function studies in mice have revealed different phenotypes for DGAT1 and DGAT2, without evidence of compensation (243). In fact, a more recent study revealed a unique role of hepatic DGAT2 in its use of *de novo*-synthesized acyl-CoA and DG as substrates (281). The TGs formed by this reaction are stored in cytosolic LDs and ultimately undergoes lipase-mediated hydrolysis. The resulting lipid intermediates (i.e., MG and DG) are available for re-esterification by overt DGAT1 activity present in the cytosolic compartment, followed by TG transport into the ER. These intermediates can also undergo translocation to the ER lumen followed by acylation catalyzed by latent DGAT1 activity (Fig. 3A). Overt and latent activity of DGAT1 contribute to lipoprotein lipidation during VLDL assembly and utilize pre-formed, but not *de novo*-synthesized acyl-CoA as preferred substrate (Fig. 3B). Therefore, DGAT2 and DGAT1 use independent pools of acyl-CoA for TG synthesis, and act in a sequential and integrated way for TG packaging into nascent VLDL particles (281).

Although hepatic TG accumulation is the major determinant of NAFLD (61), lipid and lipidomic analyses have revealed changes in the compositions of a variety of lipids, including FA, DG, LPA, PA, PL, and ceramides (78, 127, 168, 216, 260). The specific contributions of hepatic lipid species to NAFLD remain the subject of active investigation (136).

VLDL assembly and secretion

TG-rich VLDL particles represent the mechanism by which FA are exported from the liver and delivered to muscle for oxidation and adipose tissue for storage, respectively. VLDL assembly is a two-step process that begins in the ER lumen. In the first step, microsomal triglyceride transfer protein (MTP) acts to incorporate a small amount of TG in apoB100 as it is being translated by ribosomes and translocated across the ER membrane (101). In the second step, additional TG is packaged into the nascent apoB100-containing particles as they traverse from the ER to the Golgi apparatus to form mature VLDL particles (Fig. 4) (54).

The link between increased VLDL secretion and metabolic diseases, such as insulin resistance and diabetes, is well established (54, 251, 297). The development of insulin resistance leads to hepatic TG accumulation owing to both enhanced FA uptake into the liver and increased DNL. The greater availability of TG together with higher MTP activity promotes the overproduction of VLDL particles (10) and greater plasma TG concentrations (54). A positive correlation between plasma TG concentrations and the hepatic steatosis is not always observed (63). Obese leptin-deficient *ob/ob* mice exhibit decreased VLDL production rates despite marked hepatic steatosis (163). In nondiabetic obese NAFLD subjects, VLDL-TG secretion rates are elevated up to two times in comparison to normal subjects (82). In this setting, higher rates of TG accumulation fail to increase VLDL-TG proportionately, suggesting a limited capacity for increasing VLDL production and secretion (81).

Reductions in VLDL secretion can also lead to hepatic steatosis. This occurs in the setting of genetic defects in apoB100 and MTP, which lead to hypobetalipoproteinemia and abetalipoproteinemia, respectively (274). Mipomersen and lomitapide are drugs that respectively target apoB100 and MTP in the interest of reducing plasma LDL cholesterol concentrations by blocking VLDL secretion (58, 263). A mechanism-based side effect of these medications is hepatic steatosis. Finally, a polymorphism in Transmembrane 6 superfamily member 2 (TM6SF2) has been identified by human genome-wide association studies to be associated with hepatic steatosis (147, 236). The polymorphism results in reduced VLDL secretion rates, because of impaired lipidation of nascent VLDL particles (236). Whether the control of VLDL secretion can be leveraged in the management of NAFLD remains unclear.

Lipid Droplet Biology

LDs are dynamic cellular structures that transiently store lipids. Second to white adipose tissue, the liver has the next greatest capacity to store TG in LDs, such that overnight fasting leads to hepatic LD formation that accommodates FA derived from adipose tissue lipolysis (267). The neutral lipid composition of LDs differs among different hepatic cell types. Whereas hepatocytes harbor TG-enriched LD cores, stellate

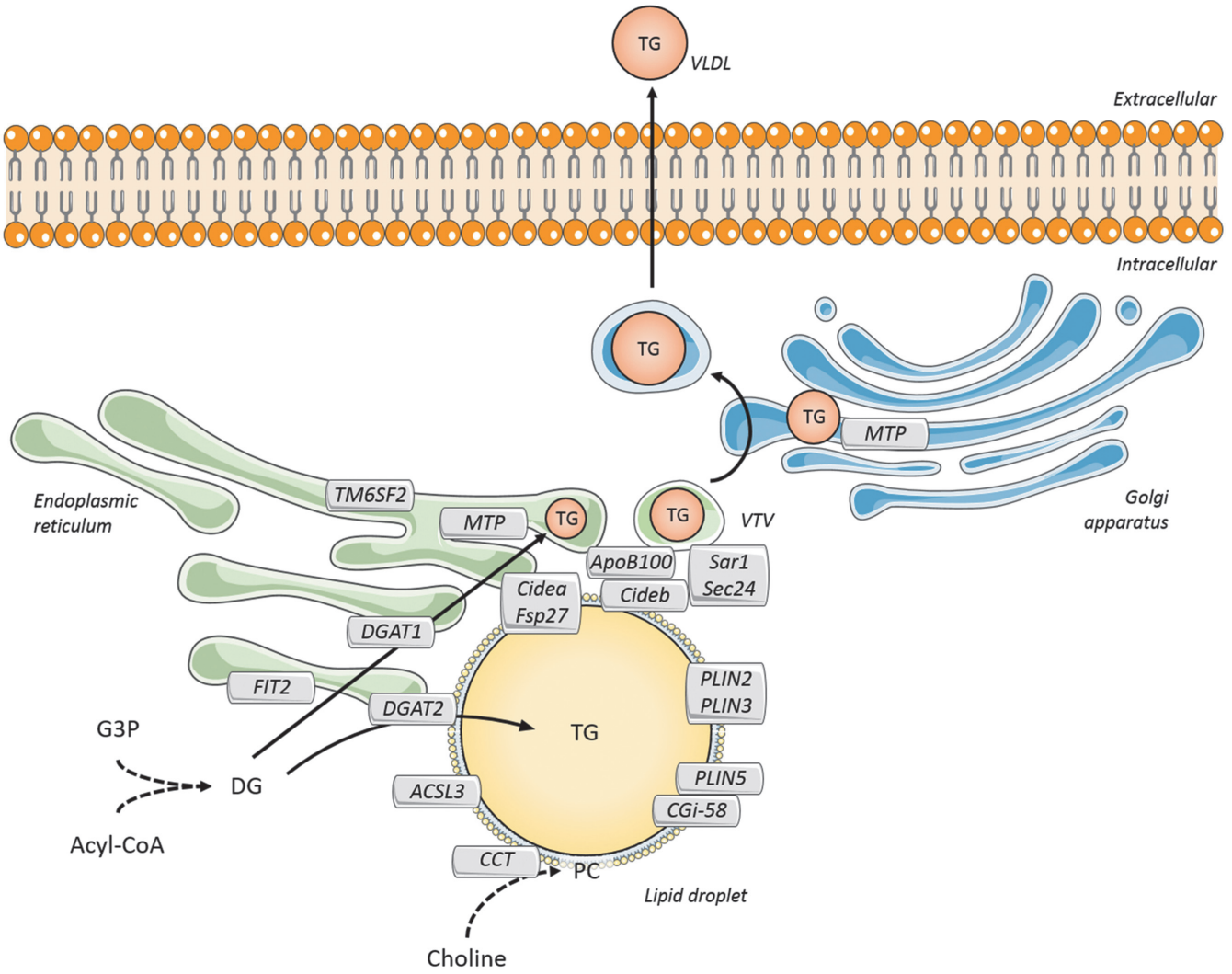

Figure 4 Triglyceride storage and secretion in hepatocytes. Triglyceride (TG) can be synthesized from diacylglycerol (DG) by diacylglycerol acyltransferases (DGAT1 and 2). DGAT1 preferably provides TG to VLDL during lipidation in the lumen of the endoplasmic reticulum. This process is mediated by microsomal triglyceride transfer protein (MTP), which facilitates the association between TG and apoB100. Transmembrane 6 superfamily member 2 (TM6SF2) may also contribute to VLDL lipidation via yet unknown mechanisms. The nascent VLDL particle is then transferred to the Golgi apparatus through the VLDL transfer vesicle (VTV), followed by a second MTP-mediated lipidation step. VLDL particles are secreted via a vesicle-mediated mechanism. TG can also be formed by the activity of DGAT2, which mainly contributes to storage in lipid droplets (LDs). LDs are delimitated by proteins and a phosphatidylcholine (PC)-enriched surfactant monolayer. Among the LD-associated proteins, perilipins (PLIN2, 3, and 5), and comparative gene identification-58 (CGI-58) contribute to LD structure and/or the regulation of LD-associated enzymes; CTP-phosphocholine cytidylyltransferase (CCT) and acyl-CoA synthetase 3 (ACSL3) may be required for the biosynthesis of lipids; DFF45-like effector (CIDE) proteins, Cidea, Cideb, and Fsp27, and the microsomal fat-inducing transmembrane protein 2 (FIT2) are required for LD formation, however their specific cellular roles are incompletely understood. Cideb also contributes to VLDL production and secretion via its interaction with Sar1 and Sec24, which are present in VTV.

cell LDs store predominantly vitamin A as retinyl esters (23). The TG-rich cores of LDs in hepatocytes are surrounded by a coat comprised of PL and proteins. The PL are primarily PC and form a monolayer around the neutral lipid core (13). CTP-phosphocholine cytidylyltransferase (CCT) is the rate-limiting enzyme in the Kennedy pathway for PC biosynthesis. Indicative of its role in LD biogenesis, CCT is recruited from the cytosol to expanding LDs in a variety of cell types to provide PC for LD surface expansion (278). However, the precise role of CCT on LD formation within hepatocytes is uncertain.

In addition to preserving the LD structure, LD-associated proteins regulate the formation, expansion, and contraction of LDs. These proteins include selected enzymes of lipid synthesis, such as ACSL3 and DGAT2 (Fig. 4). Changes in the composition and activity of the protein components of the LD have been implicated as contributing to metabolic diseases (40, 99, 100, 105). The predominant hepatocellular LD proteins are the members of perilipin (PLIN; former perilipin/ADRP/TIP47, PAT) protein superfamily (PLIN1-5). These proteins in adipocytes are involved both in the stabilization of LD

structure as well as in the control of availability of substrates for several LD-associated enzymes (141). Perilipin 1 (PLIN1; synonym Perilipin A) is normally only expressed in LDs from adipocytes, whereas PLIN2 (synonym ADRP) and PLIN3 (synonym TIP47) are associated with LDs in hepatocytes (245). *Plin1* mRNA is expressed in livers of subjects with NAFLD (104), and PLIN1 protein is found in LDs of steatotic hepatocytes (245).

A key role for PLIN2 in hepatocytes is supported by the observation that it is the most abundant LD protein in the HuH7 hepatocyte cell line (94), and its levels are also increased in steatotic livers of both humans and mice (185) in response to PPARγ activation (197). Overexpression of PLIN2 in rat hepatic stellate cells results in lipid accumulation within LDs (96), while PLIN2-deficient mice exhibit a 60% reduction in hepatic TG content and are resistant to diet-induced hepatic steatosis (46). Liver-specific ablation of PLIN2 in mice results in reduced LD size and is protective against diet-induced NASH (190). PLIN1 is frequently associated with larger LDs in the livers of NAFLD subjects while PLIN2 is predominantly found in smaller LDs (93). The specific targeting of these proteins raises the suggestion of distinct PLIN1 and PLIN2 functions.

Plin3 expression is also induced in livers of high fat-fed mice and its knockdown reduces hepatic TG content, attenuates steatosis, and improves insulin sensitivity and glucose tolerance (39). In hepatocytes, the degradation of PLIN2 and PLIN3 are steps required for lipolysis during nutrient deprivation. The removal of PLIN proteins from LD occurs via chaperone-mediated autophagy (CMA) and facilitates the LD association of components of the lipolytic machinery, including adipose triglyceride lipase (ATGL) (134). Moreover, the combined knockdown of *Plin2* and *Plin3* results in increased insulin resistance in mouse AML12 hepatocytes stimulated with oleate (17). PLIN5 is highly expressed in oxidative tissues, including liver. Hepatic *Plin5* mRNA levels are increased after fasting in response to activation of PPARα and/or PPARβ/δ (140). PLIN5 expression is also enhanced in steatotic livers of humans and obese mice, and the knockout of this protein results in decreased hepatic TG content and smaller-sized LDs (268).

In hepatocytes, LDs are tightly associated with ER membrane cisternae, allowing the physical interaction between LD components and microsomal proteins, such as apoB100 (194) (Fig. 4). The fat-inducing transmembrane proteins (FIT1 and 2) are localized in the ER and have the ability to bind DG and TG, with FIT2 showing higher affinity (108). The overexpression on FIT2, the most abundant hepatic FIT isoform, in mouse liver results in increased TG content and LD accumulation (128). These data suggest that FIT2 functions in the binding of neutral lipids synthesized within ER and their channeling to nascent LDs (99).

The DFF45-like effector (CIDE) proteins, Cidea, Cideb, and Cidec (synonym Fsp27), are associated both to the ER and LDs and have been linked to LD metabolism (100). Recent studies have demonstrated that these proteins act to promote lipid exchange and fusion among LDs (282). However, they are differentially distributed among hepatocytes based on sizes of LD that they harbor. Cideb localizes both to hepatocytes with small and with large LDs, whereas Cidea and Fsp27 specifically localize to hepatocytes containing large LDs (282).

Cidea expression is increased in livers of diabetic mice (137) and a coding region polymorphism is associated with obesity in human subjects (60, 290). Moreover, expression of both *Cidea* and *Fsp27* are upregulated in mice with hepatic steatosis induced by PPARγ activation (176, 264, 288). Fsp27 is a target gene of PPARγ and its transcription is decreased in *ob/ob* mice with liver-specific knockdown of PPARγ, accompanied by decreased TG content in the liver (175, 176). Cideb is highly expressed in the liver and *Cideb* knockout mice are resistant to obesity and liver steatosis induced by high-fat diet (160). Moreover, these animals have decreased levels of circulating TG and FA, and exhibit improved hepatic insulin sensitivity. These effects are mediated by the hepatic down-regulation of SREBP-1c, resulting in decreased lipogenesis and increased FA oxidation (160). Cideb also interacts with apoB100, and mice lacking Cideb exhibit reduced VLDL secretion and increased hepatic steatosis (284). In addition to its role in VLDL assembly, Cideb has been associated in VLDL export from ER to the Golgi. This occurs through the interaction with components of coat complex II (COPII), namely, Sar1 and Sec24, which are present in VLDL transport vesicles (257).

Because NAFLD is characterized by excessive deposition of TG within cytosolic LDs and because proteomic studies have revealed that numerous LD-associated proteins are modulated in human and mice steatotic livers (138, 250), detailed knowledge of the biology of hepatocyte LDs could reveal novel targets for therapeutic intervention.

Lipolysis

In the setting of insulin resistance, enhanced rates of lipolysis within white adipose tissue are a major contributor to the increased FFA plasma levels and hepatic steatosis (159). However, the contribution of TG hydrolysis within hepatocytes to aberrant lipid accumulation in the liver is less clear. Several lipases are present in close association with LDs in adipocytes (30, 66) (Fig. 5), but their contributions to LD TG hydrolysis remain topics of discovery.

The LD-associated ATGL (synonym PNPLA2) is the rate-limiting step in TG lipolysis within adipocytes. The resulting DG molecules are then hydrolyzed by the hormone sensitive lipase (HSL) to release monoglycerides (MG). In the final step, the monoacylglycerol lipase (MGL) cleaves MG into glycerol and FA (151) (Fig. 5). Gain and loss of function studies have revealed that hepatic ATGL is required for the lipolysis of TG stored in LDs, controls substrate availability for FA oxidation, and modulates the progression of hepatosteatosis (198, 208, 280). Full activation of ATGL depends

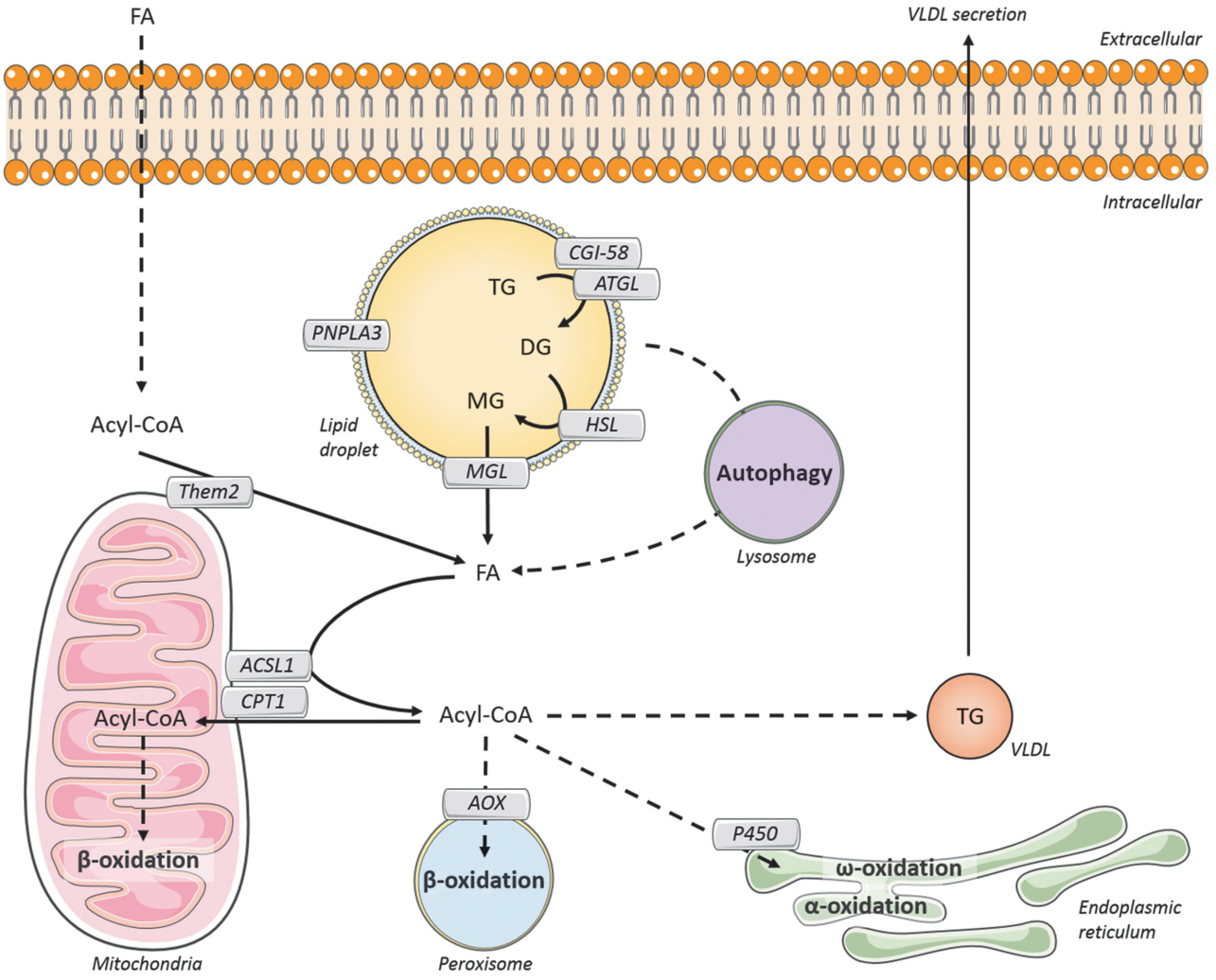

Figure 5 Lipolysis and fatty acid oxidation in hepatocytes. The initiation of hepatic lipolysis depends on the activation of adipose triglyceride lipase (ATGL) through the binding to the comparative gene identification-58 (CGI-58). ATGL catalyzes the hydrolysis of triglyceride (TG), releasing diacylglycerol (DG). This lipid is further hydrolyzed by hormone sensitive lipase (HSL), releasing monoacylglycerol (MG). Monoacylglycerol lipase (MGL) mediates the breakdown of MG into fatty acid (FA) and glycerol. Alternatively, autophagic pathways, such as macroautophagy and chaperone-mediated autophagy (CMA), promote the hydrolysis of LD. FA can also be generated from acyl-CoA by the activity of acyl-CoA thioesterase 13 (ACOT 13; synonym: thioesterase superfamily member 2, Them2). In the mitochondria outer membrane, long-chain acyl-CoA synthetase 1 (ACSL1) converts long-chain FAs to acyl-CoAs. ACSL1 interacts physically with carnitine palmitoyltransferase 1 (CPT1). It is likely that ACSL1 channels acyl-CoA to CPT1 to control the availability of substrates for mitochondrial β-oxidation. Additionally, acyl-CoAs can be directed to β-oxidation in the peroxisome, where fatty acyl-CoA oxidase (AOX) represents a rate-limiting step, or ω-oxidation and α-oxidation in the endoplasmic reticulum, mediated by P450 4A family members. In addition, part of the acyl-CoA pool can be directed for TG synthesis and VLDL assembly, enabling the transfer of hepatic lipids to other organs.

on coactivation by the comparative gene identification-58 (CGI-58) (152). Liver-specific ablation of CGI-58 results in NAFLD phenotypes in mice that include hepatic steatosis and fibrosis (109). In the setting of hepatic steatosis, lipolysis mediated by hepatic ATGL is decreased when PLIN5 binds competitively to CGI-58, displacing ATGL (268, 269). Insulin-resistant NAFLD patients who exhibit higher degrees of liver steatosis when compared to NAFLD subjects without insulin resistance, also exhibit decreased *CGI-58* mRNA levels. These observations suggest that insulin-resistance could induce hepatic TG accumulation through CGI-58-mediated reductions in ATGL-dependent lipolysis (132). Less is known concerning MGL and HSL activities in hepatocytes, although *Hsl* expression is downregulated in livers of NAFLD patients (146).

The patatin-like phospholipase domain-containing 3 (PNPLA3/adiponutrin) is a TG hydrolase/transacylase that localizes to LD, ER and cytoplasm (124, 212). A single-nucleotide variant of the *Pnpla3* gene (I148M) confers increased risk of NAFLD in humans (210). In this connection, livers from transgenic mice expressing this allele show accumulation of inactive PNPLA3 in lipid droplets and increased steatosis when fed a high-sucrose diet (235). Although the genetic association of PNPLA3 with NALFD is robust, key

details are still lacking regarding the metabolic activity of this protein.

Autophagy

Autophagy is a lysosome-dependent process that selectively targets, transports and regulates the storage of essential components, including lipids, proteins, and carbohydrates. Three types of autophagy have been described in hepatocytes (59). In macroautophagy, LDs or other organelles are engulfed to form autophagosomes, which fuse to lysosome to generate an autolysosome. After hydrolysis by lysosomal enzymes, lipid products are released to the cytosol and recycled by other cellular processes (167). The autophagosome formation relies on several autophagy receptors in addition to the autophagy-related proteins (ATGs) (241). In microautophagy, small vesicles originated from lysosomal membrane invagination mediate the engulfment of cytosolic components (167). In chaperone-mediated autophagy (CMA), lysosomal degradation of specific proteins is regulated upon recognition by CMA receptors (133).

Macroautophagy of LDs (also named lipophagy) is stimulated in hepatocytes during starvation, leading to the release of FA in the cytosol after TG breakdown (233). Additionally, products of lipid anabolism have inhibitory effects in lipophagy (167). Although the mechanisms controlling lipophagy are not well understood, this process is driven by the small GTPase Rab7 protein, which recognizes LDs and mediates their engulfment by autophagosomes (221).

Decreased autophagy has been reported in obesity-related disorders, including hepatosteatosis (219). Altered membrane lipid composition due to high-fat feeding reduces the activity of lysosomal proteins and decreases autophagic activity in mouse liver (145, 209). Impaired CMA leads to hepatosteatosis through at least three mechanisms. First, reduced CMA activity decreases mitochondrial function and β-oxidation. Second, CMA-deficient mice exhibit increased expression of lipogenic enzymes, including DGAT2 (219). Finally, CMA is required for the lipolysis of hepatic TG through hsp70-mediated degradation of PLIN2 and PLIN3 (134). Hence, impaired autophagy in the setting of steatosis exacerbates lipid accumulation within the liver.

Fatty Acid Oxidation

FA derived from hydrolysis of hepatic TG stores, circulating lipids or DNL, can be oxidized by multiple pathways. Mitochondrial β-oxidation is the primary route for the oxidation of the majority of FA found in hepatocytes, including short- (<C4), medium- (C4-C12), and long-chain (C12-C20) FA. β-oxidation of very long- (C20-C26) and branched-chain FA begins in the peroxisomes. Additional pathways for FA oxidation include α-oxidation and ω-oxidation within the ER and are mediated by cytochrome P450 4A family members (153, 187) (Fig. 5). The expression of genes involved in mitochondrial and extramitochondrial FA oxidation is regulated largely by PPARα activity. PPARα is expressed at high levels in liver, and both whole-body and hepatocyte-specific ablation of PPARα in mice lead to reduced transcription of hepatic genes related to mitochondrial β-oxidation, such as very long chain acyl-CoA dehydrogenase (VLCAD), long chain acyl-CoA dehydrogenase (LCAD), and ACSL1 (7), and genes involved in peroxisomal β-oxidation, including peroxisomal fatty acyl-CoA oxidase (AOX) and cytochrome P450 4A (156, 184). These animals also experience hypoketonemia and increased hepatic steatosis (111, 184).

It is noteworthy that AOX ablation leads to sustained activation of PPARα, likely due to accumulation of PPARα ligands and induction of cytochrome P450 4A (86). Impaired peroxisomal FA oxidation results in the accumulation of dicarboxylic acids within the liver and leads to mitochondrial damage and microvesicular steatosis (153). Alterations in dicarboxylic acid levels are also observed in conditions of FA overload and have been correlated with increased risk of NAFLD development, although the cellular mechanism remains unknown (215, 287). PGC1α and BAF60a form a complex that regulates PPARα transcriptional activity in the liver. Overexpression of BAF60a increases FA oxidation and reduces hepatic steatosis in obese mice (162). Similar phenotypes have been observed in livers of mice overexpressing the PPARα coactivator PGC1β (18).

Notwithstanding the relationship between PPARα activity and hepatic lipid metabolism, the correlation between PPARα expression levels and hepatic steatosis is inconsistent, with reports demonstrating unchanged, increased and decreased expression (16). This is also the case for rates of hepatic mitochondrial β-oxidation. The transcription of CPT1a, is downregulated in NAFLD (146). Although previous studies revealed decreased hepatic mitochondrial FA oxidation in the setting of hepatic steatosis (123, 202, 206), recent work has demonstrated increased oxidation rates in livers of subjects with hepatic steatosis, metabolic syndrome and NAFLD (16). In livers of *ob/ob* mice, mitochondrial and peroxisomal oxidative capacities are elevated (29). Mechanisms underlying increased FA oxidation in NAFLD have been reviewed (16). Hypotheses include enhanced activation of PPARα due to increased hepatic FA uptake or biosynthesis.

ER-Mediated Control of Lipid Homeostasis

Among its many functions, the ER regulates the synthesis of lipids and proteins as well as intracellular calcium storage (24). Under physiological conditions, unfolded and misfolded proteins accumulate in the ER lumen. In response to this ER stress, the cell initiates multiple lines of defense. One is the activation of ER-associated degradation (ERAD), a ubiquitin/proteasome protein degradation pathway that relies on E3 ligase complex activation (11). Second, the unfolded protein response (UPR) is initiated (229). This comprises three

main signaling pathways mediated by microsomal transmembrane proteins: PKR-like ER kinase (PERK; also known as eukaryotic translation initiation factor 2-α kinase 3, eIF2αk3), inositol requiring enzyme 1 (IRE1), and activating transcription factor 6 (ATF6) (229). UPR activation alleviates the accumulation of misfolded proteins into the ER through the inhibition of protein translation, the induction of chaperones to increase protein folding capacity, as well as expanding the ER membrane (11).

Lipid accumulation is also associated with chronic ER stress in hepatocytes (293). Lipid-mediated induction of ER stress can be triggered by multiple mechanisms. Obese mice exhibit altered microsomal membrane composition in the liver, with increased PC:PE ratios and variations in PL acyl-chain saturation (91, 121). Hence, changes in membrane fluidity leads to altered activity of membrane-associated proteins. For instance, sarco-endoplasmic reticulum Ca^{2+}-ATPase (SERCA) activity is reduced in steatotic mouse livers. In this model, decreased ER Ca^{2+} content impairs intraluminal Ca^{2+}-dependent chaperone function and is a cause for ER stress (91).

UPR-related proteins directly sense membrane lipid composition in nonhepatic cells. In mouse fibroblasts, PERK and IRE1 are responsive to increased lipid saturation even in the absence of their ER-spanning transmembrane domain (265). However, it is uncertain whether direct lipid sensing mechanisms modulate hepatic ER stress. Because ER stress in turn promotes lipid accumulation in hepatocytes, chronic ER stress may directly contribute to the pathogenesis of NAFLD (291). In cell culture systems, prolonged exposure to palmitate induces ER stress when this saturated FA is incorporated to ER membrane PL (25). A concomitant reduction in VLDL-TG secretion is attributable to enhanced apoB100 degradation by proteasomal and nonproteasomal mechanisms (43,199). In this connection, treatment with 4-phenyl butyric acid (PBA), a chemical chaperone, improves ER homeostasis and VLDL secretion after FA exposure (43). Although reduced VLDL secretion due to chronic ER stress results in TG accumulation in McA-RH7777, its potential contribution for the progression of hepatic steatosis was not demonstrated in animal models (199).

ER stress also promotes lipid accumulation in hepatocytes that is attributable to increased lipogenesis (11). In obese mice, hepatic induction of the UPR pathway reduces Insig-1 levels and leads to the activation of SREBP, increased expression of lipogenic enzymes and ultimately steatosis (129). UPR-mediated activation of PERK may also contribute to hepatic steatosis by increasing expression of VLDL receptors in hepatocytes (126). Moreover, ER stress reduces autophagy through the cross-talk with components of ERAD and UPR in different cell types, exacerbating the hepatic steatosis (11,201)

In summary, excess lipid load potentially initiates a vicious cycle of hepatic lipid accumulation due to abnormal activity of ER stress-activated pathways, leading to altered lipid homeostasis and TG deposition in the liver (11,291,293).

Conclusion

The liver is a main organ for the metabolism of FAs and TGs and altered activity of metabolic pathways in the setting of overnutrition leads to a common chronic liver disease known as NAFLD. Considering this biomedical relevance, many important questions regarding basic aspects of hepatic lipid metabolism remain unanswered. These include: (a) Which FA transporters are responsible for the increased intracellular concentration of FA in the setting of overnutrition? (b) What is the precise mechanism by which DNL is increased in obese and NAFLD subjects? (c) What is the precise molecular mechanism underlying the partitioning of FA and acyl-CoA among metabolic pathways, which presumably involve ACOT, ACSL and lipid-binding proteins? (d) How are rates of FA oxidation regulated in the setting of overnutrition? In summary, a deeper understanding is needed regarding the precise mechanisms whereby FA uptake and synthesis are tightly balanced against oxidation and secretion within the liver. This would be expected to lead to a better understanding of how TGs accumulate in many, but not all, individuals who consume excess calories and to new therapeutic strategies for the management of NAFLD.

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