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Review

1-Carbon Cycle Metabolites Methylate Their Way to Fatty Liver

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Fatty liver is a complex disease often accompanying metabolic syndrome and Type 2 diabetes mellitus (T2DM). Hepatosteatosis may have roots in multiple metabolic abnormalities. However, metabolic dysfunction in the 1-carbon cycle (1CC), which produces the methyl donor *S*-adenosylmethionine (SAM) and phosphatidylcholine (PC), induces hepatic lipogenesis in model systems. Human diseases where 1CC or PC synthesis is disrupted, such as alcoholism, congenital lipodystrophy, or cystic fibrosis, often present with fatty liver. Given that the 1CC is clearly linked to this disease, it is critical to understand how the individual metabolites drive mechanisms increasing stored hepatic lipids. In this review, I summarize evidence that ties the 1CC to fatty liver disease along with data proposing mechanisms for increased lipogenesis or decreased lipid export by phosphatidylcholine.

Introduction

Lipid accumulation in metabolic disease is not only a problem in adipose tissue. Excess fat in the liver (hepatosteatosis) also contributes to metabolic dysfunction, contributing to the insulin resistance that leads to T2DM [1]. Fatty liver occurs in multiple human syndromes, from alcoholic or nonalcoholic fatty liver disease (ALD or NAFLD) [2,3] and can also occur in patients with cystic fibrosis [4], those fed intravenously [5], or in patients with genetic lipodystrophy [6]. While the physiological roots of fatty liver are not completely understood, there is a clear connection to the 1CC (folate cycle), because mutations or drugs affecting key enzymes result in hepatosteatosis [7].

The 1CC falls at the crossroad of several anabolic processes producing amino acids, nucleotides, the redox protector glutathione (GSH), and the methyl donor SAM (Figure 1 and Table 1) [8]. Folate, a B vitamin produced by microbiota and obtained through the diet, enters the first stage of the cycle and can then progress toward purine production or produce methionine. In the next part of the cycle, this methionine is converted to SAM, the donor for nucleic acid, protein, and phospholipid methylation. The product of these methyltransferase reactions, *S*-adenosylhomocysteine (SAH), is recycled back to methionine through homocysteine (Hcy) [9]. Another B vitamin, B_{12}, can be used in this regenerative process [9]. Hcy itself can also be converted to glutathione through the *trans*-sulfuration process [9]. Multiple aspects of the 1CC, such as glutathione generation or SAM-dependent DNA or histone modification, could contribute to the overall pathology of fatty liver disease; however, the 1CC metabolite with the most direct link to the initial lipid accumulation is PC [10]. PC can be also produced *de novo* through the Kennedy pathway, independent of the 1CC [11]. In mammals, PC species generated by methylation differ in side chains from those produced by the Kennedy pathway [12]. In liver cells, where the demand for PC is high, as much as 40% of SAM goes to PC production because phosphatidylethanolamine (PE) undergoes three sequential modifications by PE methyltransferase (PEMT) [12]. Thus, limitations in the 1CC in liver can have a profound effect on PC production. While PC

Trends

Low levels of 1CC metabolites or the methylated phospholipid PC accompany lipid accumulation in invertebrate models, and rodent and human liver.

Levels of SAM, the major methyl donor, and PC have the strongest links as effectors of fatty liver.

SAM-dependent DNA or histone methylation could have effects on gene expression.

Low PC can affect signaling controlling intracellular transport, activating the lipogenic transcription factor Sterol regulatory element binding protein 1 (SREBP-1). In addition, specific PC isoforms are required for activation of a nuclear hormone receptor (liver-receptor homolog-1, LRH-1) that drives bile acid export from the liver.

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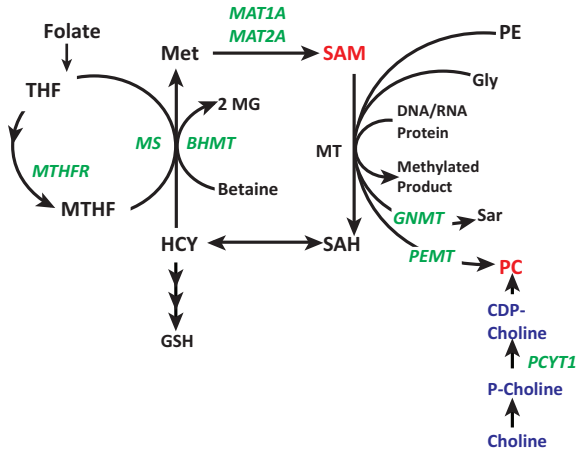

Figure 1. Schematic Diagram of the 1-Carbon Cycle (1CC). The 1CC comprises multiple interlocking pathways. Folate enters the cycle leading to methyl tetrahydrofolate (MTFH) conversion to methionine (Met) by methionine synthase (MS). Methionine is used to make *S*-adenosylmethionine (SAM), which can be used by methyltransferases (MTs) to produce methylated proteins (including histones), nucleic acids, phosphatidylcholine (PC), or metabolites, such as sarcosine (Sar). The byproduct of these reactions, *S*-adenosylhomocysteine (SAH), can be used to regenerate methionine. Homocysteine (HCY), which is produced during the reconversion process, can also be diverted to glutathione (GSH) production. Enzymes discussed in the main text are in green, and key metabolites are in red. Abbreviations: 2-MG, 2-methyl glycine; BHMT, betaine hydroxy methyltransferase; Gly, glycine; GMNT, glycine n-methyltransferase; MAT, methionine adenosyl transferase; MTHFR, methyl tetrahydrofolate reductase; PCYT1, phosphocholine cytidylyl transferase; PE, phosphatidylethanolamine; PEMT, phosphatidylethanolamine methyltransferase; THF, tetrahydrofolate.

Table 1. Important Metabolites, Processes and Physiological Effects of the 1CC

1CC Metabolite	Process	System	Tissue	Refs
SAM	PC production	Human, mouse	Liver	[10]
		Caenorhabditis elegans	Intestine	
		Drosophila	S2 cells	
	Spermidine production	Mouse	Liver, adipose	[32]
	Histone methylation	Human	Induced pluripotent stem cells, HCT116 cells	[64–66]
		Mouse	Liver	
		C. elegans	Intestine	
	DNA methylation	Mouse	Schwann cells	[67]
Methyl-tetrahydrofolate	Nucleotide synthesis			[68]

production is associated with hepatic lipid accumulation in multiple human diseases, animal models, and culture systems [10], a complete mechanistic picture of how decreased PC drives lipid production is not yet clear (see Outstanding Questions).

The 1CC, PC Levels, and Fatty Liver in Human Disease

The link between metabolic pathways tied to methylation and fatty liver disease was noted during the 1930s, when Vincent du Vigneaud found that rodents fed a diet deficient in methyl groups developed fatty liver [13]. Currently, both alcoholic and nonalcoholic steatosis have well-defined

links to the 1CC [8,14]. Alcohol consumption challenges 1CC function at multiple levels. First, ethanol inhibits the expression of key enzymes in the pathway [15], reducing functional output of the 1CC. Second, dietary folates are often not ingested at recommended levels, folate adsorption and/or storage is decreased, and excretory output is increased [15]. Dietary supplementation with betaine or SAM has been proposed as a therapeutic intervention in fatty liver disease [15].

The development of NAFLD is complex and incompletely understood. Considered to be the hepatic lesion in metabolic syndrome, hepatic steatosis increases along side decreases in response to circulating insulin [15]. Several genome-wide association studies (GWAS) have also linked hepatosteatosis to 1CC or PC synthesis in patients with fatty liver [16]. First, a variant of PEMT was linked to fatty liver development in two GWAS studies [17,18], although a linkage was not found in the Dallas Heart Study [19]. Second, methylene tetrahydrofolate reductase (MTHFR) has also been associated with fatty liver [16]. Finally, mutations in the rate-limiting enzyme for PC synthesis from the Kennedy pathway (phosphate cytidylyltransferase 1, *PCYT1*) have also been identified in two patients with congenital lipodystrophy and fatty liver disease [20]. In an elegant study, the authors found that the mutated *PCYT1* alleles were expressed at low levels in patient-derived cells and that PC synthesis was reduced. Next, corresponding mutations were used in functional studies, where one allele was shown to be deficient in association with lipid droplet membranes [20]. While multiple metabolic pathways impact the full spectrum of phenotypes altered in fatty liver disease, there are clear links between 1CC and PC production that contribute to lipid accumulation in human liver.

1CC, Low PC, and Lipid Accumulation in Animal Models

Murine Models

Hepatic lipid accumulation is a common phenotype in mice with targeted deletions in enzymes acting in the 1CC and also in mice with lesions in methylation-independent PC synthetic enzymes. Hepatosteatosis also correlates with dietary limitation in the 1CC [13] and reduction in expression of 1CC genes [21]. For example, an early study by Rinella and Green showed that a methionine-choline-deficient (MCD) diet was associated with lipid accumulation in the liver and subsequent acquisition of inflammatory markers [22]. The MCD diet was also shown to exacerbate development of ALD in a porcine model [23]. Subsequently, Lu and colleagues published a seminal paper linking the 1CC and fatty liver. Targeted deletion of the gene encoding methionine adenosyltransferase 1A (*MAT1A)*, a SAM synthase expressed predominately in the liver, resulted in mice that accumulated fat in their livers that then progressed to nonalcoholic steatohepatitis (NASH) and then hepatocarcinoma [24]. This progression occurred more rapidly when the animals were fed a choline-deficient diet [24]. As would be expected for mice with deficiencies in SAM production, the PC:PE ratio was significantly reduced in these animals [25]. Mutations in other 1CC enzymes upstream of SAM production are associated with fatty liver. MTHFR deficiency increases lipid accumulation in murine livers [26]. Interestingly, fatty liver was reduced in mice fed betaine, a 1CC metabolite that can also serve as a source for SAM in mammals [26]. Deletion of the betaine-producing enzyme (betaine hydroxymethyl transferase; BHMT) also reduces SAM and PC, induces fatty liver and leads to hepatocellular carcinoma [27]. Glycine *N*-methyltransferase (GNMT) is a SAM-utilizing enzyme that may serve as a 'sink' for excess methyl donors [28]. Perturbing 1CC function through this pathway also induces steatosis [21], although in this case PC is increased and contributes to excess stored lipid [25]. Finally, excess dietary folate has also been recently shown to reduce SAM and PC levels along with hepatic steatosis through feedback inhibition of MTHFR activity [29]. Thus, interference with 1CC function upstream of SAM is tightly correlated with reduced PC levels and development of fatty liver disease.

If PC production was a nexus for driving hepatic lipid accumulation, fatty liver should also occur in models where its synthesis is directly inhibited. Indeed, interference with PC levels through

methylation-dependent or independent synthesis pathways results in hepatic lipid accumulation (reviewed in [11,30]). As mentioned above, PC is linked to the 1CC through the activity of PEMT, which uses SAM in three sequential methylation reactions to produce PC from PE [11]. Given that methyl-dependent PC production is predominant in the liver, whole-body *PEMT* knockouts can be used to study physiological roles in mice. However, phenotypes of the $PEMT^{-/-}$ mice were complex. First, synthesis through the Kennedy pathway compensated for PC production on a chow diet [12]. By contrast, challenge of these animals with a choline-deficient diet led to hepatic lipid accumulation and rapid liver failure [12].

PC synthesis through the Kennedy pathway is essential for viability, given that mice lacking the rate-limiting enzyme choline-phosphate cytidylyltransferase A (PCYT1a, also known as CCT/CTa) die early during embryonic development [31]. However, mice with tissue-specific deletions in PCYT1a also accumulate liver lipids. Jacobs *et al.* created a liver-specific knockout of PCYT1a and found that, although PEMT-dependent PC production increased twofold, PC decreased while triacylglycerols (TAGs) increased [31]. Thus, both direct blocks of PC synthesis or decreases in PC downstream of effects on SAM and 1CC function have a profound connection with lipid accumulation in mouse models of liver disease.

The 1CC may also be connected to lipid accumulation in adipocytes through alternative mechanisms. Kahn and colleagues identified changes in several metabolites linked to the 1CC in white adipose tissue (WAT) from obese mice. They found that utilization of SAM by the enzyme nicotinamide *N*-methyltransferase (NMNT) impacts polyamine metabolism. Synthesis of polyamines, which are important for energy metabolism, also consumes SAM and their levels fall as NMNT uses available methyl donors [32]. The authors showed that reduction of NMNT in WAT and hepatic tissue increased energy expenditure in obese mice as SAM levels increased and polyamine synthesis recovered [32].

Invertebrate Models

Interactions between the 1CC, PC, and lipogenesis have also been intensely studied in invertebrate models, where genetic screens or other unbiased tools have greatly contributed to our understanding. In a groundbreaking study, Guo *et al.* performed a small interfering (si) RNA screen in Drosophila S2 cells for modulators of lipid droplet formation, and classified results according to their size and morphology [33]. From this screen, PC biogenesis enzymes were identified as key players in the formation of large lipid droplets, along with genes with important roles in lipogenesis (*Drosophila melanogaster* SREBP) and those that were later shown to have important mechanistic roles in lipid droplet formation (ARF1/COP1 components) [33].

Caenorhabditis elegans has also proved to be an important model for understanding links between the 1CC and lipogenesis, although two aspects of the mammalian pathway are incompletely conserved. First, methylation steps producing PC occur earlier in the pathway, converting phosphoethanolamine to phosphocholine, as in plants [34]. Second, *C. elegans* appears to lack a BHMT ortholog, suggesting that choline can not be used as a methyl donor [35] and would only contribute to PC synthesis through the Kennedy pathway. However, blocking the 1CC through inactivation of the SAM synthase sams1 or interfering directly with PC production by RNAi of the phosphoethanolamine methyltransferase *pmt-1* or *pmt-2* have clear effects on lipogenesis, namely increasing visible lipid droplets and stored TAGs, which can be readily rescued when the Kennedy pathway is supported through dietary choline [36]. Interestingly, *C. elegans* must synthesize all the PC necessary for growth and reproduction, because the laboratory food source, *Escherichia coli*, lacks this membrane phospholipid [37]. The ability to use unbiased genetic screens in *Drosophila* and *C. elegans* has enhanced our understanding of mechanisms inducing lipid accumulation when the 1CC is dysfunctional.

Mechanisms of Low PC Action on Lipogenesis: PC Itself or Downstream Metabolites?

Metabolites are small molecules that are modified and assembled to build the components of the cell. Their levels may also influence the activity of the molecular machines that transcribe genes, respond to growth factors, or control intracellular transport [38]. However, it can be difficult to describe the molecular mechanisms tying an abundant metabolite to a specific cellular process. This question is critical, because understanding the underlying molecular mechanism is key to designing therapeutics. However, metabolites are not amenable to the traditional structure–function studies used to determine mechanistic relations between proteins. Furthermore, metabolites such as lipids have many different molecular species, depending on which side chains are added and how they are modified. Thus, determining which molecular processes are directly affected by metabolite levels and not a downstream or indirect consequence is difficult [39]. In the case of connections between the 1CC and lipogenesis, the two metabolites more closely correlated with hepatic lipid accumulation, SAM and PC, could both affect a variety of mechanisms driving lipogenesis (Figure 2, Key Figure). As the major methyl donor, SAM has the potential to change gene expression through histone or DNA methylation [38,40]. Many reports cite such alterations [7,14,15,41,42]. However, it is difficult to show which changes are sufficient to cause lipid accumulation, although histone or DNA modification could contribute to lipogenic phenotypes driven by other mechanisms. Blocking PC synthesis from the 1CC or independently though the Kennedy pathway both cause fatty liver, suggesting that low PC is sufficient to drive hepatic lipogenesis [10]. Nevertheless, whether it is PC itself or a downstream metabolite that exerts these effects, or which cellular pathways are impacted to drive lipogenesis, is incompletely understood.

As a structural component of cellular membranes, metabolic precursor to other lipids, a substrate for phospholipases, and a ligand for nuclear hormone receptors, PC levels could impact diverse cellular mechanisms [30]. Early studies suggested that reductions in dietary methionine or choline affected secretion of lipoprotein particles, such as VLDL [11]. Later murine models targeting PC production through the Kennedy pathway [31] or through methylation of PE [43] showed that VLDL secretion decreased in both cases, trapping lipoprotein particles loaded with TAG in the liver. The authors showed in a separate study that VLDL particles had abnormally low PC:PE ratios and observed degradation in a post-endoplasmic reticulum (ER) compartment [44]. However, it was not clear whether low PC effects were due to incomplete membrane formation in the lipoprotein particles, or whether changes in this phospholipid could also affect signaling components controlling secretory processes.

Recent invertebrate studies have provided additional mechanisms that could increase the potential for hepatic lipid storage when the 1CC or PC production is limited. Using *C. elegans*, it was recently shown that animals with a knockdown in *sams-1* contained large lipid droplets in the intestine [36] (which serves both endocrine and digestive functions) [45]. This increase in stored fat was driven by the transcription factor Sterol regulatory element binding protein (SBP-1/SREBP-1) [36], a master regulator of genes required for lipogenesis [46] and, importantly, this regulatory circuit was conserved in mammals [36]. The SREBP family of transcription factors can be regulated at the transcriptional levels by insulin signaling or Peroxisome proliferator-activated receptor gamma (PPARγ) regulation, activated by proteolytic processing in a feedback loop responding to low cholesterol, and modified by phosphorylation, acetylation, or ubiquitination during the transcription cycle [47]. Newly translated SREBPs are stored in the ER as intrinsic membrane proteins [47]. When cholesterol levels drop, chaperones escort SREBPs to the Golgi, where proteases release the N-terminal transcriptionally active domain [36]. Importantly, SREBP-1c overexpression is sufficient to induce fatty liver in murine models [48,49]. It was found that, when PC was low, mRNA levels of *sbp-1* (in *C. elegans*) or *Srebf1* (in mouse liver) did not increase; however, more of the active transcription factor entered the nucleus due to

Key Figure

Diagram Illustrating Pathways Affected by Low *S*-Adenosylmethionine (SAM) or Low Phosphatidylcholine (PC) that Could Lead to Fatty Liver

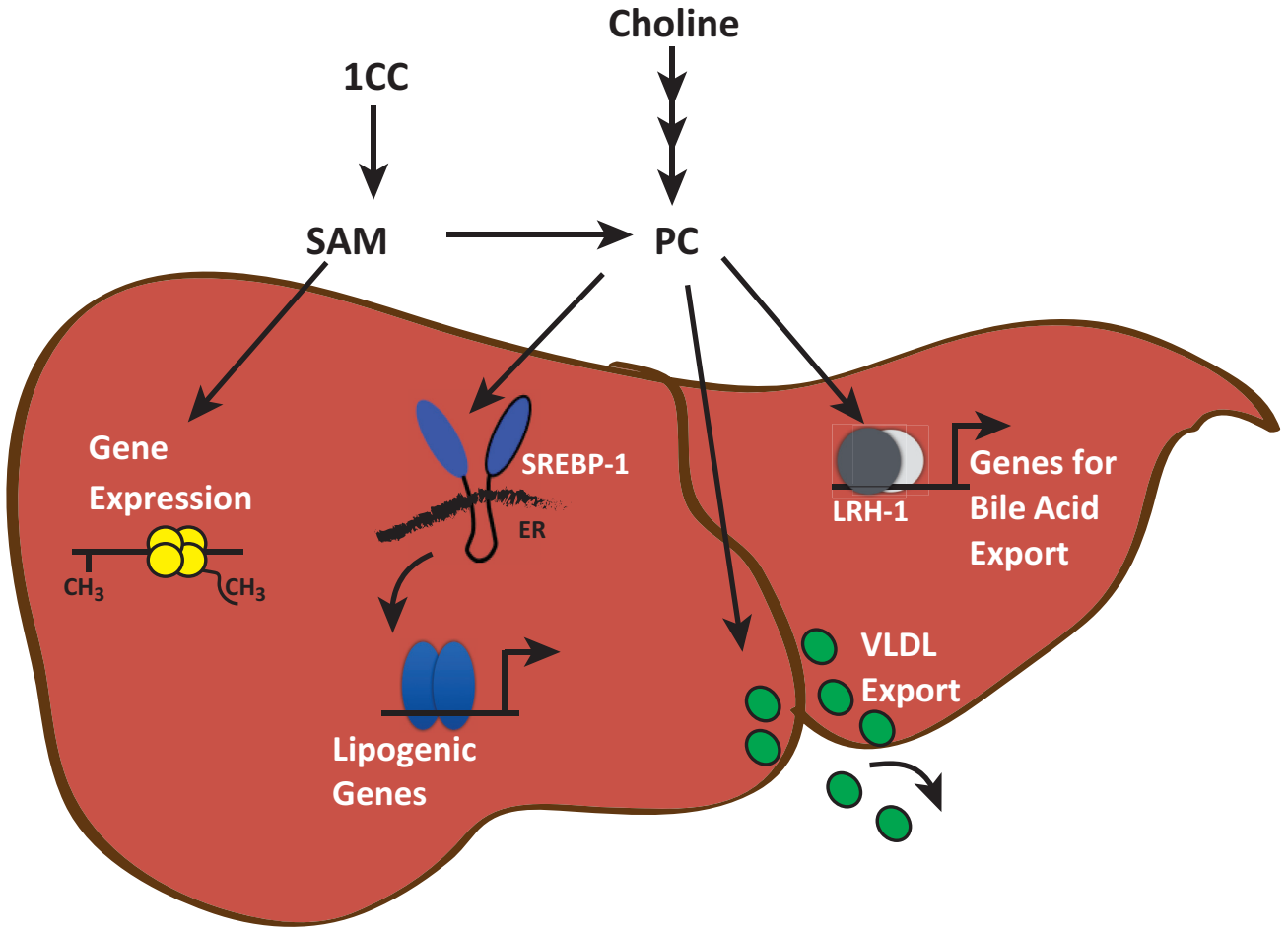

Figure 2. The 1-carbon cycle (1CC) has multiple links to lipid accumulation in the liver. SAM, the major methyl donor, can potentially directly affect gene expression if histone or DNA methylation patterns are sensitive to SAM levels. Furthermore, through changes PC levels, activation of sterol regulatory element binding protein 1 (SREBP-1)-dependent genes for lipogenesis or liver receptor homolog 1 (LHR-1)-dependent genes for bile export could impact lipid accumulation in the liver. Finally, lower levels of PC could also limit export of lipoprotein particles, increasing total hepatic lipid levels. Abbreviation: VLDL, very low-density lipoprotein.

increased proteolytic processing [36]. When the rate-limiting enzyme for PC production, PCYT1, was knocked down by RNAi in HepG2 cells, Golgi-resident proteins, such as the SREBP-activating proteases, assumed an ER-like pattern, allowing SREBP-1 maturation without transit to the Golgi [36]. While these studies uncovered important details of the mechanism, they did not reveal how the mislocalization of Golgi proteins occurred or what might link PC to this process.

Changes in PC levels in the ER or Golgi could have structural effects, altering membrane-bending properties, availability for synthesis of downstream lipids, or phospholipase-dependent signaling cascades. While it is difficult to directly target PC to identify mechanistic links, proteins

whose activity could be changed by membrane properties or PC availability can be analyzed. An RNAi screen in *C. elegans* identified factors that were necessary and sufficient for low PC activation of SBP-1/SREBP-1 and determined which of these candidates was also relevant to low PC processing in mammals [50]. Interestingly, it was found that depletion of Lipin 1 (*lpin-1*/LPIN-1) and *arf-1.2*/ARF1 was sufficient to activate SBP-1 in *C. elegans* or SREBP-1 in mammalian liver-derived cells. LPIN-1 is a phosphatidic acid phosphatase that produces the diacylglycerol (DAG) used in TAG and PC synthesis [51]. ADP-ribosylation factor 1 (ARF1) is a GTPase critical for Golgi–ER trafficking, whose activation and cycling require membrane association [52]. Guo *et al.* also identified *Drosophila* ARF in a S2 cell screen for lipid droplet modifiers [33], and subsequent work from the Walther and Farese labs found that ARF1 itself had an important role in lipid droplet formation [53]. Interestingly, in mammalian cells, the levels of active GTP-bound ARF1 were decreased in cells treated with siRNA to *LPIN1* or *PCYT1*, which may be due to decreases in association of the ARF-GEF, GBF1, to these membranes [50]. Supporting the idea that levels of multiple lipids in PC synthesis pathways were disrupted, microsomal membranes from *sams-1* or *lpin-1* (RNAi) animals were shown to have altered lipid profiles; showing a relative increase of some PA species to PC [50]. This suggests that the mechanistic effects of low PC occur upstream, because changes in PA, DAG, and PC limit ARF-GTPase cycling and disrupt COP-I transport. Thus, defects in 1CC function or PC production appear to change membrane properties, allowing for activation of SBP-1/SREBP-1. SBP-1/SREBP-1 activation is an important part of a lipogenic program that could contribute to lipid storage as fatty liver develops.

SREBPs are important regulators of lipogenic gene expression; however, other transcription factors also regulate lipogenic genes and may be affected by PC through distinct mechanisms. Nuclear hormone receptors, such as liver-receptor homolog 1 (LRH-1), are intracellular receptors that become active transcriptional regulators upon ligand binding [54]. NHRs may bind a variety of ligands; however, two NHRs that can act in the liver have been shown to bind specific PC species. PPARα has a well-established role as a regulator of fatty acid oxidation, lipid transport, and gluconeogenesis [55], and it is the target of therapeutics [56]. The Semenkovich lab used tandem mass spectrometry to identify endogenous ligands for PPARα in the mouse liver and found that a specific species of phosphatidylcholine (16:0/18:1 GPC) was associated with the active transcription factor [57]. Generation of this ligand required not only fatty acid synthase (FAS), but also the intact Kennedy pathway, demonstrating that PC synthesis was required. Given that impairment of PPARα reduces β-oxidation and shifts the cell toward stored energy [57], reduced levels of this ligand could also contribute to hepatic lipid storage.

LRH-1 is important for controlling lipid levels in the liver and has a complex relation with the 1CC. LRH-1 activates genes involved in bile acid transport, and targeted deletion results in fatty liver in mice [58]. Moore and colleagues determined that a PC moiety with two saturated fatty acid side chains [dilauroyl phosphatidylcholine (DLPC)] could serve as a ligand for LRH-1, allowing activation of genes promoting bile acid flux [59]. Interestingly, the gene encoding SREBP-1c along with its target genes in fatty acid biogenesis, were also decreased. To investigate the protective effects of this PC species, the authors administered DLPC to mice fed a high-fat diet and found that insulin sensitivity was improved, serum glucose levels fell, and hepatic lipids decreased [59]. Importantly, the authors showed that these effects did not occur in LRH-1-deficient mice. Structural studies in the Ortland lab have provided additional mechanistic insights into the relation between DLPC and LRH-1 by determining the structures of the PC-bound receptor [60]. The authors found that DLPC interacts with amino acids outside the classic ligand-binding pocket and, importantly, that ligand binding alters interactions between coactivators and corepressors. Many nuclear hormone receptors are partially unfolded in the unbound state, and ligand binding allows additional intramolecular interactions, thus stabilizing the active structure [61]. However, Musille *et al.* found that the phospholipid backbone bridges

the intramolecular interactions, stabilizing and activating the receptor [60]. Finally, the authors also found that interaction with coactivator peptides are favored by the ligand-bound receptor, but that a corepressor (SMRT) can directly interact with the unbound receptor [60]. The detailed molecular mechanism in this study provides a powerful example of how a phosphatidylcholine moiety could change the potential to store lipids in the liver by switching a nuclear hormone receptor from inactive to an active state.

A recent study showed that LRH-1 is also critical for the expression of 1CC genes, impacting the development of hepatosteatosis in mice fed a methyl donor-depleted diet [62]. Wagner *et al.* reported that LRH-1-deficient mice fed methionine-choline-deficient diets were protected from fatty liver and that decreases in SAM and PC occurring on these diets was blunted [62]. Furthermore, the authors found that LRH-1 was a direct transcriptional regulator of Gmnt1 [62], an enzyme that consumes excess methyl groups, and that LRH-1 binding sites were located in the promoters of many 1CC genes [63]. Thus, LRH-1 may be an important regulator of methyl donor levels in the liver. Lastly, the authors stated that the ligand for LRH-1, DLPC, is not likely to be synthesized through the 1CC–PEMT pathway [62], suggesting that a feedback loop is not likely in this instance. However, LHR-1 represents an intriguing link between levels of 1CC metabolites and lipid accumulation in the liver.

Concluding Remarks and Future Perspectives

The 1CC has many roles in the cell, contributing to nucleotide synthesis, redox protection, histone/DNA methylation, and phospholipid synthesis [8]. Changes in 1CC function are strongly associated with hepatic lipid accumulation [11]; however, an understanding of which 1CC metabolites affect this process and how they directly impact cellular mechanisms is only now emerging. Deciphering the molecular mechanisms driving the development of metabolic disease has the added challenge of determining how metabolites directly impact signaling and transcriptional mechanisms or organelle function. Studies examining 1CC function and lipogenesis have identified multiple mechanisms that may contribute to excess lipid storage in the liver when 1CC metabolites or PC are low, from control of nuclear hormone receptors essential for lipid export, to activation of transcription factors activating the program of *de novo* lipogenesis. It is critical to add more mechanistic details to these models, to understand other physiological effects of 1CC dysfunction, and to determine whether these mechanisms act in concert or are sufficient to cause liver disease on their own. In this way, we can begin to understand how hepatosteatosis develops and how to develop new tools to treat it.

Outstanding Questions

How do individual metabolites affect molecular mechanisms? In the case of lipids, such as PC, are primary effects through membrane structure or interaction of specific PC species with transcriptional regulators, or do effects occur as levels of lipids derived from PC are altered? In the case of SAM, what changes in methylation are tightly linked to SAM levels? Furthermore, which changes in histone or DNA methylation are sufficient to alter expression of lipogenic genes?

Which physiological effects of PC are linked to overall changes in this class of lipids, or large groups of species, and which are due to changes in a few or single species?

What are the processes that regulate 1CC production or PC levels? Are nutritional changes sufficient to alter SAM or PC levels in a physiologically relevant way? What environmental changes may also cause perturbation?

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