Hepatic Fatty Acid Trafficking: Multiple Forks in the Road[1–3]

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ABSTRACT

The liver plays a unique, central role in regulating lipid metabolism. In addition to influencing hepatic function and disease, changes in specific pathways of fatty acid (FA) metabolism have wide-ranging effects on the metabolism of other nutrients, extra-hepatic physiology, and the development of metabolic diseases. The high prevalence of nonalcoholic fatty liver disease (NAFLD) has led to increased efforts to characterize the underlying biology of hepatic energy metabolism and FA trafficking that leads to disease development. Recent advances have uncovered novel roles of metabolic pathways and specific enzymes in generating lipids important for cellular processes such as signal transduction and transcriptional activation. These studies have also advanced our understanding of key branch points involving FA partitioning between metabolic pathways and have identified new roles for lipid droplets in these events. This review covers recent advances in our understanding of FA trafficking and its regulation. An emphasis will be placed on branch points in these pathways and how alterations in FA trafficking contribute to NAFLD and related comorbidities. *Adv. Nutr. 4: 697–710, 2013.*

Introduction

Hepatic steatosis is defined by the accumulation of TG in lipid droplets (LDs)[4], commonly referred to as nonalcoholic fatty liver disease (NAFLD) in humans when alcohol is not a contributing factor. NAFLD can comprise simple steatosis or the more advanced nonalcoholic steatohepatitis, which is characterized by inflammation and hepatocyte injury that often is accompanied by fibrosis (1). The reported prevalence of NAFLD varies widely among limited studies with estimates showing that ~20–30% of the general population have NAFLD (1,2), although numerous risk factors such as obesity (3–5), type 2 diabetes (6,7), Hispanic ethnicity (2,8), and male gender (9) increase its prevalence. NAFLD increases the risk of type 2 diabetes, dyslipidemia, hypertension, cardiovascular disease, chronic kidney disease, liver cirrhosis, hepatocellular carcinoma, and mortality (10–15). Unfortunately, there are limited effective treatment options for NAFLD. Lifestyle intervention involving weight loss, insulin-sensitizing agents, and vitamin E are the most recommended treatment options, but efficacy is limited due to patient adherence and safety concerns (16). Additionally, hepatic steatosis is not limited to humans and occurs in numerous other species. The most prevalent examples include obese cats, periparturient ruminants, especially dairy cows in negative energy balance, and poultry force-fed grain for foie gras production (17–19).

Ultimately, hepatic steatosis occurs due to an imbalance of TG synthesis and breakdown. An inherent difficulty in understanding the etiology of NAFLD is that there is no single pathway universally responsible for the development of

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[4] Abbreviations used: ACC, acetyl-CoA carboxylase; ACSL, long chain acyl-CoA synthetase; AGPAT, sn-1-acyl-glycerol-3-phosphate acyltransferase; AMPK, AMP-activated protein kinase; ATGL, adipose triglyceride lipase; CES, carboxylesterase; CHREBP, carbohydrate response element binding protein; CIDEB, cell death-inducing DFF45-like effector B; CPT1, carnitine palmitoyl-transferse 1; DAG, diacylglycerol; DGAT, diacylglycerol acyltransferase; DNL, de novo lipogenesis; ER, endoplasmic reticulum; FA, fatty acid; FAS, fatty acid synthase; FATP, fatty acid transport protein; FOXA/O, forkhead box protein; FXR, farnesoid X receptor; GPAT, glycerol-3-phosphate acyltransferase; HMGCS2, HMG-CoA synthetase 2; HNF-4α, hepatocyte nuclear factor-4α; LD, lipid droplet; LFABP, liver fatty acid binding protein; LXR, liver X receptor; MGAT, monoacylglycerol acyltransferase; mTORC1, mammalian target of rapamycin complex 1; MTP, microsomal triglyceride transfer protein; NAFLD, nonalcoholic fatty liver disease; PGC-1α, peroxisome proliferator-activated receptor gamma, coactivator 1-α; *PNPLA3*, phospholipase domain containing A3; SCD1, stearoyl-CoA desaturase 1; SREBP, sterol regulatory element binding protein.

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steatosis. Rather, there is most likely heterogeneity in flux through individual pathways that ultimately results in the development of steatosis. These pathways are highly influenced by genetics, diet, environment (toxins, drugs, etc.), and the presence of specific diseases; thus, it is difficult to identify the cause of or optimal treatment for NAFLD.

Current Status of Knowledge

Pathways of fatty acid accretion

The liver derives fatty acids (FAs) from 3 primary sources: uptake of FFAs from the blood, chylomicron remnant uptake, and de novo lipogenesis (DNL). Each of these pathways is highly regulated; therefore, their contribution to the total liver FA pool is variable. In addition to these external or de novo sources of FAs, intracellular pathways such as TG hydrolysis and autophagy can also contribute to endogenous release of FAs. Alterations in any of these pathways influence both hepatic and whole-body energy metabolism and may contribute to disease development.

FA uptake and transport. The liver extracts exogenous FFAs proportional to their concentration in the blood (20,21). Studies employing arterial-venous balance and perfused rodent livers reveal that first-pass extraction of FFA is typically 20–30% (20,21). There appears to be no difference in uptake among FA species with the exception of stearic acid (18:0), which has low or no hepatic net uptake, a finding that is conserved across numerous animal species (20,22–25). Stable isotope studies in humans show that uptake of exogenous FFA is the single largest source of FA in stored hepatic TG and this contribution is further increased with fasting and NAFLD (26,27). Increased visceral fat content is correlated with hepatic TG content (28–30) and has been implicated in NAFLD etiology. According to the portal vein theory, visceral adipose tissue releases FFA and cytokines into the portal vein, thereby exposing the liver to high concentrations of adipose-derived products. Yet visceral depots account for only 5–30% of hepatic FFA exposure (31) and the liver does not discriminate between FFA derived from different adipose depots. The importance of FFA derived from visceral adipose tissue to the portal vein theory and development of NAFLD remains to be determined. Clearance of chylomicron-remnant TG also contributes to the hepatic FA pool. In humans, chylomicron remnant uptake accounts for 15% of the liver FA pool during fasting and 25% during the fed state (26), and this contribution decreases with meal feeding compared with continuous feeding (32). **Figure 1** illustrates the different possible routes of FA uptake and overviews the major pathways that contribute to hepatic FA trafficking.

The regulation of FA transport and uptake into the liver is complex and often debated. Clearly, there is strong evidence for protein-mediated transfer of FA across the plasma membrane. There are numerous proteins involved in the membrane transport of FA, including the family of FA transport proteins (FATPs) and the scavenger receptor CD36. Hepatic FA uptake is decreased in mice lacking FATP2 or FATP5 in the liver (33,34). In addition, overexpression of CD36 in the liver increases hepatic FA uptake in vivo (35) and CD36 expression is increased in animal models of NAFLD and in humans with NAFLD (35–37). Surprisingly, CD36 knockout mice have normal rates of FA uptake, suggesting that CD36 is not required for FA uptake under normal conditions (38). Membrane proteins such as caveolins are also important contributors to FA influx, but their role has not been sufficiently characterized in the liver (39). An important factor that influences FA uptake is the subsequent activation of FAs to their acyl-CoA derivatives, thereby preventing efflux (40). Both ACSL and FATP family members possess acyl-CoA synthetase activity and play key roles in channeling exogenous FAs into specific metabolic pathways as discussed below. In addition to FA activation, impairments in downstream utilization of

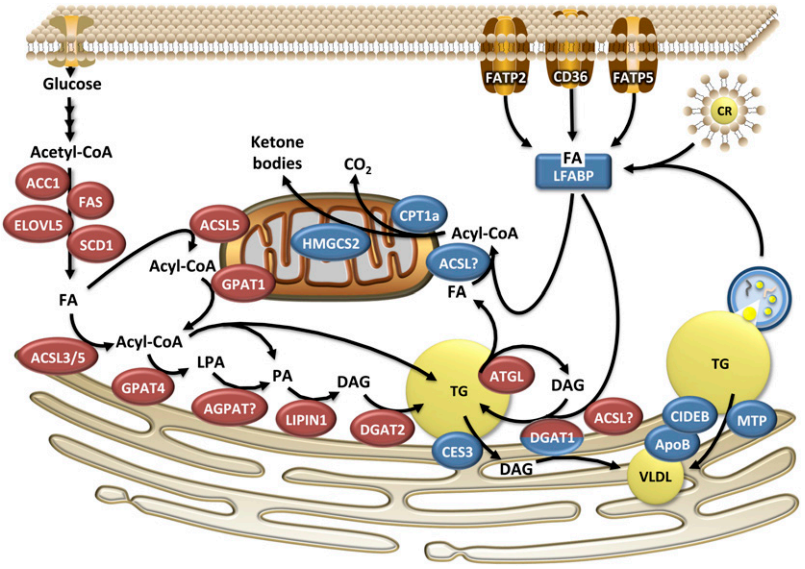

FIGURE 1 Pathways and enzymes involved in hepatic FA trafficking. Enzymes in red indicate catabolic pathways, whereas blue enzymes are involved in FA disposal. DGAT1 is bicolor, because it may be involved in the synthesis of cytosolic TG from exogenous FA and in VLDL synthesis. ACC1, acetyl-CoA carboxylase 1; ACSL, long chain acyl-CoA synthetase; AGPAT, sn-1-acyl-glycerol-3-phosphate acyltransferase; ATGL, adipose triglyceride lipase; CES, carboxylesterase; CIDEB, cell death-inducing DFF45-like effector B; CPT1a, carnitine palmitoyl transferase 1; CR, chylomicron remnant; DAG, diacylglycerol; DGAT, sn-1,2-diacylglycerol acyltransferase; ELOVL5, long chain fatty acid elongase 5; FA, fatty acid; FAS, fatty acid synthase; FATP, fatty acid transport protein; GPAT, glycerol-3-phosphate acyltransferase; HMGCS2, HMG-CoA synthetase 2; LFABP, liver fatty acid binding protein; LPA, lysophosphatidic acid; MTP, microsomal triglyceride transfer protein; PA, phosphatidic acid; SCD1, stearoyl-CoA desaturase 1.

acyl-CoAs also can lead to reduced FA uptake [see (41) for review]. It should be noted that passive diffusion also contributes to hepatic uptake of FAs, but this process is a minor contributor to total FA influx (42,43). Regardless of the exact mechanism, uptake of exogenous FAs is a major source of intracellular FAs in the liver.

FAs are intrinsically hydrophobic and, as a result, do not readily diffuse into the aqueous cytosol. Thus, intracellular binding proteins are responsible for shuttling FAs between organelles and facilitating their uptake and metabolism. The FA binding protein (FABP) family member that is the most highly expressed isoform in the liver is liver fatty acid binding protein (LFABP) (44). Ablation of LFABP in mice does not alter initial rates of FA uptake in hepatocytes, but after 30 s, FA uptake is reduced (45). This delayed uptake is suggestive of a mechanism whereby altered downstream metabolism, perhaps activation of FAs to acyl-CoAs, becomes impaired in the absence of LFABP. Once activated, acyl-CoAs are subsequently bound by liver acyl-CoA binding protein (46), although the contribution of this protein to hepatic FA uptake has not been well defined. The fate of FA taken up by the liver is dependent on a host of factors, including the structure of the FA, the rate of uptake, and influenced by the activity of the hepatocytes, which is influenced by activity of the enzymes involved in FA metabolism.

DNL. The liver is a major site of DNL, but its contribution to whole-body lipogenesis varies across species. The liver is thought to be the primary source of DNL in poultry, fish, and humans (47–49); the latter is still controversial. Hepatic DNL has a moderate contribution to whole-body DNL in rodents and rabbits (50) and is low in ruminants, dogs, and pigs (50–52). DNL accounts for ~5% of hepatic FA during fasting and up to 30% after a meal (27,53), although rates of DNL are highly variable among people (54).

Acetyl-CoA carboxylase (ACC) and FA synthase (FAS) are responsible for carrying out the committed steps of DNL. Two isoforms of ACC exist, with each differing in its physiological function. ACC1 is primarily cytosolic and produces malonyl-CoA that is largely used as a substrate by FAS for DNL. In contrast, ACC2 is localized on the mitochondria and produces a local pool of malonyl-CoA that inhibits carnitine palmitoyl-transferse 1 (CPT1; discussed below), although it may also be able to compensate for ACC1 when the latter is ablated (55–57). The terminal elongation of FAs by FAS produces palmitate (16:0) as its primary product (58). Subsequently, elongase and desaturate enzymes can act on de novo synthesized 16:0 to generate 16:1, 18:0, or 18:1. In the liver, stearoyl-CoA desaturase 1 (SCD1) is the primary desaturase responsible for the generation of C16:1 and C18:1 from SFAs (59), whereas long-chain FA elongase 5 appears to be the primary hepatic elongase for generating C18:0 and C18:1 (60). Additional elongases and desaturases likely also contribute to the elongation and desaturation of longer PUFAs (e.g., C18:2 and C18:3) (61,62). Long chain acyl-CoA synthetase (ACSL) 3 and ACSL5 are involved in the activation of de novo synthesized FAs and regulate their subsequent incorporation into TG and phospholipids (63,64).

TG synthesis. The initial step in the formation of TG is the formation of lysophosphatidic acid from glycerol-3-phosphate and acyl-CoA catalyzed by sn-1-glycerol-3-phosphate acyltransferase (GPAT). Subsequently, sn-1-acyl-glycerol-3-phosphate acyltransferase (AGPAT) catalyzes the formation of phosphatidic acid, which can be further metabolized to generate phosphatidylinositol and phosphatidylglycerol or can be dephosphorylated by LIPIN proteins to produce 1,2-diacylglycerol (DAG). DAG is an important branch point in glyceroplipid synthesis, because it can be further acylated by sn-1,2-diacylglycerol acyltransferase (DGAT) to form TG, or phosphoethanolamine or phosphocholine head groups can be added to form phosphatidylethanolamine and phosphatidylcholine, respectively.

These pathways become more complex when the number of isoforms of each enzyme is considered. For example, there are 4 known isoforms of GPAT, 10 putative AGPATs, 3 LIPINs, and 2 DGATs that differ in tissue expression pattern, intracellular location, and substrate preference (65). As an example of the latter point, GPAT1, a major hepatic GPAT isoform, prefers SFAs as a substrate resulting in the high prevalence of SFAs in the sn-1 position of many glycerolipids (66,67). Thus, the relative activity of different isoforms along with availability of specific FAs dictates the final composition of TG and phospholipids. Additionally, each of these enzymes most likely utilizes a physically distinct pool of substrate and forms a distinct pool of product due to the enzyme's intracellular location and interaction with upstream/downstream proteins in a given pathway. Although both GPAT1 and GPAT4, the 2 major liver isoforms, can utilize exogenous FAs as substrates, only GPAT1 is required for the esterification of de novo synthesized FAs (68). Similar differences exist among the DGAT isoforms with DGAT2 utilizing FAs derived from DNL as its substrate, whereas DGAT1 preferentially reesterifies exogenous FAs onto DAG generated from lipolysis (69–71). This selective formation of TG also appears to have ramifications on VLDL secretion as discussed below.

It should also be noted that the liver expresses all 3 isoforms of monoacylglycerol acyltransferase (MGAT) and the expression of MGAT3 increases in individuals with NAFLD (72). The monoacylglycerol pathway was thought to exist primarily in enterocytes; however, these recent data suggest that the MGATs may contribute to hepatic FA metabolism as well.

FATP and ACSL isoforms also play an important role in activating FAs to be used for TG synthesis. Liver ablation of FATP2 or FATP5 lowers hepatic TG in models of NAFLD (33,34). However, it has not been determined if this effect is due to decreased FA uptake or altered TG synthesis or hydrolysis. ACSL5, which is located on mitochondria and the endoplasmic reticulum (ER), increases FA uptake and partitions FAs toward TG and phospholipid synthesis in hepatocytes (64,73).

A single mutation (I148M) in the gene patatin-like phospholipase domain containing A3 (*PNPLA3*) is the best known genetic predictor of NAFLD (74). This variant is more prevalent in certain minorities, including Hispanics, and likely accounts for the increased rates of NAFLD in this population (74,75). *PNPLA3* encodes the protein adiponutrin, which shares the greatest homology to the TG lipase adipose triglyceride lipase (ATGL; encoded by *PNPLA2*). Given the similarity to a known TG lipase, previous studies identified adiponutrin to have TG hydrolase activity; therefore, it was presumed that the I148M mutation would cause NAFLD as a result of reduced TG hydrolysis (76). However, mice lacking adiponutrin do not have hepatic steatosis or any overt phenotype, suggesting that other mechanisms are involved (77,78). Subsequent work has revealed that adiponutrin is a lysophosphatidic acid acyltransferase (i.e., catalyzes the same reaction as the AGPAT enzymes discussed above) with a preference for unsaturated FAs (79). The I148M variant has increased acyltransferase activity compared with wild-type adiponutrin, suggesting that this mutation results in a gain-of-function leading to enhanced formation of phosphatidic acid, and subsequently TG, rather than a loss of function influencing TG hydrolysis (79). Given its prominent role as a genetic predictor of NAFLD, future work is needed to definitively elucidate the mechanism through which adiponutrin regulates TG metabolism and FA trafficking.

FA incorporation into TG serves an important role in preventing the accumulation of intracellular FAs or lipid metabolites that can cause liver injury and dysfunction (80). For example, DGAT2 overexpression causes steatosis in the absence of liver injury or insulin resistance (81), whereas DGAT2 knockdown reduces steatosis yet causes oxidative stress and fibrosis (82). The accumulation of intermediates involved in glycerolipid synthesis, such as phosphatidic acid and DAG enriched in SFAs, also impairs insulin signaling (83,84). Moreover, the inability of FAs to be esterified into TG is responsible for the lipotoxicity associated with high levels of SFAs (85). Thus, dysregulation through one or more steps of the TG synthetic pathway can have detrimental effects on hepatic function.

LDs

Once formed, TGs are stored primarily in cytosolic LDs. The formation of LDs occurs largely on the membrane of the ER, although enzymes responsible for TG synthesis are also present on LDs and may play a role in LD expansion (86). Numerous models of LD biogenesis have been proposed, but the exact mechanism of LD formation and maturation is still under investigation [see (87) for review]. The hydrolysis of TG from LDs has received much attention in adipose tissue, but more recent research has shed light on the importance of this metabolic pathway in the liver. Studies from our laboratory and others have characterized several lipases, including ATGL, carboxylesterase 3 (CES3; also known as TGH) and carboxylesterase 1 (CES1) as important hepatic TG hydrolases with divergent effects on LD metabolism and FA partitioning. LD-associated proteins play an important structural role, but many are also directly involved in regulating lipolysis or interacting with other proteins to coordinate LD formation and lipolysis. The composition of the LD proteome changes with NAFLD and likely contributes to changes in FA metabolism (88,89). Because of the divergence in effects of specific TG lipases and LD proteins, we will discuss them in the appropriate sections that follow.

Pathways of FA disposal

The liver disposes of FAs through either oxidation or secretion as VLDL. FA oxidation is highly variable and largely influenced by FA influx, whereas VLDL secretion exhibits much more subtle changes in response to FA influx and hormonal oscillations. Multiple branch points exist regarding the partitioning of FAs between these 2 pathways.

FA oxidation. FAs can be catabolized through 3 distinct pathways of oxidation: α, ω, and β. ω-Oxidation occurs exclusively in microsomes and α- and β-oxidation can occur in peroxisomes and mitochondria. Mitochondrial β-oxidation is the primary catabolic pathway in the liver for most FAs, but peroxisomal β-oxidation is largely responsible for the initial oxidation of very long-chain (>20) FAs (90). Although mitochondrial β-oxidation is a major pathway of FA disposal in the liver, complete oxidation to CO_2 is a minor contributor, with incomplete oxidation to ketone bodies being the primary end product of hepatic FA catabolism (91).

FAs must be activated to their acyl-CoA derivatives prior to their subsequent metabolism. ACSL1 is located on mitochondria and plays a major role in activating FAs destined for β-oxidation in heart and adipose (92,93). In contrast, ACSL1 ablation in the liver results in only a small reduction in FA oxidation (94); the ACSL or FATP isoforms responsible for activating FAs for hepatic mitochondrial β-oxidation remain unknown. LFABP appears to play an important role in carrying exogenous FAs to the mitochondria. Mice that lack LFABP have reduced FA oxidation and impaired ketogenesis during fasting (95). LFABP also interacts with mitochondria (96) and, more specifically, CPT1a, the rate-limiting step in mitochondrial FA oxidation (97,98). Malonyl-CoA, an intermediate in DNL, allosterically inhibits CPT1a to prevent FA oxidation during times of increased flux through DNL (99). This regulation forms the basis of the classic Randle Cycle that links carbohydrate metabolism and DNL to FA catabolism (100). There is also evidence that CPT1a reduces VLDL secretion, perhaps due to the disposal of FAs through oxidation, thereby limiting the availability of FAs for VLDL synthesis (101–103).

There are numerous studies showing that inhibiting esterification of FAs diverts them toward oxidation. ACSL5 knockdown diverts FAs from incorporation into TG and phospholipids toward β-oxidation (64). Similar to ACSL5, hepatic GPAT1 (68,104,105) and DGAT2 (106,107) ablation results in increased partitioning of FAs toward oxidation at the expense of esterification. Thus, the flux through TG

synthesis plays an important role in determining the rate of FA oxidation.

In addition to oxidation of exogenous FAs, hydrolysis of intracellular TG is also a source of FAs that can be used for energy production. Research from our laboratory and others shows that gene ablation or shRNA knockdown of hepatic ATGL reduces TG hydrolase activity and increases TG accumulation (108,109). In addition, ATGL channels hydrolyzed FAs toward oxidation but has no effect on VLDL secretion (108,109). The lack of effect of ATGL on VLDL secretion is surprising and clearly highlights LD catabolism as a branch point in hepatic FA trafficking. Consistent with the effects of ATGL, ablation of the LD proteins perilipin 2 or 5, which results in enhanced lipolysis, increases the oxidation of hydrolyzed FAs, whereas ablation of the ATGL coactivator comparative gene identification-58 impairs FA oxidation (110–112). ATGL-mediated lipolysis also regulates PPARα signaling as a means to coordinate downstream FA oxidation (108–110). PPARα is a transcription factor that is activated by FAs and in turn increases transcription of genes involved in FA oxidation (discussed below). Surprisingly, administration of the PPARα agonist, fenofibrate, did not normalize PPARα target gene expression or liver TG concentrations in mice treated with adenovirus harboring ATGL shRNA (108), suggesting that a more complex mechanism linking ATGL-catalyzed lipolysis to PPARα activation may exist. It is unclear if ATGL primarily drives FA oxidation through selective channeling, through increased oxidative capacity (driven by PPARα), or if both these mechanisms contribute to enhanced FA oxidation.

VLDL secretion. Although considerable progress has been made in recent years into our understanding of VLDL synthesis and secretion, much remains to be determined. Current models suggest that there are distinct steps of TG addition during VLDL synthesis. The first step occurs in the ER where lipidation of the primordial VLDL particle is mandatory to prevent apoB degradation (113–115). This step requires microsomal triglyceride transfer protein (MTP), which facilitates the transfer of lipids onto the developing VLDL particle (116). The second step involves bulk lipidation of the VLDL from cytosolic LDs. The exact mechanism of how cytosolic lipids are transferred across the membrane is not known and the importance of MTP in this process is also unclear (117,118). Early studies demonstrated that <30% of exogenous FAs are directly incorporated into VLDL, with the majority first transiting through the cytosolic LD droplet pool (70,119,120). TG incorporated into VLDL are not completely hydrolyzed from cytosolic LDs, but instead they are partially hydrolyzed to DAG (119, 121), pointing toward a role for TG lipases in regulating VLDL assembly.

In contrast to the effects of ATGL on the partitioning of FAs to oxidative pathways, CES3 appears to be a major lipase involved in VLDL secretion. Global or hepatic ablation of CES3 impairs VLDL secretion without causing steatosis (122,123). The lack of effect of CES3 on steatosis may be explained by increased rates of hepatic FA oxidation (123). Additionally, hepatic CES3 knockout mice have altered LD morphology, with more but smaller LDs and a delayed transfer of newly formed LDs to preformed LDs (124). CES3 localizes to the ER and appears in proximity to cytosolic LDs (124,125). Although the exact mechanism defining how CES3 alters LD metabolism and VLDL synthesis remains unknown, it is clearly an important regulator of FAs destined for VLDL secretion. CES1, another member of the CES family, has recently been shown to play an important role in the hydrolysis of PUFAs from TG. Mice with global deletion of CES1 are obese, develop hepatic steatosis, and have increased lipogenesis and VLDL secretion (126). Mice lacking CES1 have TG that is specifically enriched in long-chain PUFAs, especially EPA (20:5) and DHA (22:6). The authors further show that the block in EPA and DHA hydrolysis from TG results in an upregulation of sterol response element binding protein-1c (SREBP-1c), a master transcriptional regulator of lipogenesis that is normally suppressed by long-chain PUFAs. Taken together, these data identify a novel role for CES1 in generating a specific pool of long-chain PUFAs that inhibit SREBP-1c to suppress lipogenesis and VLDL secretion. Thus, in addition to the channeling of hydrolyzed FAs, members of the CES family of lipases may also serve an important role in FA signaling to coordinate downstream metabolism.

Despite the fact that most cytosolic TG is hydrolyzed prior to its incorporation into VLDL, it is unclear if there are ever truly cytosolic LDs that are independent of the ER. Indeed, Wolinski (127) showed that all LDs in yeast are associated with the ER. If this model holds true, it would help our understanding of how ER luminal lipases involved in VLDL formation would have access to the LD to facilitate hydrolysis. Cell death-inducing DFF45-like effector B (CIDEB, also known as FSP27) is a protein that localizes to LDs and the ER (128) and has been shown to play a role in VLDL assembly. As evidence, CIDEB directly interacts with apoB and ablation of CIDEB reduces VLDL secretion (128). Additional studies show that CIDEB also localizes to the golgi and may be involved in the second lipidation step of VLDL formation (129).

DGAT1 is thought to be the primary acyltransferase responsible for the reesterification of DAG to TG that is destined for secretion (69,70,130). Indeed, hepatocytes possess DGAT activity on both the cytosolic and luminal sides of the ER (70,131). DGAT1 also selectively uses FAs derived exogenously, rather than through DNL, as substrates and selectively channels these FAs into a pool of TG that is preferentially secreted as VLDL (70,71). This finding may also explain why de novo synthesized FAs have a slower rate of secretion into VLDL compared with exogenous FAs in humans (132). The finding that FAs formed from DNL are more slowly secreted is surprising given that rates of DNL are typically associated with increased VLDL secretion, an observation noted across multiple species (133). It is still unclear, however, which ACSL or FATP isoform is responsible for the activation of FAs prior to DGAT1. Additionally, the

role of carnitine acyltransferases in shuttling acyl-CoAs across the ER membrane for use by lumen DGAT activity has not been extensively characterized (134,135).

Autophagy and FA metabolism

Autophagy, which becomes activated in response to cellular stress such as starvation, is an evolutionarily conserved mechanism to degrade and recycle damaged organelles. A major breakthrough in the field was the discovery that autophagy is also involved in the disposal of LDs, a process known as macrolipophagy (136). Accordingly, ablation of hepatic autophagy results in hepatic steatosis through decreased TG turnover (136). Although autophagy results in the catabolism of complex lipids, there is no net gain or loss of FAs, because FAs released by lysosomes remain within cells. Although autophagy is clearly an important regulator of hepatic metabolism, many questions remain regarding its role in lipid metabolism. For example, the metabolic fate of FA liberated by lysosomal lipases is largely unknown as are the factors that regulate this process; ablation of hepatic autophagy decreases FA oxidation but also suppresses VLDL secretion (136,137). Also, it remains to be determined how LDs are recognized by autophagosomes to be endocytosed. It is presumed that specific LD-associated proteins target the LD for degradation, but these proteins have yet to be identified. Regardless, future research into autophagy and NAFLD and related diseases undoubtedly will advance our understanding of yet another pathway controlling hepatic FA trafficking.

Regulation of FA trafficking

Transcriptional regulation. PPARα is perhaps the most well-characterized transcription factor involved in governing hepatic FA metabolism; see **Figure 2** for an overview of hepatic FA trafficking regulation. PPARα induces the expression of many of the enzymes involved in peroxisomal and mitochondrial β-oxidation in addition to proteins involved in FA uptake (CD36), transport (LFABP, acyl-CoA binding protein), and activation (ACSL1) (138). PPARα binds numerous ligands, but long-chain PUFAs such as EPA appear to be the most robust activators (139). Formation of 16:0/18:1 phosphatidylcholine, which requires DNL for its synthesis, is also an activator of PPARα (140). PPARα is coactivated by peroxisome proliferator-activated receptor gamma, coactivator-1α (PGC-1α, which drives mitochondrial biogenesis and oxidative capacity through its interaction with other transcription factors such as estrogen-related receptor-α and nuclear respiratory factors 1 and 2 (141). Forkhead box protein O1 (FOXO1) is another transcription factor that binds PGC-1α and has been shown to induce ATGL expression, although the physiological consequences of this regulation on downstream FA metabolism have not been studied (142). In addition to its regulation by PPARα, hepatic FA uptake is also promoted by the liver X receptor (LXR) via changes in CD36 expression (143) and inhibited by FOXA1 via reduced FATP2 expression (144). There is also evidence that FOXA1, FOXA2, and hepatocyte nuclear factor-4α (HNF-4α) increase transcription of genes that promote hepatic FA oxidation and ketogenesis as a means to decrease hepatic TG content (144–148).

In general DNL and TG synthesis run in parallel and, as such, are controlled by similar mechanisms. Following a meal, nutrient flux to the liver and the insulin response to the meal signal to upregulate DNL and TG synthesis. SREBP1c is responsive to insulin signaling and upregulates ACC1/2, FAS, GPAT1, and other genes, including glycolytic genes that support lipid anabolism (149,150). ER stress also increases SREBP1c activity leading to elevated DNL and may be an important contributor to the elevated DNL observed in individuals with NAFLD (151,152). Glucose and/or fructose drive DNL by providing substrates for DNL and by upregulating carbohydrate response element binding protein (CHREBP), which in turn increases the transcription of genes involved in glycolysis, DNL, and TG synthesis (153–155). Fructose is especially lipogenic due to its very rapid flux into the liver and its ability to bypass key regulatory

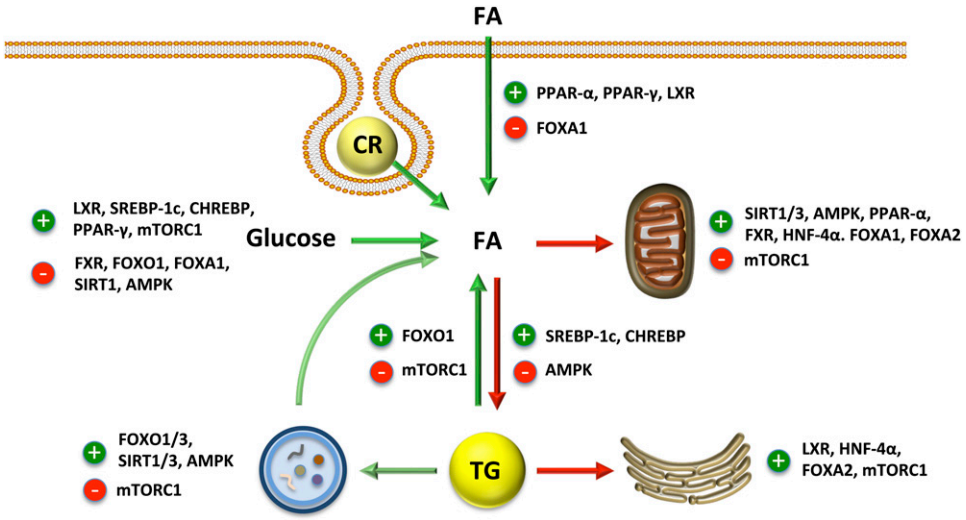

FIGURE 2 Transcriptional and post-transcriptional regulation of hepatic FA trafficking. Green arrows indicate sources of intracellular FA and red arrows indicate routes of FA disposal. AMPK, AMP-activated protein kinase; CHREBP, carbohydrate response element binding protein; CR, chylomicron remnant; FA, fatty acid; FOXA/O, forkhead box protein; FXR, farnesoid X receptor; HNF-4α, hepatocyte nuclear factor-4α; LXR, liver X receptor; mTORC1, mammalian target of rapamycin complex 1; SIRT1, sirtuin 1; SREBP, sterol regulatory element binding protein.

steps in glycolysis [see (156) for review]. In support, high-carbohydrate diets or sugars, especially fructose, induce DNL (54,157). Both SREBP1c and CHREBP are target genes of LXR, thus making it an important regulator of DNL as well (158,159). Farnesoid X receptor (FXR), which is activated by bile acids, antagonizes SREBP1c to decrease DNL (160) and promotes PPARα-mediated FA oxidation (161). Additional work has also shown PPARγ plays an important role in upregulating DNL especially in NAFLD (162–164). In contrast, FOXO1 and FOXA1 antagonize DNL (144,165,166).

The transcriptional regulation of VLDL secretion is less clear. LXR, HNF-4α, and FOXA2 promote VLDL secretion (145,148,167). Although SREBP1c and CHREBP do not upregulate genes involved in VLDL secretion, they do promote DNL, which contributes substrates for VLDL synthesis. As such, SREBP1c is required for increased VLDL production in diabetic or high-carbohydrate diet-fed mice (168). FOXO1 is a transcription factor that regulates both DNL and VLDL secretion. FOXO1 increases the expression of MTP and apoCIII and promotes VLDL secretion in some studies (169,170). However, FOXO1 also inhibits DNL and has been shown to reduce VLDL secretion in other studies (171,172). These studies employ mouse models that use different constitutively active forms of FOXO1, which may be a contributing factor to the discrepancies.

Transcriptional activation of autophagy is mediated largely by stress-responsive transcription factors such as p53 and nuclear factor-κB (173). The FOXO family of transcription factors is also induced by cellular stress and accordingly have been shown to promote autophagy (174,175). However, little is known regarding the transcriptional regulation of lipophagy and how it contributes to hepatic FA metabolism.

Post-translational regulation. As expected, FA metabolism is highly regulated by cellular energy status. AMP-activated protein kinase (AMPK) acts as a cellular energy gauge and in response to low cellular energy ($\uparrow$AMP/ATP), it phosphorylates target proteins to increase or decrease their activity. AMPK-mediated phosphorylation of ACC1 and ACC2 (176–178) results in reduced activity and a lowering of malonyl-CoA levels. In addition to lowering rates of DNL, this modification of ACC2 also enhances FA oxidation through the blunting of malonyl-CoA–mediated inhibition of CPT1. Therefore, targeting one enzyme can reciprocally regulate both DNL and FA oxidation. Although direct phosphorylation has not been shown, AMPK increases malonyl-CoA decarboxylase activity, thus providing another mechanism through which AMPK lowers malonyl-CoA levels (179). AMPK inhibits the activity of GPAT1 in hepatocytes, resulting in a shuttling of FAs toward oxidation rather than esterification into TG (180). Finally, AMPK also regulates FA metabolism through changes in transcription thought largely to emanate from its phosphorylation and activation of PGC1α (181). Thus, taken as a whole, AMPK acts as a critical node in partitioning hepatic FA between anabolic and catabolic pathways.

The sirtuin family of deacetylases has garnered considerable research attention regarding their role in regulating energy metabolism. Similar to AMPK, the sirtuins function as sensors of the cellular energy state. The activity of sirtuins is increased by NAD^+, which is required for their activity. Of the family of sirtuins, SIRT1 and SIRT3 have been the most characterized in regards to their roles in hepatic FA metabolism. SIRT1-mediated deacetylation results in inactivation of SREBP1c (182,183) and activation of FXR (184). The net result of these modifications is a decrease in lipogenic gene expression leading to reduced flux through the DNL pathway. SIRT1 also increases PGC1α/PPARα signaling. SIRT1 deacetylates and activates PGC1α (185) and interacts with and regulates PPARα as well (186); direct SIRT1-mediated deacetylation of PPARα has yet to be shown. SIRT1 also activates FOXA1, to promote FA oxidation and ketogenesis (187), and FOXO1, which increases ATGL expression to increase lipolysis (142,188). In addition, SIRT1 induces autophagy in numerous cell types to further promote FA catabolism (189), but this has not been studied in the liver. Consistent with its role in promoting FA oxidation, SIRT1 expression is inhibited by CHREBP and induced by the cAMP-response element binding protein, which is activated by β-adrenergic signaling (e.g., fasting or exercise) (190). The role of specific targets of SIRT3 has been less characterized, but one known target involved in FA metabolism is HMG-CoA synthetase 2 (HMGCS2), the rate-limiting step in ketogenesis. Upon activation, SIRT3 deacetylates and activates HMGCS2 to increase ketone body synthesis (191). The importance of this regulation in hepatic and whole-body lipid metabolism has not been characterized; however, absence of HMGCS2 causes hypoketotic hypoglycemia and fatty liver in humans fed low-carbohydrate diets (192,193), suggesting that this enzyme plays a major role in FA disposal and linking FA catabolism to gluconeogenesis.

In opposition to AMPK and SIRT1/3, mammalian target of rapamycin complex 1 (mTORC1) is activated in response to insulin signaling and nutrients, especially amino acids. Activation of mTORC1 increases phosphorylation of LIPIN1, which blocks the LIPIN1-mediated inhibition of SREBP-1c, resulting in enhanced DNL (194). Recent studies also suggest that mTORC1 inhibits PPARα activity through its activation of nuclear receptor co-repressor 1, a PPARα repressor (195). In addition, mTORC1 inhibits ATGL-mediated lipolysis in adipose tissue, which is mediated by the transcription factor early growth response protein 1 (196); the importance of this regulation in the liver has not been determined. Finally, mTORC1 is a potent inhibitor of autophagy adding yet another layer through which it antagonizes FA catabolism (197).

Insulin signaling also regulates VLDL secretion. Acute insulin signaling inhibits VLDL secretion in part through enhanced apoB degradation (198). In contrast, elevated and sustained insulin signaling, such as in insulin resistance, promotes VLDL secretion (199). There is much debate regarding the specific nodes of insulin signaling that regulate this process, although recent data suggest that mTORC1

may be involved in the rise in VLDL secretion during insulin resistance (200).

Clinical importance of intracellular hepatic FA trafficking

NAFLD is closely associated with obesity, insulin resistance, and type 2 diabetes (2,201). A hallmark of insulin resistance is elevated serum FFA concentrations (202). As a result of increased FFA exposure, the liver of insulin-resistant individuals has increased rates of FFA uptake, which is likely a major contributor to the development of NAFLD (203,204). Coinciding with changes in FFA uptake, individuals with NAFLD or NASH exhibit altered hepatic FA composition. A consistent finding is that PUFAs, especially n–3 long-chain FAs such as EPA and DHA, in total hepatic lipids or TG are reduced in individuals with NAFLD or NASH (205–207) the mechanisms leading to these changes in FA content remain unknown. Given its importance in FA catabolism, impairments in mitochondrial β-oxidation have been implicated as a causative factor in the development of steatosis (208–210). Undoubtedly, there are situations where this is the case; however, additional evidence suggests that NAFLD coincides with increased rates of FA oxidation in humans and mice (211–216). It is also well established that participants with NAFLD or insulin resistance have increased rates of VLDL secretion (199,217,218). Taken together, these data suggest that the liver attempts to compensate for the increased FA flux by increasing FA disposal through both secretion and oxidation. In addition, a growing body of literature shows that DNL is increased in the liver and reciprocally decreased in adipose tissue in insulin-resistant and type 2 diabetic individuals (157,219–222). Finally, autophagy is downregulated by high-fat–induced hepatic steatosis (136), suggesting that the decrease in this route of lipid disposal may also contribute to NAFLD etiology.

With so many pathways that contribute to steatosis, it is unclear which pathway should be targeted. Numerous studies that have targeted DNL inhibition have observed marked improvements in hepatic steatosis, insulin resistance, and obesity resistance (223). Increasing the expression of wild-type or a malonyl-CoA–insensitive CPT1a mutant enhances hepatic FA oxidation and decreases steatosis (101–103). Enhancing autophagy alleviates steatosis and liver injury following high-fat feeding in mice (224). Is decreasing hepatic FFA uptake a good idea, or will it divert FFA to other tissues such as heart and muscle, where dysfunction will ensue? Is it a good idea to increase VLDL secretion as a means of disposing hepatic FA, or would the consequences of increasing serum TG outweigh the benefits of decreasing steatosis? Perhaps the pathway that should be targeted depends upon the specific disease (e.g., NAFLD alone or with complications). Little is known in regards to how the above individual pathways contribute to the progression of NAFLD to steatohepatitis or hepatocellular carcinoma or more systemic disease like Type 2 diabetes. Thus, a more tailored approach of targeting individual pathways based on the desired outcomes is warranted.

TG defines NAFLD and is tightly correlated with the comorbidities of steatosis; however, TG itself is widely considered to be inert and not involved in the complications of NAFLD. Instead, FAs may directly trigger hepatic dysfunction through their incorporation into and accumulation of metabolites such as DAG and ceramides (83). Alternatively, products resulting from FA metabolism, such as reactive oxygen species, may also link NAFLD to its comorbidities (225). Although FA metabolites are important signaling molecules, it is important to recognize that FAs, or their acyl-CoA derivatives, are also signaling molecules. FAs/acyl-CoAs can serve as ligands for transcription factors and as allosteric regulators of numerous enzymes involved in energy metabolism (65). These functions are dependent upon the structure of the FA/acyl-CoA and the pathway and enzyme responsible for its formation.

Clearly, there is no single metabolite or pathway that is responsible for NAFLD or its progression to more detrimental manifestations. Rather, variability in diet (e.g., carbohydrate content), genetics (e.g., *PNPLA3* variants), metabolic state (e.g., insulin resistance), and other environmental factors all converge and lead to the single, but very heterogeneous, disease known as NAFLD.

In conclusion, hepatic FA trafficking is a complex process with many levels of regulation. Although tremendous progress towards our understanding of hepatic FA metabolism has been made in recent years, many pathways and enzymes that contribute to FA trafficking remain understudied or perhaps even unknown. Additionally, more studies in humans evaluating the flux through each pathway and its contribution to hepatic and whole-body FA trafficking in different metabolic states and disease models are needed. Given the prevalence of NAFLD and its associated comorbidities and the lack of effective treatment options, hepatic FA metabolism will remain an important research focus as we strive toward effective preventative or therapeutic options.

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