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Insulin Regulation of Hepatic Lipid Homeostasis

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Abstract

The incidence of obesity, insulin resistance, and type II diabetes (T2DM) continues to rise world-wide. The liver is a central insulin-responsive metabolic organ that governs whole-body metabolic homeostasis. Therefore, defining the mechanisms underlying insulin action in the liver is essential to our understanding of the pathogenesis of insulin resistance. During periods of fasting, the liver catabolizes fatty acids and stored glycogen to meet the metabolic demands of the body. In postprandial conditions, insulin signals to the liver to store excess nutrients into triglycerides, cholesterol, and glycogen. In insulin-resistant states, such as T2DM, hepatic insulin signaling continues to promote lipid synthesis but fails to suppress glucose production, leading to hypertriglyceridemia and hyperglycemia. Insulin resistance is associated with the development of metabolic disorders such as cardiovascular and kidney disease, atherosclerosis, stroke, and cancer. Of note, nonalcoholic fatty liver disease (NAFLD), a spectrum of diseases encompassing fatty liver, inflammation, fibrosis, and cirrhosis, is linked to abnormalities in insulin-mediated lipid metabolism. Therefore, understanding the role of insulin signaling under normal and pathologic states may provide insights into preventative and therapeutic opportunities for the treatment of metabolic diseases. Here, we provide a review of the field of hepatic insulin signaling and lipid regulation, including providing historical context, detailed molecular mechanisms, and address gaps in our understanding of hepatic lipid regulation and the derangements under insulin-resistant conditions.

Introduction

Glucose and lipid processing are under the control of hepatic insulin action. Past and current research continues to focus on the mechanisms of insulin signaling within metabolic tissues, such as the liver, to understand how these mechanisms are dysregulated in disease

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conditions. In response to nutrients, insulin is secreted from the pancreas and signals to the liver to control the synthesis and storage of fat molecules. Insulin controls postprandial liver metabolism by increasing *de novo* lipogenesis (DNL), suppressing fatty acid oxidation (FAO), and promoting triglyceride (TAG) esterification and secretion (173). During periods of fasting, DNL is suppressed, hepatic TAG is elevated due to increased uptake of circulating free fatty acids from the breakdown of fat tissue, and glycogen stores are broken down to produce glucose (42). These robust physiological responses to insulin are lost during the progression of metabolic disease. As first suggested by Howard Root, “such great variations in the amount of insulin required to reduce hyperglycemia are at times observed that the use of the term insulin resistance seems justified” (269). Following this seminal observation, Himsworth published findings indicating that there is a distinction between insulin-sensitive and insulin-insensitive people based on the lowering of blood glucose upon a bolus of insulin, or the lack thereof, respectively (128). Since the initial observations of insulin insensitivity, current research describes a paradox of hepatic insulin resistance in which insulin promotes DNL but fails to suppress glucose production. Taken together, the uncontrolled production and decreased utilization of substrates lead to the disease state’s hallmark hyperglycemia and hyperinsulinemia (324). Though numerous pathways are well-defined, the exact mechanisms through which insulin-mediated lipid synthesis is preserved yet hepatic gluconeogenesis is unrestrained remains a strong topic of interest for the field (41).

Metabolic disorders, including obesity and insulin resistance, continue to rise worldwide, with over 40% of the US population suffering from obesity, correlating with high percentage of individuals with diabetes and insulin resistance (133). Many patients with type II diabetes mellitus (T2DM) present with hyperinsulinemia, hyperglycemia, and hypertriglyceridemia. Notably, this condition correlates with a higher risk of developing other metabolic disorders, such as atherosclerosis, cardiovascular disease (CVD), and nonalcoholic fatty liver disease (NAFLD) (72). Deficiencies in insulin signaling, substrate utilization, hormone levels, and cytokine secretion, including contributions of increased inflammation, are all implicated in the pathogenesis of insulin resistance. Although the specific underlying mechanisms of NAFLD and NASH are unclear, patients with these diseases are insulin-resistant, revealing a clear association between liver diseases and insulin resistance (280).

Studies dating more than five decades ago observed a correlation between hyperinsulinemia and hypertriglyceridemia with carbohydrate intolerance, proposing the role of insulin in regulating lipids in addition to carbohydrates (87). In 1974, Reaven and coworkers suggested that insulin plays a major role in regulating the synthesis of TAG and its secretion from the liver (239). Following decades of research, insulin-mediated regulation of lipid metabolism remains a primary target for the development of effective therapeutics for NAFLD, of which an FDA-approved treatment does not currently exist. Patients with NAFLD have increased lipid accumulation in the liver, known as hepatic steatosis (51). In cases of T2DM and hyperinsulinemia, two major risk factors of NAFLD, there is a marked increase in TAG turnover, providing evidence that aberrant insulin levels dramatically affect lipid metabolism (309). Elevated hepatic DNL is a key feature of NAFLD patients, which correlates with an increase in circulating insulin levels (174, 297). Prominently, 25% to 30% of hepatic TAG is produced through DNL in individuals with NAFLD (77, 174, 297). Furthermore, human

loss-of-function mutations in the insulin receptor (IR) are severely insulin-resistant and lipodystrophic; however, these individuals are protected from fatty liver, further implicating that intact insulin signaling is required for hepatic lipid accumulation in humans (288). Therefore, it is critical to identify the underlying mechanisms of how insulin regulates hepatic lipid metabolism in a healthy liver for the development of therapeutic strategies for metabolic disorders. To fundamentally understand the detailed mechanisms of insulin's regulation of lipid homeostasis in humans, researchers continue to couple cell culture-based systems with transgenic mouse models to perform reductionistic experiments with the goal of identifying the signal transduction pathways underlying insulin action.

Hepatic Insulin Signaling Cascade

Insulin controls numerous metabolic pathways through coordinated regulation of gene expression and posttranslational modifications. Upon feeding, insulin is released from specialized cells in the pancreas called beta cells (169). Once insulin is released into the portal vein, the liver is the primary organ to respond as it receives the highest concentrations of insulin in circulation and serves an important role in regulating insulin clearance. Unsurprisingly, congenital deletion of the IR, in mice, leads to an early neonatal death due to diabetic ketoacidosis (5). Ronald Kahn and coworkers have generated unique mouse models to further define the tissue-specific actions of insulin *in vivo*. As a result, the liver insulin receptor knockout (LIRKO) mouse model serves as a valuable tool to understand the direct actions of hepatic insulin in a living organism. In support of insulin's key role in hepatic lipid and glucose metabolism, LIRKO mice develop severe insulin resistance and glucose intolerance, which is attributed to a failure of the liver to suppress glucose production (223). Interestingly, LIRKO mice are protected from hepatic TAG deposition and do not develop fatty liver disease when subjected to a high-fat diet (29). Similar experiments in mice lacking the hepatic IR selectively in adulthood further validate these findings (323). Therefore, hepatic insulin action is a central regulator of both systemic glucose and lipid homeostasis. Due to its key role in maintaining whole-body homeostasis, a major focus in the field has been to unravel the downstream signaling mechanisms coordinating liver insulin action and lipid metabolism.

In the liver, the insulin signal transduction cascade begins with hormone binding to the endogenous IR, a receptor tyrosine kinase that undergoes a conformational change leading to its auto-phosphorylation and the recruitment of the insulin receptor substrates (IRS) (Figure 1) (341). The IRS family of proteins functions as scaffolds for multiple signaling complexes. Though there are many members of the IRS family, IRS-1 and IRS-2 mediate the hepatic response to insulin action (341). Deletion of IRS-1 in mice results in mild glucose intolerance, providing evidence that IRS-1 has an important role in peripheral insulin action (19). The residual insulin action that occurs in IRS-1-null mice is attributed to the activation of the second IRS, IRS-2 (250, 315). Unlike IRS-1, disruption of IRS-2 protein in mice leads to striking changes in glucose homeostasis, providing evidence that IRS-1 and IRS-2 are not interchangeable, but play distinct roles in regulating insulin action (345). Deletion of both IRS-1 and IRS-2 in mouse livers renders the liver completely unresponsive to insulin action leading to hyperglycemia by 5 weeks of age (76). Moreover, liver-specific loss of IRS-1 and IRS-2 are glucose intolerant and fail to suppress hepatic

glucose production in response to insulin (320). Once phosphorylated, IRS proteins recruit and activate another class of proteins critical to insulin action, phosphatidylinositol-3-kinase (PI3K), which subsequently phosphorylates phosphatidylinositol-4,5-biphosphate (PIP_2) to generate phosphatidylinositol-3,4,5-triphosphate (PIP_3) (93, 106). The generation of these second messenger phosphoinositols is necessary for the execution of further downstream signaling, thus PI3K has a key role in mediating insulin action in all tissues including the liver.

There are three classes of PI3K, classified by the substrates they phosphorylate, in which class I is divided into class I_A and class I_B (46). Class I_A PI3K is activated via receptor tyrosine kinases, such as insulin and insulin-like growth factor (IGF) stimulation, while class I_B kinases are activated through G-protein coupled receptors (83). Insulin regulates class I_A kinases, which consist of two subunits: p85 regulatory subunit and p110 catalytic subunit (271). The p85α unit positively regulates the phosphatase and tensin homolog (PTEN), an antagonist of the PI3K pathway (319). PTEN facilitates the dephosphorylation of PIP_3 to promote the conversion to PIP_2, thereby decreasing both the PIP_3 pool and AKT phosphorylation (318). Consistent with having a key role in liver insulin action, hepatocyte-specific loss of PTEN results in hepatic steatosis, indicating that PIP_3 generation, downstream of insulin, promotes lipogenesis (130). Accordingly, PTEN deficiency improved glucose tolerance in mice, confirming its role in glucose metabolism in addition to regulating lipid metabolism (130). Mice lacking p110α specifically in hepatocytes have impaired insulin-stimulated AKT phosphorylation and are resistant to diet-induced steatosis, revealing the importance of PI3K catalytic subunit for insulin action (53, 300). Additionally, there are four isoforms of PI3K: PI3Kα, PI3Kβ, PI3Kδ, and PI3Kγ (163), though most insulin-responsive actions were found to be mediated through PI3Kα and PI3Kβ redundant activities (228).

Upon PIP_3 generation, 3-phosphoinositide-dependent protein kinase-1 (PDK1) is recruited leading to the phosphorylation of AKT, otherwise known as protein kinase B (PKB), specifically at the Thr308 site (318). AKT is a central regulator for many cellular processes including cell growth, survival, lipid synthesis, and glucose production. AKT serves as an important node in the insulin signaling pathway, regulating a wide range of processes such as glucose uptake, protein synthesis, and lipid metabolism (207). AKT phosphorylation is often regarded as a molecular sensor for insulin stimulation. Although both IRS are required for insulin-stimulated PDK1 phosphorylation of AKT1 at Thr308/Thr309 (AKT2), phosphorylation of both Thr308/Thr309 and Ser473 (AKT1)/Ser474 (AKT2) is required for maximal activity of AKT (Figure 1) (13, 76). While PDK1 phosphorylates AKT at Thr308/Thr309, phosphorylation of Ser473/Ser474 is facilitated primarily by the mechanistic target of rapamycin complex 2 (mTORC2) (Figure 1) (282). The Ser473/Ser474 phosphorylation stabilizes Thr308/Thr309 phosphorylation, leading to the activation of AKT kinase ability (351).

There are three main isoforms of AKT, from three different genes, AKT 1-3. Mice bearing a whole-body deletion of AKT1 are growth retarded and have shorter lifespans, but these mice do not develop a diabetic phenotype (56). Additionally, AKT1-deficient mice respond normally to glucose and insulin, suggesting AKT1 is dispensable for glucose homeostasis,

but critical for development and growth (59). Loss of AKT3 in mice does not affect carbohydrate metabolism, but these mice present with smaller brain sizes than control mice and other AKT isoform knockout models (79). This is consistent with the finding that AKT3 is expressed most significantly in the brain and testes (352). However, mice deficient of AKT2 have a significantly impaired ability to lower blood glucose in response to insulin and display a diabetic-like phenotype (58). This is consistent with human mutations reported in the *AKT2* gene that result in severe insulin resistance and T2DM (100). Along these lines, AKT2 inhibition in PTEN-deficient mice leads to the development of glucose intolerance and hyperglycemia, indicating the importance of AKT2 activity downstream of PTEN and PDK1 (123). Furthermore, deletion of AKT1&2 from mouse liver results in a dramatic reduction in DNL as measured by gene expression and newly synthesized palmitate, providing genetic evidence for its central role in DNL regulation (325).

Downstream of AKT lies the forkhead box protein O, FOXO, and transcription factors that are key effectors of hepatic glucose and lipid regulation (Figure 1) (43). There are three main hepatic isoforms of the FOXO transcription factors (FOXO1, FOXO3, FOXO4), and all three are implicated in regulating cell growth and insulin-stimulated glucose and lipid metabolism (118, 132). FOXO transcription factors are regulated by phosphorylation events, which induce FOXO nuclear exclusion thus inhibiting transcriptional activity (4). Expression of the FOXO1 isoform is dysregulated under conditions of insulin resistance, implicating its key role in the pathogenesis of insulin resistance (16). AKT mediates the phosphorylation and inhibition of FOXO1 at Thr32, Ser253, and Ser3125 to induce FOXO1 association with 14-3-3 proteins and inactivation via exclusion from the nucleus (43). FOXO1 activation promotes hyperglycemia in mice, emphasizing a role for FOXO1 activity in regulating glucose homeostasis (76). In IRS-deficient mice, hyperglycemia is observed and attributed to the loss of AKT phosphorylation and unrestrained activity of FOXO1 (76). To define the consequences of constitutive nuclear localization and activation of FOXO1, the Accili group developed a series of FOXO1 mutants, which are unable to be posttranslationally modified at key serine, threonine, and lysine residues, preventing its exclusion from the nucleus in response to AKT activation (231, 262). A constitutively active FOXO1 mutation induces hypertriglyceridemia in mouse models, highlighting the additional significance of the AKT-FOXO1 axis in maintaining lipid homeostasis under normal physiological conditions (16). In mice, concomitant deletion of FOXO1 in an AKT-deficient liver is not sufficient to drive DNL but interestingly completely restores insulin sensitivity and glucose tolerance, suggesting there are other pathways downstream of AKT that contribute to DNL (323).

In addition to the AKT-FOXO pathway, the AKT-TSC-mTORC1 (mechanistic target of rapamycin complex 1) pathway is an essential axis in maintaining lipid homeostasis (Figure 1) (207). mTORC1 is a nutrient-sensing, serine/threonine kinase complex that regulates anabolic processes, such as promoting lipid synthesis, protein synthesis, and cell growth (37, 275, 276). mTORC1 has three main components: mTOR, mLST8, and Raptor, which distinguishes it from complex 2 that contains the Rictor protein (mTORC2) (74). mTORC1 is activated by nutrients and amino acids via Rag proteins (157). However, insulin-stimulated activation of mTORC1 requires GTP-bound Rheb, which is tightly regulated by the tuberous sclerosis complex (TSC) (279, 350). Structural studies reveal

that lysosomal docking elements are required for mTORC1 activation (267). As such, TSC localizes to the lysosome in nutrient-depleted conditions, preventing mTORC1's lysosomal localization and activation by Rheb, defining TSC as the negative regulator of mTORC1 (220). In response to insulin stimulation, AKT phosphorylates tuberin (TSC 2), allowing its dissociation from the lysosome and for Rheb activation of mTORC1 (206). Therefore, insulin signaling promotes mTORC1 activation via inhibition of TSC and subsequent activation of Rheb.

In addition, a key homeostatic feedback loop exists between mTORC1 and proximal insulin signaling. Constitutive mTORC1 activation dampens phosphorylation of AKT, whereas inhibition of mTORC1 using rapamycin, the well-known mTORC1 inhibitor, restores AKT phosphorylation and downstream signaling (Figure 1) (205). Rapamycin was discovered several decades ago from the soils of Easter Island (336). Rapamycin interacts with FK506 binding protein 12 kDa (FKBP12) that binds and allosterically inhibits mTORC1, and not mTORC2 (253, 281). Although mTORC1 activity is sensitive to rapamycin, mTORC2 kinase activity is not acutely affected by rapamycin treatment. Prolonged treatment with high concentrations of rapamycin can affect mTORC2 activity and subsequently, AKT phosphorylation (283). Chronic treatment of rapamycin leads to insulin resistance and glucose intolerance, of which are mediated by mTORC2 (175). However, not all mTORC1 targets are sensitive to rapamycin, but most are sensitive to active site mTOR inhibitors like Torin1 (135).

mTORC1 is required but not sufficient for the induction of DNL, a topic fully discussed later in this article (189, 325, 338, 354). In addition to its role in lipid metabolism, mTORC1 directly promotes anabolic processes, such as protein synthesis, through the phosphorylation of the eukaryotic translation initiation factor 4E (eIF4E)-binding protein 1 (4EBP1) to promote cap-dependent translation (45). mTORC1 also coordinates translation through its phosphorylation and activation of ribosomal S6 kinase 1 (S6K1), which phosphorylates ribosomal S6 protein, machinery required for protein translation (202). Phosphorylation status of S6K and S6 protein are regularly used as direct read-outs of mTORC1 activity. Interestingly, inhibition of S6 kinase does not block the induction of lipogenic program in response to insulin, suggesting that there are other pathways downstream of mTORC1 that regulate DNL gene programming (189). Not only does mTORC1 promote cell proliferation through protein translation mechanisms, but it also negatively regulates catabolism through inhibition of autophagy and ketogenesis (176, 289). Autophagy promotes the degradation and recycling of cellular machinery, which is inhibited by mTORC1-dependent phosphorylation of ULK1 complex (99). Likewise, ketogenesis is essential for the generation of substrates, including acetyl CoA under fasting and nutrient-limited conditions, a process stimulated upon mTORC1 inhibition (63, 289).

Insulin Induces Hepatic *De Novo* Lipogenesis

Insulin regulates hepatic lipid metabolism by balancing lipid synthesis with breakdown of hepatic TAG stores (Figure 2). Lipogenesis is decreased in LIRKO mice in response to high carbohydrate diet refeeding, indicating the requirement of insulin for hepatic lipid synthesis (116). Genetic deletion of AKT from mouse liver results in a dramatic reduction

in postprandial DNL, demonstrating a requirement of AKT for insulin-stimulated induction of DNL (325). Moreover, ectopic activation of AKT in cells causes an increase in cell size due to increased glucose uptake and accumulation of fatty acids (258). Along these lines, adenoviral delivery of a constitutively active myristoylated AKT (myr-AKT) is sufficient to stimulate DNL in the absence of insulin stimulation (354). Therefore, AKT serves as an important nexus point for hepatic insulin signaling and the control of liver lipid metabolism, as it is required and sufficient for the stimulation of DNL. In the next section, we discuss the lipogenic genes and their regulation by insulin signaling.

Hepatic lipogenic gene program

Sterol regulatory element binding proteins (SREBPs) are a family of membrane-bound proteins that regulate transcription of genes involved in cholesterol uptake and lipid synthesis (Figure 3) (278). SREBPs are activated by binding of the SREBP cleavage-activating protein (SCAP), causing a conformational change in SCAP to initiate site-1 cleavage of SREBP, which allows their translocation to the nucleus (Figure 4) (36, 40, 278). Once in the nucleus, SREBPs bind to sterol regulatory elements and activate the transcription of genes promoting cholesterol uptake and fatty acid synthesis (Figure 3) (278). There are three isoforms of SREBP: SREBP-1a, SREBP-1c, and SREBP2. In the liver, SREBP-1c is the predominant isoform, where SREBP-1a and SREBP2 are expressed in lower amounts. SREBP-1a is a splice variant of SREBP-1 and has a minor role in activating transcription of cholesterol and lipogenic genes (292). On the other hand, SREBP-1c is required for lipid synthesis, as observed in dominant-negative studies in cultured hepatocytes (95). Both SREBP-1a and SREBP-1c are repressed in fasting states and induced by refeeding (221). Notably, deletion of liver IRs abolishes the feeding-induced expression of *Srebp-1a* and *Srebp-1c* (gene name *Srebf1*) (221). Furthermore, LIRKO mice fail to accumulate nuclear SREPBP-1c, suggesting the necessity of insulin for SREBP-1c activation and subsequent initiation of lipogenesis (116). Thereby, upon refeeding, insulin stimulates the transcription of SREBP-1c to stimulate DNL, while glucagon suppresses its expression (95).

Many studies described below utilize the leptin-deficient mouse model, termed *ob/ob* mouse ($Lep^{ob/ob}$) (78). These mice lack the gene encoding leptin, a neuroendocrine starvation signal (9, 92). These mice are often applied for studies examining the effects of insulin resistance, obesity, and fatty liver disease. Whole-body loss of SREBP-1 prevents hepatic steatosis in $Lep^{ob/ob}$ mice indicating that SREBP-1 proteins are required for TAG accumulation in the liver (349). In primary rat hepatocytes, Foretz et al. found that insulin stimulation enhances the transcription of SREBP-1c (95). Insulin increases the posttranslational processing of SREBP-1c through mTORC1 via S6K (245). Moreover, primary rat hepatocytes from streptozotocin (STZ)-treated, type 1 diabetic rats, have reduced basal transcription of lipogenic genes (293). Insulin stimulation of the isolated STZ-treated rat hepatocytes results in an increase in *Srebp-1c* mRNA, selectively, consistent with the notion that insulin stimulates lipid synthesis in the liver (293). Furthermore, gene expression and protein expression of active SREBP-1c is significantly downregulated in LIRKO mice, further suggesting insulin is the main regulator of SREBP-1c protein activation (29, 116).

SREBP-cleavage activating protein (SCAP) is the enzyme responsible for cleavage of SREBP-1 and SRBEP-2, releasing them from the membrane of the Golgi, and allowing for their nuclear translocation (Figure 4) (136). Indeed, loss of SCAP in the liver results in an 80% decrease in fatty acid synthesis, along with a reduction in cholesterol synthesis (216). Furthermore, SCAP is a sterol sensor, such that sterols suppress SCAP-mediated activation of SREBPs (110). A D443N mutation in SCAP renders it resistant to sterol suppression leading to constitutive SREBP cleavage and activation, revealing a mechanism in which SCAP and SREBPs are regulated by sterol (136, 166).

Insig proteins are important regulators of lipid synthesis. Insig-1 and Insig-2 can bind SCAP, preventing the SCAP-dependent cleavage of SREBPs and its subsequent translocation to the nucleus (Figure 4) (347). Insig-1 expression is regulated by SREBPs, whereas Insig-2 is not (347). It is well-known that insulin stimulates processing of SREBP-1c, and this is associated with a downregulation of Insig-2a transcription (348). However, knockdown of Insig-2 in LIRKO mice only modestly increases nuclear SREBP-1c, suggesting that insulin regulates SREBP-1c processing through additional mechanisms other than Insig-2 transcriptional regulation (116). Posttranscriptionally, insulin additionally regulates SREBP-1c in part through the depletion of Insig-2a protein, via decay of *Insig2a* mRNA (355). Downstream of insulin, inhibition of hepatic TSC, which leads to constitutive activation of mTORC1, results in increased expression of *Insig2a* and protein, but reduced expression of *Insig1* (354). siRNA-mediated knockdown of *Insig2a* restores *Srebp1c* expression in L-TSC-KO hepatocytes (354). Furthermore, ectopic activation of AKT, via myr-AKT expression, in TSC-deficient hepatocytes rescues the suppression of *Insig2a* (354). Therefore, AKT signaling is required for suppression of *Insig2a*.

Liver X receptors (LXR) are nuclear receptors that are highly expressed in the liver and have an important role in maintaining lipid homeostasis (343). LXR agonists, such as dietary cholesterol as well as synthetic agonists, increases mRNA expression of *Srebp1c* via an LXR-binding site (266). Although activation leads to upregulation of *Srebp1c* transcription, LXR activation alone does not sufficiently lead to cleavage of SREBP-1c protein (124). Although, activation of SREBP-1c occurs upon acute insulin stimulation, LXR activation fails to rescue lipogenesis in LIRKO mice, consistent with other studies that indicate insulin is required for activation of SREBP-1c (29, 124). Tian et al. uncovered the mechanism in which LXRα and C/EBPβ form a complex that binds to *Srebp1c* promoter region to induce transcription upon insulin stimulation (322). Furthermore, deletion of both LXR isoforms (α and β) in mice impairs induction of SREBP-1c lipogenic targets by 75%, thus LXR activation has a key role in activation of lipogenesis (266).

Carbohydrate-responsive element-binding protein (ChREBP) is a glucose metabolizing and lipid synthesis promoting transcription factor that responds to changes in carbohydrate levels. In particular, ChREBP contains a low-glucose inhibitory domain (LID) that renders it inactive when glucose concentrations are low (330). ChREBP induction occurs independently of insulin, as determined by unchanged *Chrebp* (gene name *Mlxipl*) mRNA expression in LIRKO mice (116). However, ChREBP activation nuclear translocation are blunted in LIRKO mice, likely due to the decreased expression of glucokinase and reduced generation of glucose-6-phosphate in the absence of insulin signaling (116). ChREBP-

deficient mice have decreased lipogenesis as well as reduced expression of ACC, ACLY, and FASN, but unchanged levels of SREBP-1c, suggesting that ChREBP and SREBP proteins are both required for the regulation of lipid synthesis genes (137). In *ob/ob* mice, ChREBP mRNA and nuclear protein are upregulated (71). *Ob/ob* mice lacking ChREBP, via adenovirus-mediated shRNA or congenital deletion, have improved glucose tolerance and improved hepatic steatosis due to decreased hepatic fatty acid synthesis and plasma TAG, as compared to *ob*/+ controls (71, 138). Overall, ChREBP is an essential transcription factor coordinating the regulation of lipid synthesis in response to carbohydrates. Together, these transcription factors coordinate DNL gene expression to respond to the liver's metabolic demands.

Lipid synthesis requires the production of acetyl-CoA, the main substrate composing lipids (Figure 3). Therefore, an important regulator of DNL is the ATP-citrate lyase (ACLY) enzyme, which catalyzes the synthesis of acetyl-CoA and oxaloacetate from citrate transported into the cytosol from the TCA cycle in the mitochondria (Figure 3) (304). Following feeding, *A*cly transcription is stimulated (82, 96). Additionally, the ACLY promoter contains a sterol response element (SRE), indicating its transcription is regulated by SREBP-1 (Figure 3) (284). Posttranscriptionally, ACLY enzymatic activity is regulated via phosphorylation, specifically, phosphorylation at Ser454 activates the enzyme (259). mTORC2-AKT signaling is recognized as the primary pathway for the phosphorylation of ACLY at Ser454 in primary rat hepatocytes and adipocytes (14, 28). Additionally, acetylation is suggested to promote ACLY protein stability, and this modification is implicated in NAFLD. ACLY acetylation at Lys540, Lys546, and Lys554 are detected at higher levels in NAFLD, promoting ACLY stability and DNL (114). Whole-body knockout of *Acly* is embryonically lethal, revealing that ACLY-dependent lipid synthesis is essential for development (26). Deletion of liver ACLY in genetically obese mice improves hepatic steatosis via inhibition of DNL (340). Cultured ACLY-deficient mouse embryonic fibroblasts have impaired viability due to a lack of acetyl-CoA pools, a phenotype that is rescued by acetate supplementation to replenish acetyl-CoA (361). The significance of ACLY in DNL and glucose metabolism makes the enzyme an attractive target for reducing lipid synthesis. Thus, many groups aimed to identify potential inhibitors of ACLY. Hydroxycitrate is a competitive inhibitor of ACLY that is known to inhibit enzymatic activity and suppress DNL *in vivo* (314). Bempedoic acid directly inhibits ACLY to block fatty acid synthesis, ultimately leading to the upregulation of low-density lipoprotein (LDL) receptors, decreasing circulating LDL-cholesterol, and attenuating atherosclerosis (255, 256). Recently, the FDA approved the ACLY inhibitor, bempedoic acid (Nexetol), for the treatment of heterozygous familial hypercholesterolemia (FH) and atherosclerotic CVD (210).

Following the production of acetyl-CoA from citrate, acetyl coenzyme A carboxylase (ACC) is the first major enzyme involved in lipid synthesis (101). ACC catalyzes the carboxylation of acetyl-CoA to generate malonyl-CoA that is shuttled within the mitochondria to FASN for further processing (159). In the liver, insulin and refeeding promote the transcription of ACC (gene name *Acaca*) (151). Two isozymes of ACC are present in mammals, ACC1, which is present in the cytosol, and ACC2, which is located at the mitochondrial membrane (101, 102). The production of malonyl-CoA generated by either ACC1 or ACC2

has distinct roles, respective of the compartments they are produced in. There are two isoforms of cytosolic ACC1, ACC-α, and ACC-β, where ACC-α is the rate-limiting enzyme in long-chain fatty acid synthesis (121). ACC-α is regulated posttranslationally, being activated through a series of phosphorylation events (159). Whole-body deletion of ACC1 in mice is embryonically lethal, providing evidence for the importance of ACC1-derived malonyl-CoA for embryonic development (3, 121). Multiple groups have studied the effects of liver-specific deletion of ACC1 (L-ACC1-KO). Some report that L-ACC1-KO has no significant effect on hepatic DNL, which is attributed to the compensatory increase of ACC2 mRNA and protein expression (121), while other groups report L-ACC1-KO decreases DNL and is protective of hepatic steatosis (208). ACC2 is the mitochondria-anchored isozyme of the ACC enzyme, which regulates the production of malonyl-CoA at the mitochondrial membrane, potently regulating the influx of fatty acids into the mitochondria for β-oxidation (3). It is well-known that malonyl-CoA is a negative regulator of FAO, due to the allosteric inhibition of the carnitine palmitoyltransferase 1 (CPT1) shuttle that regulates the transferring of long-chain acyl-CoAs into the mitochondria for oxidation (218). Deletion of ACC2 in mice results in fewer hepatic TAG accumulation and improved glucose metabolism due to the unrestrained FAO in the mitochondria (3, 299). On the other hand, a study from Olson et al. detected very little changes in lipid metabolism following ACC2 deletion (240). Based on the role of ACC and its product, malonyl-CoA, as a critical regulator balancing synthesis and breakdown of lipids, many pharmaceutical companies target ACC1 and ACC2 for the treatment of NAFLD. Pharmacological inhibition of both hepatic ACC1 and ACC2 lowered hepatic TAG by inhibiting DNL and increasing FAO but ultimately led to hypertriglyceridemia from increased VLDL-TAG export and SREBP-1c activation (156, 299).

Fatty acid synthase (FASN) is the second enzyme in the pathway contributing to lipogenesis, thereby it catalyzes the production of palmitate from acetyl-CoA and malonyl-CoA (337). Insulin stimulates transcription of *Fasn* in a PI3K-AKT-dependent manner (339). Deletion of FASN in the liver reduces DNL and increases malonyl-CoA pools in the cytosol, due to the lack of flux through palmitate synthesis (50). As another key enzyme in DNL, inhibitors of FASN are currently being tested for their therapeutic efficacy in reversing hepatic steatosis. A selective and reversible FASN inhibitor, TVB-2640, has shown strong potential for reducing DNL in human studies, with minor hair loss side effects (317).

Stearoyl-CoA desaturase 1 (SCD1) is the membrane-bound rate-limiting enzyme in the conversion of saturated fatty acids to monounsaturated fatty acyl-CoAs, which is used in the production of TAG, amongst other fats (115). Upon refeeding of a high carbohydrate diet, *Scd1* expression is substantially increased in mice, suggesting nutrients may regulate expression of the desaturase (147, 235). Additionally, insulin stimulates *Scd1* mRNA expression through the interaction of an insulin-responsive element and this mechanism is dependent on PI3K and mTORC1 activation (217). Correlating with decreased SREBP-1c, expression of lipogenic genes is decreased in LIRKO mice, such as *Scd1* and *Fasn* (29). Loss of SCD1 activity enhances liver AKT phosphorylation and improves glucose tolerance in diet-induced insulin resistance (115).

Interplay of carbohydrates and lipid synthesis

One of the main roles of the liver is to maintain consistent blood glucose levels between fluctuating periods of nutrient deprivation and abundance. Since dietary sugar intake correlates with lipogenesis in adults; it is important to understand the metabolic link between carbohydrate metabolism and lipogenesis (Figure 3) (248). Glucokinase (GCK) is the predominant glucose sensor in the liver and is activated when blood glucose rises, driving an increase in glucose utilization. GCK is a hexokinase in the liver that catalyzes the phosphorylation of glucose to generate glucose-6-phosphate (G6P), either to be consumed for energy or stored as glycogen or TAGs (213). Hepatic GCK activity is tightly regulated to the nutritional status of the liver, particularly regarding glucose homeostasis. First, *Gck* transcription requires stimulation by insulin, denoting an increase in blood glucose levels (142). Second, glucose induces a structural shift in GCK into a more active conformation, making the protein more active as glucose concentrations increase (126). Lastly, when glucose concentrations are low (e.g., during extended fasting), GCK is sequestered in the nucleus by the liver-specific protein, glucokinase regulatory protein (GKRP) (332). GKRP inactivates GCK, allowing the liver to shift from glucose uptake to hepatic glucose production to meet the systemic metabolic demands of the body (73). When glucose levels rise, this pool of GCK can be quickly released back into the cytoplasm, allowing the liver to switch swiftly back to glucose uptake. Along with glucose, other carbohydrates, such as fructose-1-phosphate (F1P), break the GCK-GKRP interaction, signaling to the liver that a mixed carbohydrate meal has been consumed (73, 334). Fructose is processed in cells by ketohexokinase (KHK) to generate F1P (125). Whereas, when glucose is processed through glycolysis, fructose-6-phosphate (F6P) is generated, and F6P interacts with GKRP, promoting its association with GCK, and inhibiting GCK kinase activity (333). Therefore, F6P presence acts as an inhibitor of GCK activity, but the generation of F1P promotes its activation. Thereby, fructose feeding potently activates lipogenic pathways, such that fructose feeding partially rescues hepatic lipogenesis in the absence of insulin signaling in the liver (116). Together, these regulatory mechanisms help ensure that the liver can respond quickly and efficiently to the body's constantly fluctuating blood glucose levels.

Insulin activates hepatic *Gck* transcription, via AKT signaling (141, 197, 242). Deletion of AKT1 and AKT2 in the liver eliminates liver GCK mRNA and protein expression in mice (197). Conversely, mice expressing the constitutively active myr-AKT display a marked increase in *Gck* gene expression (242). AKT promotes *Gck* expression in part via suppression of FOXO, a negative regulator of *Gck*. Hepatocyte-specific deletion of FOXO1 in AKT-deficient mice partially restores GCK expression, glycemia, and insulin sensitivity (323, 325). Similarly, loss of the three FOXO isoforms expressed in the liver FOXO1, 3, and 4) blocked the decrease in GCK expression during fasting (118). GCK re-expression in AKT-deficient livers improves glycemia and increases insulin sensitivity (325). Therefore, *Gck* expression is regulated by AKT in part via FOXO-dependent mechanisms.

SREBP-1c is commonly considered to be a major regulator of hepatic *Gck* transcription. Expression of a dominant negative form of SREBP-1c blocked insulin-stimulated *Gck* expression in rat primary hepatocytes, whereas expression of an active form of SREBP-1c increased expression of *Gck* independently of insulin stimulation (94). However, other

studies demonstrate that SREBP-1c inhibition in mice is not sufficient to suppress insulin-mediated or AKT-mediated induction of *Gck*, suggesting SREPB-1c alone is not sufficient for *Gck* expression (193, 242). Additional data demonstrate that several other factors, such as ChREBP, LXR, hypoxia-induced factor 1 (HIF1), hepatic nuclear factor 4 (HNF4), and peroxisome proliferator-activated receptor gamma (PPAR-γ) also have roles in the insulin-dependent induction of GCK (161, 270).

The significant role of GCK in maintaining hepatic glucose homeostasis has made it an appealing target for pharmaceutical companies to develop drugs to lower blood glucose levels in diabetic patients (214). However, because of the role of GCK in other tissues, namely its role as the glucose sensor controlling insulin secretion in the pancreatic β-cell, global activation of GCK leads to hyperinsulinemia, resulting in potentially severe hypoglycemia (232). As such, companies have aimed to develop liver-specific GCK activators to promote glucose uptake while minimizing the effects on insulin secretion from the β-cell (20, 25). Many of the liver-specific GCK activators improved blood glucose levels; however, the majority have failed clinical trials due to adverse side effects, which typically include increased blood lipid levels and/or fatty liver, suggesting that prolonged and chronic activation of hepatic GCK adversely affects hepatic lipid metabolism (69, 84). These findings are complemented by the identification of several single-nucleotide polymorphisms (SNP) in GKRP, the protein that inhibits and sequesters GCK during fasting, that strongly associate with lower fasting glucose and insulin resistance coupled with increased blood and liver TAGs (243, 296). Together, these data suggest that GCK activity promotes lipogenesis in the liver and that inappropriate timing of GCK activation may lead to increased lipid synthesis.

The mechanisms by which glucose and GCK contribute to lipid metabolism are still ill-defined. The predominant mechanism is presumed to be through controlling glycolytic flux of G6P into acetyl-CoA, the building block of fatty acids in DNL (214, 236, 252). However, recent liver-specific GCK activators have challenged this hypothesis, as they are capable of decreasing blood glucose without such an effect on hepatic lipid metabolism (335). This suggests that increased hepatic glucose flux *per se*, due to activated GCK, is not sufficient to drive hepatic lipid metabolism. In addition to metabolic flux, glucose can play other roles in the regulation of DNL (196, 335). Like SREBP, ChREBP promotes the expression of many genes involved in DNL, as previously described. ChREBP is activated by G6P and migrates to the nucleus where it activates the lipogenic gene program. Mice with ChREBP deleted in the liver show reduced glycolytic and lipogenic gene expression following a high-carbohydrate diet (196). Further, restoring SREBP-1c signaling in a liver-specific ChREBP knockout mouse partially restored expression of lipogenic gene expression without affecting glycolytic gene expression. Conversely, when SREBP is inactivated by deletion of SCAP, ChREBP is insufficient to restore lipogenic gene expression (196). Altogether, these data indicate that, *in vivo*, SREBP is sufficient to regulate lipogenesis while ChREBP is not. Therefore, even though it is known that glucose can regulate DNL via activation of ChREBP, whether this activity is responsible for the increased TAG levels observed when GCK is inappropriately activated is still to be determined.

Another mechanism by which glucose has been proposed to regulate lipogenesis is through LXR modulation. Glucose and G6P are proposed to be direct activators of LXR, which in turn would lead to the activation of SREBP-1c to promote the expression of lipid and cholesterol synthesis genes (226). However, these data are not consistent with LXR ligand binding favoring small hydrophobic compounds (177, 226). Moreover, this study is controversial as it was not able to be reproduced by others (70, 113). In a separate study, Grønning-Wang's group proposed a glucose-mediated posttranslational regulation of LXR (17). In response to glucose, LXRs undergo *O*-linked β-*N*-acetylglucosamine (*O*-GlcNAc) and act as a glucose-*O*-GlcNAc sensor, enhancing *Srebp1c* transcription via upregulation of GCK, upstream of ChREBP (17, 30).

In addition to glucose, another carbohydrate with an increasingly important role in regulating lipogenesis is fructose. Studies have shown that fructose consumption, and not glucose consumption, led to a potent stimulation in DNL and decreased insulin sensitivity (85, 305). Further, fructose consumption is associated with a more severe development of fibrosis in patients with NAFLD (1). It is necessary to consider the role of fructose in lipogenesis given its increasing presence in human consumption. From 1978 to 2004, fructose consumption in the United States, measured as a percentage of total energy intake, increased from 8.1% to 9.1% (204). Unlike glucose, very little fructose makes it to systemic circulation, being primarily absorbed by the intestine and liver (321). Until recently, the liver was believed to be the main site of fructose clearance, but isotope-tracing experiments with ^{13}C-labeled fructose in mice found that most of the fructose is cleared by the intestines while any excess passes through the portal vein and is taken up by the liver (146, 360). Unlike glucose, circulating fructose does not trigger insulin secretion and hepatic fructose uptake occurs largely independently of insulin (64). Upon uptake by the liver, fructose is phosphorylated by the fructokinase, KHK, into F1P (7, 125). Fructose is broken down into acetyl-CoA via a similar pathway to glycolysis but bypasses several of the rate-controlling enzymes of glycolysis, including GCK and phosphofructokinase. Fructokinase can phosphorylate fructose at a rate 10 times faster than GCK can phosphorylate glucose, allowing fructose a faster rate of entry into further metabolic pathways (298). Without these regulatory steps that glucose metabolism requires, fructose can proceed more readily, and studies have demonstrated that consuming fructose resulted in larger amounts of circulating lactate than glucose (81).

Despite fructose metabolism itself being largely insulin independent, fructose regulates many facets of the insulin-dependent arms of DNL. A high fructose diet can partially stimulate SREBP-1c expression and activity in LIRKO mice, demonstrating that fructose is sufficient to induce lipogenesis, but insulin is still required for a maximal effect (116). Additionally, F1P is a potent activator of GCK, promoting the dissociation of GCK from its regulatory protein, GKRP, and thus stimulating glucose phosphorylation and activation of ChREBP (8, 251). Fructose, itself, is also a potent activator of ChREBP and mice on a high fructose diet demonstrate a higher increase in ChREBP than mice on a high dextrose diet. In addition, ChREBP is required for fructose to exert its effects on hepatic gene expression (160). One of its gene targets encodes FGF21, a protein thought to regulate glucose tolerance and FAO in the liver (21, 209). Knockdown of hepatic FGF21 causes hepatic steatosis and a reduction in serum ketones in mice fed a ketogenic diet (22). Fructose

promotes FGF21 expression and secretion in a manner completely dependent on ChREBP, as deletion of ChREBP eliminates fructose-stimulated FGF21 secretion (91). Taken together, carbohydrates provide more than just the fuel for lipid synthesis and regulate many aspects of DNL in the liver, including gene expression.

Insulin control of lipogenic gene expression

Acute loss of liver IR results in reduced expression of the lipogenic genes *Srebp1c* and *Fasn*, highlighting the importance of insulin signaling for postprandial induction of DNL (116). Downstream of insulin, AKT is required to stimulate lipogenesis. Inhibition of hepatic AKT activity leads to a significant reduction in DNL and lipogenic gene expression (325). Treatment of primary rat hepatocytes with a specific AKT inhibitor, AKTi-1/2, blocked the induction of insulin-stimulated SREBP-1c, indicating that insulin increases SREBP-1c through AKT (189). Loss of AKT2 in *ob/ob* mice or high fat diet-fed (HFD) mice prevents hepatic TAG accumulation, and subsequently leads to reduced body and liver weight in both models (179). Consistent with insulin action on driving lipogenesis, loss of PTEN in the liver induces SREBP-1c, as PTEN deficiency leads to PIP_3 accumulation (130). Thus, PTEN deficiency in hepatocytes drives DNL through promotion of lipid genes, *Fasn* and *Acc* (130). However, simultaneous deletion of PTEN and AKT2 reduced lipogenic gene expression to similar levels of the control, including *Scd1* (123). In contrast, constitutive activation of AKT led to increased glucose uptake and accumulation of hepatic TAGs (242). Therefore, AKT is both required and sufficient to stimulate SREBP-1c and DNL.

AKT-dependent activation of SREBP1 is blunted by rapamycin treatment, indicating that mTORC1 is additionally required for SREBP1 activation and lipogenesis (258). Accordingly, pretreatment of isolated hepatocytes with rapamycin suppressed the ability of insulin to stimulate *Srebp1c* and *Fasn*, which subsequently inhibited DNL (189). Therefore, AKT and mTORC1 activation are required for insulin-stimulated induction of SREBP-1c and DNL. To define the effects of constitutive mTORC1 activation, several studies utilize the liver-specific knockout of TSC, leading to destabilization of the TSC complex and mTORC1 activation. Interestingly, loss of TSC in the liver protected mice from age-induced and high fat diet-induced hepatic steatosis as compared to floxed controls (354). The improved hepatic steatosis from TSC deficiency is, in part, a result of modulation in DNL, FAO, and VLDL secretion. In terms of hepatic lipogenic gene expression, *Srebp1c, Fasn*, and *Scd1* are reduced, accompanied by a decrease in the ratio of active SREBP-1c to full-length protein (354). Interestingly, S6K inhibition in the hepatocytes did not block the induction of *Srebp-1c* mRNA in response to insulin but did impair insulin-stimulated processing and activation of SREBP-1c, suggesting that downstream of mTORC1, S6K regulates DNL at the protein expression level (189, 245). In livers deficient of AKT activity, constitutive mTORC1 activation fails to induce lipogenic genes *Srebp1c* and *Fasn*, and DNL (325). Taken together, mTORC1 is required but not sufficient to stimulate DNL downstream of hepatic insulin signaling.

Activation of hepatic AKT is regulated in part via a negative feedback loop from mTORC1 activity (145, 153, 264, 354). Though the mechanisms in which AKT activity is inhibited by mTORC1 are still unclear and may occur at multiple levels in the signaling cascade,

it is clear that disruption of TSC negatively impacts insulin-stimulated PI3K and AKT activation. Initial observations demonstrated that TSC1-TSC2 deficiency results in decreased activation of the IRS proteins, causing a defect in insulin-mediated activation of PI3K and AKT (327). However, other groups describe a mechanism through mTORC1 and S6K in the regulation of IRS in both mRNA transcription and in protein activation and turnover (122, 290). TSC-deficient cells were found to have depleted IRS-1 mRNA, an effect that is reversed by treatment with rapamycin, which inhibits unrestrained mTORC1 activation (122, 290). These effects of TSC deletion are attributed to the enhanced activation of S6K, as RNA knockdown of S6K1 and S6K2 restores IRS-1 mRNA levels (122). Nonetheless, mice with constitutive mTORC1 activity in the liver, via TSC1 deletion, display decreased AKT phosphorylation and subsequently decreased phosphorylation of FOXO1 (329, 354). Due to decreased phosphorylation and inhibition of FOXO1, TSC-deficient mice have increased gluconeogenic genes, *Pepck* and *Igfbp*, both targets of FOXO1 (354). Expression of an active AKT isoform in hepatocytes with unrestrained mTORC1 activity rescued the defect in lipogenesis (354). Thus, AKT is required for the induction of lipogenesis, in addition to its regulation of the mTORC1 signaling pathway. Moreover, AKT signals through FOXO1, independent of mTORC1, to regulate lipid synthesis (118). Interestingly, simultaneous deletion of FOXO1 and TSC1 from livers lacking AKT completely restored lipogenesis *in vivo*, independent of increased *Insig2a* expression, indicating that the AKT-FOXO1 signaling axis may additionally regulate DNL (325).

Insulin Regulates Hepatic Triglyceride Content

Triglycerides, or triacylglycerol (TAG), are neutral lipids utilized as storage forms for fatty acids (FA). TAG are composed of a glycerol backbone and three fatty acyl chains. Aside from *de novo* synthesis of TAG by the liver, dietary TAGs are packaged in chylomicrons and released into circulation for uptake by metabolic tissues (Figure 2). The liver facilitates the synthesis of TAG from free fatty acids (FFA), as well as the export of packaged TAG to be taken up by peripheral tissues. In the liver, insulin stimulates TAG synthesis (326, 358). Dysregulation of insulin-regulated TAG pathways is implicated in the pathophysiology of metabolic diseases, such as in NAFLD, of which insulin resistance is highly correlative. A hallmark feature of NAFLD is the accumulation of TAG in the liver, which is hypothesized to cause lipotoxicity and further progression of NAFLD (68). Additionally, hyperinsulinemia, through chronic insulin infusion, accelerates TAG turnover (309). Further, the rate of TAG secretion from the liver is decreased in mice with loss-of-function IRs, as measured by injection of a lipase-inhibiting detergent, which suggests that insulin regulates TAG secretion from the liver (29). Therefore, the synthesis, clearance, and degradation of TAG are essential for maintaining hepatic lipid homeostasis.

Fatty acid esterification and triglyceride synthesis

During periods of fasting, TAGs are broken down from adipose tissue and released into circulation as FFA. FFA are taken up by hepatocytes, diffusing freely via membrane-associate proteins, including FA-binding protein (FABP-pm) and FA-translocase (FAT) (294, 313). In humans TAG synthesis is mainly fueled by circulating FA, which highlights the impact of FA uptake in the contribution toward hepatic steatosis in NAFLD (77).

Interestingly, FAT and FABP-pm mRNA expression are upregulated in genetically obese *ob/ob* mice (219). However, in primary hepatocytes from LIRKO mice, cells retain normal FFA uptake, normal rates of FAO, and normal fasting hepatic TAG levels, suggesting other factors may regulate FA uptake and TAG synthesis in addition to insulin (116). Amongst FAT and FABP-pm, another family of proteins involved in trafficking exists, named FA-transport proteins (FATP) (285). Though there are six isoforms of FATP involved in long-chain fatty acid activation for uptake and re-esterification, only FATP2 and FATP5 are detected in the liver (75, 86). Knockdown of FATP2 in the murine liver caused an approximately 40% reduction in long-chain FA uptake, and an overexpression of FATP2 in immortalized HepG2 cells reciprocally increased FA uptake (86, 168). Deletion of FATP5 led to a reduction in long-chain FA uptake and hepatic TAG content (75). Taken together, FATPs have a significant role in FA uptake in the liver.

Upon being taken up by hepatocytes, FA must be activated for further processing and utilization. Long-chain FA undergo thioesterification to form fatty acyl-CoA, facilitated by the long-chain acyl-CoA synthetases (ACSLs). There are five members of the mammalian ACSL family, ACSL1, ACSL3, ACSL4, ACSL5, and ACSL6 (211). The ACSL isoforms vary in terms of their localization and substrate preference, which aligns with their individually distinct functions in regulating FA fates (158, 184, 331). ACSL1 interacts with the outer mitochondrial membrane and carnitine palmitoyltransferase 1 (CPT1) and is a target of peroxisome proliferator-activated factor α (PPARα) in rat livers (180, 287). Deletion of ACSL1 from mouse livers led to a reduction in the oxidation of FA but did not affect total hepatic TAG levels (187). Interestingly, overexpression of ACSL1 in rat hepatocytes does not increase TAG either (188). The ACSL3 isoform is upregulated in *ob/ob* mice and knockdown of ACSL3 reduces expression of DNL genes (44). ACSL5 is the only isoform found associated with the mitochondria, suggesting it may have a role in regulating FAO (184). However, overexpression of ACSL5 in cells increases FA uptake and flux into TAG synthesis (212). Additionally, ACSL5 mRNA expression is induced upon insulin stimulation and SREBP-1c activation, suggesting this isoform contributes to the anabolic FA uptake cascade (6).

After the generation of long-chain acyl-CoAs, TAG synthesis primarily occurs through the glycerol-3-phosphate (G3P) pathway. G3P acyltransferase (GPAT) enzymes control the esterification of acyl-CoA chains to G3P, producing lysophosphatidic acid (LPA), which is then acylated to phosphatidic acid. Phosphatidic acid is further converted to cytidine diphosphate diacylglycerol (CDP-DAG) or diacylglycerol (DAG) (154). There are multiple fates of DAG, one of which is the acylation by DAG acyltransferase (DGAT) to produce TAG (60). There are two named DGAT enzymes, DGAT1 and DGAT2. DGAT1 deficiency does not significantly affect TAG metabolism (54). However, whole-body deletion of DGAT2 reveals a requirement of DGAT2 for survival and TAG synthesis (312). Therefore, DGAT2 is an essential DGAT that is responsible for the majority of hepatic TAG synthesis. DGAT2 is an integral endoplasmic reticulum (ER) membrane protein, where it resides to facilitate TAG synthesis (311). Antisense oligonucleotide (ASO) silencing of DGAT2 in diet-induced obese and genetically obese *ob/ob* mice results in a decrease in hepatic TAG content (357). Reduction in hepatic TAG corresponds to a decrease in TAG synthesis and simultaneous increase in FAO (357). Shulman and coworkers demonstrate a mechanism

by which DGAT silencing prevents TAG synthesis, increases DAG levels, and prevents activation of protein kinase C epsilon (PKCε), which suppresses DNL and increases oxidation (57). Indeed, a liver-specific deletion of DGAT2 prevents diet-induced hepatic steatosis, in which hepatic TAG was approximately 70% lower than DIO controls (107).

Insulin facilitates hepatic triglyceride export

Insulin-regulated liver secretion of TAG occurs via very low-density lipoproteins (VLDL), and this step is essential for maintaining systemic lipid homeostasis. Blockade of VLDL-TAG secretion causes hepatic steatosis, demonstrating the importance of TAG secretion for lipid homeostasis (225). In healthy males, hyperinsulinemia suppresses VLDL-TAG production as well as reduces circulating FFAs (185). Elevated FFA levels additionally stimulate hepatic VLDL production, which modestly attenuates insulin's inhibitory effects (185). This is consistent with the observation that LIRKO mice retain normal FFA uptake (116). During periods of fasting, approximately 70% of VLDL-TAG pools are derived from adipose FA (24). VLDL production and its subsequent secretion from the liver are regulated at multiple levels, one of which being the synthesis of apolipoprotein B (ApoB) (105).

ApoB is an important structural protein integrated in chylomicron, LDL, VLDL, and intermediate-density lipoprotein (IDL) (31). In humans, liver-derived VLDL particles primarily contain apoB100, but rodent hepatocytes synthesize both apoB100 and apoB48 for VLDL production, therefore lipoprotein data from mice must be evaluated cautiously (31, 260). Interestingly, secretion of apoB100 and apoB48 and its association with VLDL and LDL are increased in LIRKO mice (29). However, insulin has no effect on apoB mRNA (67). Though it is well characterized now that *ApoB* gene is constitutively expressed and any changes in its protein levels do not directly correlate to mRNA expression (105). ApoB is regulated through a co-translational mechanism, being readily degraded, in which multiple mechanisms are proposed (32, 117). Labeling in primary hepatocytes shows that insulin inhibits the synthesis of apoB and stimulates the degradation of newly translated apoB to inhibit newly synthesized apoB secretion (249, 303). Consistently, in rat livers, insulin inhibits TAG secretion (358). Serum FFA levels are also linked to plasma apoB protein content, where exogenous FFA decreased the rate of apoB degradation, thus promoting VLDL secretion (203).

Apolipoproteins E and C regulate uptake of chylomicrons by the liver. In the liver, apoE mediates uptake of chylomicrons and TAG-containing lipoproteins, and this process is inhibited by apoC-III (263). apoC-III is an apolipoprotein that inhibits uptake of chylomicrons (344). Therefore, apoC-III expression is correlated with elevations in plasma TAG (48). Further, overexpression of apoC-III leads to hypertriglyceridemia (140). Therefore, it is no surprise that insulin regulates chylomicron uptake, where insulin dose-dependently suppresses transcription of *apoC-III* (gene name *Apoc3*) gene (55). Genetic variation in insulin response element of the apoC-III promoter impairs insulin's control of apoC-III transcription, and patients with this variation often present with hypertriglyceridemia (190). FOXO1 activity is also linked to apolipoprotein C-III expression, such that expression of constitutively active FOXO1 stimulates apoC-III expression and the opposite is true when FOXO1 is deleted in hepatocytes (16). Altogether,

insulin suppresses apoC-III expression through its inhibition of FOXO1 to promote uptake of chylomicrons.

Another component of lipoprotein production is microsomal triglyceride transfer protein (MTTP). MTTP is a lipid transfer protein that regulates the synthesis of apoB-containing lipoproteins, such as VLDL and chylomicrons (112). Impaired MTTP activity is linked to abetalipoproteinemia, a genetic disease characterized by low levels of apoB lipoproteins (291). Insulin negatively regulates MTTP mRNA in an acute, dose-dependent fashion, which was elucidated in cultured HepG2 cells (195). However, further investigation into the mechanisms of apoB regulation demonstrates that insulin acutely suppresses apoB secretion independently of its regulation of MTTP mRNA (302). Dong and coworkers demonstrate a mechanism by which insulin suppresses MTTP expression through FOXO1 inhibition (148). In mice that express a constitutively active form of FOXO1, MTTP expression is induced, and VLDL production and its subsequent secretion are enhanced (148). Interestingly, mutations in MTTP (rs2306986) are associated with a higher susceptibility of developing NAFLD, attributed to a deficiency in VLDL-TAG secretion (65, 134). Accordingly, MTTP inhibitors in mouse models lead to hepatic steatosis, via decreased hepatic TAG export (183).

VLDL secretion is also regulated by the rate of biosynthesis of phosphatidylcholine (PC), the main phospholipid coating lipoproteins (144). Vance and coworker have elucidated the mechanisms in which inhibition of PC synthesis decreased the rate of VLDL secretion (88). In the liver, PC is primarily produced through the Kennedy (CDP-choline) pathway and regulated by the rate limiting enzyme, CTP:Phosphocholine cytidylyltransferase (CCT) (154). DAG concentration is an important regulator of PC synthesis, as it is required to produce PC from CDP-choline (154). CCTα is the major isoform expressed and accounts for most PC production in liver (149). Congenital loss of liver CCTα protein, in mice, results in hepatic steatosis due to reduced PC synthesis with no effect of liver TAG synthesis (143). Further, CCTα loss-of-function mutations in humans lead to a reduction in PC synthesis and development of fatty liver disease (272). Thus, CCTα activity is essential for maintaining hepatic lipid homeostasis. Recently, our laboratory demonstrated that loss of mTORC1 activity suppressed hepatic VLDL-TAG secretion (264). Inhibition of VLDL secretion was linked to defects in CCTα activity, suggesting a mechanism in which mTORC1 regulates PC synthesis through CCTα protein stabilization (264, 329). Furthermore, activation of hepatic mTORC1 promotes PC synthesis and VLDL-TAG secretion in mice fed NASH-promoting diets, which prevents and reverses NASH in mice (329). These data are consistent with findings that the rate of TAG secretion is decreased in LIRKO mice, as measured by injection of a lipase-inhibiting detergent to monitor TAG secretion *in vivo* (29). Serum VLDL particles isolated from LIRKO mice contained less TAG as compared to control, but had normal levels of cholesterol, indicating that lack of liver insulin signaling leads to impaired TAG secretion (29).

Sortilin also has a role in regulating apolipoproteins and VLDL secretion (61). Sortilin is proposed to be involved in intracellular trafficking (10). A Genome-wide association study (GWAS) identified a novel locus on chromosome 1p13 harboring *SORT1*, the gene encoding sortilin, that is associated with reduced LDL cholesterol (150). Indeed, knockdown

of sortilin in primary murine hepatocytes increases apoB secretion, while its overexpression decreases secretion, suggesting that sortilin is involved in regulating degradation of apoB (230). In immortalized AML12 liver cells, insulin upregulated sortilin protein content (186). In mice, treatment with wortmannin, a PI3K inhibitor, or an AKT1/2 inhibitor decreased sortilin protein levels and increased plasma cholesterol and TAG (186). Downstream of insulin and AKT, mTORC1 suppresses sortilin, leading to an increase in apoB secretion (10).

The balance between the synthesis and secretion of TAG may represent a target for the treatment of NAFLD, in which no effective therapeutic exists yet. Decreased levels of hepatic PC are correlated with liver damage, implicating PC in the progression of NAFLD (192). The I148M loss-of-function mutation in patatin-like phospholipase domain-containing protein 3 (PNPLA3) correlates with an increased risk of developing NAFLD. Carriers of I148M mutation have decreased PC synthesis and increased hepatic TAG content (199, 328). Additionally, protective NASH variants correspond with increased hepatic PC content. Carriers of the protein-truncating variant (rs72613567T > TA), which encodes a hepatic lipid droplet protein hydroxysteroid 17-β dehydrogenase 13 (HSD17B13) protein, have an upregulation in liver PC and are protected from fibrosis (200, 257). Next, a coding variant (rs2642438G > A) in mitochondrial amidoxime reducing component 1 (MARC1) gene is associated with decreased severity of NAFLD, presenting with increased plasma TAG and an enrichment of hepatic PC content (198, 286). Lastly, a human genome-wide association study identified the E167K variant in the transmembrane 6 superfamily 2 (TMS6F2) gene correlated with defects in apoB trafficking and VLDL secretion, leading to an increased risk of NAFLD and progression to NASH (80, 167, 201). Collectively, there are an increasing number of studies supporting a role for hepatic PC content and VLDL-TAG secretion in NAFLD progression. Taken together, hepatic TAG export is an essential regulator of lipid homeostasis that is controlled by hepatic insulin action via multiple complementary mechanisms.

Insulin Inhibits Fatty Acid Oxidation

Regulation of lipid homeostasis requires a coordinated balance between fatty acid synthesis and fatty acid breakdown, otherwise known as FAO, or β-oxidation. Though there are other pathways of FA breakdown, named α-oxidation and ω-oxidation; β-oxidation is the primary pathway in the liver. While insulin plays a key role in regulating lipid synthesis, here we discuss insulin's role in regulating the breakdown of fats, an essential provider of energy during periods of fasting and nutrient limitations. Under fasting conditions, adipose tissue activates lipolysis, breaking down TAG into FFA. FFA are released into circulation by the adipose and taken up by the liver. Upon the uptake of FFA into the cytosol of hepatocytes, carnitine palmitoyltransferases (CPT) and carnitine-acylcarnitine translocase (CACT), allow for the transferring of acyl chains into the mitochondria for β-oxidation and complete breakdown of lipids (170). Deficiencies in mitochondrial enzymes involved in FAO result in inborn fasting-induced hypoketotic hypoglycemia, indicating the necessity of FAO in maintenance of metabolic homeostasis (246). The oxidation of acyl chains in the mitochondria leads to the production of acetyl-CoA and ketone bodies. The products of β-oxidation contribute to TCA cycle and the electron transport chain to release

energy via ATP. Ketogenesis is key in the processing of liver lipid content, regulating the breakdown of nearly two-thirds of lipids in the liver (342). Under periods of fasting, the liver facilitates breakdown of glycogen stores and secretion of TAG to supply peripheral tissues. However, during prolonged fasting, characterized by low blood glucose and insulin levels, with increased glucagon, the liver utilizes the FFA, resulting in the generation of ketone bodies. Ketone bodies supply energy to the brain, being a monocarboxylate that may be transported through the blood-brain barrier. Several mitochondrial enzymes are required for the metabolism of lipids and are discussed in detail below.

β-Oxidation in the mitochondria

Among the first enzymes required for acyl chain breakdown are the CPTs, required to transport long-chain acyls to the mitochondria. First, CPT1 sits at the outer mitochondrial membrane awaiting acyl-CoA chains (103, 229). CPT1 facilitates the transferring of acyl groups from acyl-CoA to carnitine to produce long-chain acyl-carnitines that may translocate the inner mitochondrial membrane, via CACT, encoded by the gene SLC25A20, while releasing CoA as a byproduct (34, 129). Deficiencies in SLC25A20 are correlated with early infancy death, likely occurring from decreased fasting FAO, causing a decrease in ATP production required for fueling gluconeogenesis, leading to hypoglycemia and hypoketonemia (306). Within the mitochondrial matrix, CPT2 transfers the acyl group from acyl-carnitine to CoA to reform acyl-CoA for breakdown in the mitochondria (353). However, medium-chain fatty acids can transport into mitochondria independent of CPT1/2. As previously mentioned, CPT1 may be allosterically inhibited by malonyl-CoA, a product of ACC (Figure 3). A key regulator of ACC activity is AMP-activated protein kinase (AMPK), a kinase activated during low-energy states, such as fasting. AMPK activation promotes catabolic, energy-producing pathways like β-oxidation (47, 139, 237). AMPK phosphorylates both isoforms of ACC at Ser79 on ACC1 and Ser221 on ACC2 leading to inhibition of ACC and subsequent reduction of malonyl-CoA production (152, 238, 247). Insulin inhibits AMPK activity and promotes dephosphorylation of these AMPK-mediated sites, among others, activating ACC and inhibiting FAO (346). Thus, the synthesis of new fatty acids prevents mitochondrial oxidation of lipids. Genetic CPT1A deficiency is a very rare occurrence, but patients generally present with hypoketotic hypoglycemia and hepatic encephalopathy due to defects in long-chain FAO (27).

Once in the mitochondria, dehydrogenases act on the acyl-CoA chains. There are numerous acyl-CoA dehydrogenases, all of which are flavoenzymes, that facilitate β-oxidation (97, 119). The acyl-CoA dehydrogenases are bound to flavin adenine dinucleotide (FAD) and are reduced to $FADH_2$ upon dehydrogenation of acyl chains (97). The bound $FADH_2$ is reoxidized by the electron-transferring flavoprotein (ETF) which shuttles electrons to the inner mitochondrial membrane (307, 308). An ETF:ubiquinone oxidoreductase complex carries electrons from ETF to ubiquinone in the electron respiratory chain to generate ATP (273). Acyl-CoA dehydrogenases (ACADs) desaturate the acyl-CoA esters in the mitochondria. There are 11 known members of ACADs in the human genome (316). Five of the 11 ACADs are involved in mitochondrial β-oxidation: very long-chain acyl-CoA dehydrogenase (VLCAD), long-chain acyl-CoA (LCAD), medium-chain acyl-CoA dehydrogenases (MCAD), short-chain acyl-CoA dehydrogenase (SCAD), and ACAD9

(316). VLCAD and ACD9 have similar homology and specificity for long acyl chains, palmitoyl-CoA (C16:0) and stearoyl-CoA (C18:0) (301, 359). LCADs play an essential role in oxidizing C8-C16 chains (178). Mice that lack LCAD present with defects in FAO, leading to hypoglycemia, elevated FFA in circulation, and some cases of cardiomyopathy, suggesting the importance of LCAD in β-oxidation (171). MCADs act on medium-chain acyl-CoA chains, specifically C4-C16 (90). SCAD catalyzes the breakdown of short-chain fatty acids, with specificity for butyryl-CoA (C4:0) and is the final step of oxidation for an acyl-chain (90).

Upon dehydrogenation, enoyl-CoA hydratases hydrate the substrate, 2-*trans*-enoyl-CoA to L-3-hydroxyacyl-CoA (97, 98). In the next step, L-3-hydroxylacyl-CoA is dehydrogenated, producing 3-ketoacyl-CoA, specifically with NAD+ (244). This leads to the last reaction in β-oxidation, in which 3-ketoacyl-CoA is thiolytically cleaved to produce acetyl-CoA and the acyl-CoA chain that is two carbons shorter (224). Two thiolases exists, where thiolase I may cleave any length of acyl-CoA, but thiolase II, otherwise known as acetoacetyl-CoA thiolase, exclusively cleaves acetoacetyl-CoA (227).

Transcriptional regulation of β-oxidation

Regulation of the FAO-associated genes is essential for maintaining lipid homeostasis. For example, genetic abnormalities in FA shuttling and FAO genes in the mitochondria are associated with lipid storage defects, hypoglycemia, lactic acidosis, and low ketones (164). Many cases of CPT1A deficiency result in fasting-induced hypoglycemia and infant mortality (33). A functional mutation in MCAD leads to low plasma carnitines and fasting hypoglycemia (215). Therefore, the expression of FAO genes is essential for survival. Many of the FAO enzymes are regulated in part by nuclear receptors like peroxisome proliferator-activated receptor (PPAR). Though there are three forms of PPARs identified, PPARα is highly expressed in the liver (162). Mice with whole-body loss of PPARα do not respond to peroxisome proliferators, indicating that PPARα is the main isoform for PPAR actions (181). Additionally, PPARα-deficiency in mice results in decreased expression of VLCAD, LCAD, and ACSL1 (18). Under high fat diet conditions, deletion of PPARα induces hepatic steatosis (155).

PPAR-γ coactivator-1 (PGC-1) is a family of transcriptional coactivators that are known for promoting cellular energetics, but also have a role in regulating hepatic lipid and glucose homeostasis (89). They are well-known for their role in activating transcription of genes involved in gluconeogenesis and FAO (89). In mice, PGC-1 expression is induced by fasting and repressed in refed conditions (356). PGC-1 mRNA and protein expression are highly increased in both *ob/ob* and LIRKO mice (356). Unsurprisingly, insulin suppresses PGC-1 transcription in an AKT-dependent manner (66, 127) in addition to the regulation of the FOXO pathway (191). However, FOXO1 can bind PGC-1 to activate gluconeogenic gene program, a mechanism reversed by insulin stimulation (261). Adenoviral expression of PGC-1 in isolated rat hepatocytes stimulates the transcription of gluconeogenic genes, in a dose-dependent manner, and results in enhanced glucose output (356). Further, PGC-1 knockdown reduces basal hepatic glucose output and improves liver insulin sensitivity, which was attributed to an increase in insulin-stimulated phosphorylation of AKT in *db/db*

diabetic mice (165). Therefore, AKT controls both FOXO and PGC-1α to regulate FAO and gluconeogenesis. Adenoviral knockdown of PGC-1 in mice increased hepatic TAG, highlighting the essential role for PGC-1 activity in maintaining lipid homeostasis as well (165). Increase in liver TAG is due to impaired induction of FAO genes in the fasted state, including genes *Cpt* and *Mcad* (165). Montminy et al. elucidated the mechanism in which PPARα and PGC-1 induces *tribbles* homolog, TRB-3, during fasting, which in turn is an inhibitor of AKT signaling (165). Knockdown of liver-specific TRB-3 yields improvements in glucose tolerance (165). Thus, fasting induces PGC-1 and induces TRB-3 that contributes to decreased AKT signaling in the fasted state. To determine the role of PGC-1α more definitively in regulating glucose homeostasis, Kelly and others generated a whole-body mouse knockout of PGC-1α. In this model, mice have a wide range of abnormalities, but specifically in the liver, a 24-h fast-induced hepatic steatosis, with no change in serum TAG levels (182). PGC-1α-deficient hepatocytes demonstrate reduced rates of FAO, which contribute to the hepatic steatosis induced in fasting, providing evidence of the importance of PGC-1α in FAO (182). Of note, PGC-1α knockout mice are protected against insulin resistance when challenged with a HFD (182). Along these lines, *ob/ob* mice have increased expression of gluconeogenic genes, such as *G6pc, Pck1*, and *Pgc-1α* (222). Overall, PGC-1α is activated at a high level in a diabetic liver; however, PGC-1 coactivators have various roles depending on tissue context (89).

PGC-1β is also expressed in the liver and is suggested to play a different role than PGC-1α (89). Surprisingly, mice fed a HFD have an increased expression of PGC-1β, as compared to mice fed normal chow, but have no effect on PGC-1α (194). To elucidate the mechanism of this effect, Spiegelman and coworkers treated isolated hepatocytes with trans-fat and found an enhanced expression of PGC-1β and not PGC-1α (194). Ultimately, it was revealed that PGC-1β activates lipogenesis through transcriptional coactivation of SREBP-1c, LXR, and ChREBP (52, 194). Overall, both PGC-1α and PGC-1β play a key role in regulating both hepatic glucose and lipid homeostasis.

Insulin Promotes Cholesterol Metabolism

T2DM patients present with insulin resistance and dyslipidemia, amongst other metabolic defects. Dyslipidemia is often defined by abnormal levels of cholesterol, including decreases in HDL, increase in VLDL and LDL, and as well as increases in inflammation (265). During insulin resistance, dysregulation of lipid and cholesterol metabolism leads to CVD. Insulin resistance is correlated with high cholesterol synthesis and decreased cholesterol absorption in men (254). Initial observations implicate insulin as an important regulator of LDL cholesterol degradation, further identifying a role for insulin action in the control of serum cholesterol (49). T2DM associates closely with increased cholesterol synthesis and decrease cholesterol absorption (295). Of significance, familial hypercholesterolemia (FH) is a genetic condition causing elevated plasma LDL cholesterol levels (12). When cholesterol in the diet is high, cholesterol synthesis is decreased, whereas the deficiency of cholesterol in the diet promotes cholesterol synthesis (131). However, cholesterol synthesis is enhanced in obesity (233). Thus, it is essential to understand the pathogenesis of dyslipidemia and how insulin regulates lipid and cholesterol processes.

Cholesterol is a 27-carbon steroid that is essential for cellular function and survival. Cholesterols have many roles in the cell, including membrane structure, and production of bile acids, hormones, and oxysterols. Cholesterol synthesis occurs through several processes starting with acetyl-CoA and acetoacetyl-CoA substrates. Isoprenoids are generated, involving hydroxymethylglutaryl-CoA (HMG CoA) synthase and HMG CoA reductase, which is used to produce squalene. Squalene undergoes cyclization and further conversion to lanosterol and eventually generates cholesterol (15). The activity of HMG CoA reductase is a rate-limiting step in the synthesis of cholesterol and is negatively regulated by cholesterol (38). Cholesterol synthesis is suppressed in the fasting state and induced under feeding conditions. When IR is deleted in hepatocytes, refeeding-induced cholesterol synthesis is blunted (221). Therefore, insulin is the key regulator for initiating the fasting and refeeding cholesterol synthetic response.

Cholesterol is transported throughout the body through two major pathways, HDL and LDL (241). Initial studies observed that increased plasma LDL correlated with disposition for myocardial infarction (39). In FH, patients present with elevated plasma LDL cholesterol levels because of increased cholesterol synthesis and decreased clearance (108). LDL is taken up by hepatocytes through the LDL receptor (LDLR) (108). LDLR resides at the cell membrane and binds LDL to undergo endocytosis and degradation at the lysosome (Figure 5) (109, 111). In the lysosome, LDL is degraded to amino acids, and cholesterol is released into the cell. The LDL-derived cholesterol was found to be a negative regulator of HMG CoA reductase by decreasing its gene transcription and promoting its degradation (104). Additionally, LDL-derived cholesterol promotes the activation of acyl CoA:cholesterol acyltransferase (ACAT) to upregulate the conversion of cholesterol to cholesterol esters (310).

In the liver, SREBP1 and SREBP2 are the master regulators of cholesterol gene programming. As previously mentioned, insulin regulates transcription and activation of SREBPs. In mice lacking liver-specific IR, the nuclear active form of SREBP2 protein is drastically reduced in all feeding conditions, highlighting the dependency on insulin signaling for protein activation (221). Subsequently, gene expression of SREBP2 targets was downregulated in LIRKO mice, including HMG-CoA reductase (*Hmgcr*), farnesyl diphosphate synthetase (*Fdps*), squalene synthase (*Fdft1*), and LDL receptor (*Ldlr*) (29). LIRKO mice develop hypercholesterolemia after 10 weeks on a cholesterol-rich, high fat diet, suggesting insulin signaling is required for maintaining cholesterol homeostasis in the presence of excess fat (29). Shimano et al. developed a mouse model that expresses active SREBP2 in the liver and adipose tissues, in which the C-terminal regulation domain is truncated, leading to constitutive translocation to the nucleus (131). They demonstrate that active SREBP2 increases mRNA expression of many cholesterol synthesis genes, including *Ldlr, Hmgcr*, and *Sqle*. The overexpression of the truncated SREBP2 in the liver dramatically enhances cholesterol synthesis with a modest induction of lipid synthesis (131). Accordingly, hepatocyte-specific deletion of *Srebf2*, the gene encoding SREBP2, reduced cholesterogenic gene transcription and cholesterol synthesis (268). Surprisingly, SREBP2 deletion also leads to decreased fatty acid synthesis, attributed to the reduction in sterol synthesis and subsequent reduction in SREBP-1c stimulation, demonstrating the

requirement of SREBP2 for cholesterol and lipid synthesis (268). Thus, SREBP2 is the main gene regulating hepatic cholesterol metabolism.

In early studies, insulin action was noted to control LDL cholesterol degradation, supporting the notion that insulin stimulation acts to reduce serum cholesterol (49). LDL half-life is increased in LIRKO mice, consistent with a reduction in LDLR protein when mice were challenged to a high fat diet for 10 weeks (29). Further, when subjected to atherogenic Paigen diet (15% dairy fat, 1% cholesterol, 0.5% cholic acid), LIRKO mice, developed severe atherosclerosis in 12 weeks, suggesting liver insulin action is required for handling cholesterol and loss of insulin signaling increases susceptibility to atherosclerosis (29). The requirement of insulin for LDLR-mediated cholesterol clearance was tested using an siRNA-mediated knockdown of hepatic IR in an LDLR-deficient liver, which displayed decreased LDLR protein, as expected, and decreased secretion of apoB, and VLDL-TAG and -cholesterol (120). Consequently, mice that lack hepatic insulin signaling have 50% less HDL cholesterol, but a threefold increase in VLDL cholesterol (29). Along these lines, LIRKO mice have a reduction in serum TAG, which is attributed to reduced TAG in serum VLDL and LDL particles (29). Therefore, insulin is an essential regulator of lipoproteins and the LDL receptor.

Although LDLR mRNA increases with insulin stimulation, LDLR protein levels are modulated by proprotein convertase subtilisin/kexin type 9 (PCSK9) (Figure 5) (277). Mutations in *PCSK9* are linked to autosomal dominant hypercholesterolemia (ADH) (2). A loss of function variant in PCSK9 is associated with lower LDL-cholesterol and reduced risk for CVD (274). Overexpression of PCKS9 in isolated LDLR-deficient hepatocytes does not affect apoB protein secretion, thereby providing evidence that PCSK9 does not affect apoB and only LDLR protein (277). Horton et al. demonstrate, through rigorous parabiotic experiments, that PCSK9 is secreted from the liver and binds to LDLR proteins to promote its endocytosis and subsequent degradation (Figure 5) (172). Therefore, PCSK9 is a posttranslational, negative regulator of LDLR protein, and increases in PCSK9 reduce hepatic LDLR protein, resulting in an increase in plasma LDL cholesterol. Due to its essential role in inhibiting LDLR and LDL-cholesterol degradation, PCSK9 remains a therapeutic target for lowering plasma cholesterol.

Interestingly, insulin increases transcription of *Ldlr*, yet paradoxically promotes degradation of LDLR protein. Thereby, loss of IR in liver decreased PCSK9 and LDLR mRNA expression (222). Mice that undergo 24 h fasting have approximately 70% decrease in PCSK9 mRNA, which is restored upon refeeding, suggesting a correlation between nutritional status and PCSK9 expression (62). STZ-induced diabetic rats have higher serum cholesterol levels, correlating to a decrease in *Ldlr* expression (234). In primary rodent hepatocytes, insulin stimulates the induction of *Pcsk9* mRNA and protein (62). Further, Biddinger et al. elucidated the mechanism in which insulin stimulates PCSK9 transcription to promote the degradation of LDLR protein (222). Indeed, liver insulin signaling is required for LDLR and PCSK9 expression, as LIRKO mice display decreased *Pcsk9* and *Ldlr* mRNA (222). However, LIRKO mice are still able to induce *Pcsk9* mRNA in response to feeding stimulus, but not to the same extent as control mice (222). Further, there is no difference in plasma PCSK9 levels between human patients with normal glycemia and T2DM, suggesting

there are other factors regulating PCSK9 and perhaps these factors could be therapeutically attractive (35).

Downstream of insulin action, mTORC1 is linked to PCSK9 and LDLR protein regulation. Constitutive mTORC1 activity impairs the induction of *Srebp2, Ldlr*, and *Hmgcr* in response to feeding (354). Administration of rapamycin results in enhanced expression of PCSK9 and a subsequent reduction in LDLR protein (11). Tall et al. describe a mechanism in which mTORC1 activates PKCδ to suppress the activity of HNF4α which negatively regulates PCSK9 (11). *Pcsk9* gene expression is induced in fasting conditions, where glucagon is high, and insulin is low. Upon refeeding, insulin suppresses PCSK9, thereby promoting LDLR protein degradation. Thus, another mechanism downstream of insulin signaling is the coordination of PCSK9, LDLR, and cholesterol homeostasis.

Conclusion

In Sir Frederick Banting's 1925 Nobel Lecture, he states, "Insulin is not a cure for diabetes; it is a treatment," opening a Pandora's box for the endless pathways and mechanisms to explore in our goal to understand and treat metabolic disorders, including diabetes (23). As the world celebrates the 100 years since the discovery of insulin, we take a closer look at the studies from the past century that identified and elucidated the key components of insulin's action on hepatic lipid metabolism. Understanding these specific molecular mechanisms of hepatic insulin action will likely reveal new regulatory pathways governing hepatic metabolism in health and disease. Although extensive research has unraveled key insulin-dependent mechanisms, much is still unknown about the molecular pathogenesis of metabolic diseases like insulin resistance and NAFLD. Thus, many questions remain to be addressed in the future such as:

- What is the molecular explanation for sustained DNL by insulin during hepatic insulin resistance?
- What are the transcription-independent mechanisms controlling hepatic lipid synthesis and how do they contribute to NAFLD?
- How does reducing excess lipid burden (i.e., suppression of DNL) alleviate NAFLD progression to inflammation and fibrosis?
- Are there specific lipid species that are particularly pro-inflammatory, pro-fibrotic in hepatic insulin resistance?

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References

1. Abdelmalek MF, Suzuki A, Guy C, Unalp-Arida A, Colvin R, Johnson RJ, Diehl AM, Nonalcoholic Steatohepatitis Clinical Research Network. Increased fructose consumption is associated with fibrosis severity in patients with nonalcoholic fatty liver disease. Hepatology 51: 1961–1971, 2010. [PubMed: 20301112]

2. Abifadel M, Varret M, Rabès J-P, Allard D, Ouguerram K, Devillers M, Cruaud C, Benjannet S, Wickham L, Erlich D, Derré A, Villéger L, Farnier M, Beucler I, Bruckert E, Chambaz J, Chanu B, Lecerf J-M, Luc G, Moulin P, Weissenbach J, Prat A, Krempf M, Junien C, Seidah NG, Boileau C. Mutations in PCSK9 cause autosomal dominant hypercholesterolemia. Nat Genet 34: 154–156, 2003. [PubMed: 12730697]
3. Abu-Elheiga L, Matzuk MM, Abo-Hashema KAH, Wakil SJ. Continuous fatty acid oxidation and reduced fat storage in mice lacking acetyl-coa carboxylase 2. Science 291: 2613–2616, 2001. [PubMed: 11283375]
4. Accili D, Arden KC. FoxOs at the crossroads of cellular metabolism, differentiation, and transformation. Cell 117: 421–426, 2004. [PubMed: 15137936]
5. Accili D, Drago J, Lee EJ, Johnson MD, Cool MH, Salvatore P, Asico LD, José PA, Taylor SI, Westphal H. Early neonatal death in mice homozygous for a null allele of the insulin receptor gene. Nat Genet 12: 106–109, 1996. [PubMed: 8528241]
6. Achouri Y, Hegarty BD, Allanic D, Bécard D, Hainault I, Ferré P, Foufelle F. Long chain fatty acyl-CoA synthetase 5 expression is induced by insulin and glucose: Involvement of sterol regulatory element-binding protein-1c. Biochimie 87: 1149–1155, 2005. [PubMed: 16198472]
7. Adelman RC, Ballard FJ, Weinhouse S. Purification and properties of rat liver fructokinase. J Biol Chem 242: 3360–3365, 1967. [PubMed: 6029444]
8. Agius L, Peak M, Van Schaftingen E. The regulatory protein of glucokinase binds to the hepatocyte matrix, but, unlike glucokinase, does not translocate during substrate stimulation. Biochem J 309: 711–713, 1995. [PubMed: 7639682]
9. Ahima RS, Prabakaran D, Mantzoros C, Qu D, Lowell B, Maratos-Flier E, Flier JS. Role of leptin in the neuroendocrine response to fasting. Nature 382: 250–252, 1996. [PubMed: 8717038]
10. Ai D, Baez JM, Jiang H, Conlon DM, Hernandez-Ono A, Frank-Kamenetsky M, Milstein S, Fitzgerald K, Murphy AJ, Woo CW, Strong A, Ginsberg HN, Tabas I, Rader DJ, Tall AR. Activation of ER stress and mTORC1 suppresses hepatic sortilin-1 levels in obese mice. J Clin Invest 122: 1677–1687, 2012. [PubMed: 22466652]
11. Ai D, Chen C, Han S, Ganda A, Murphy AJ, Haeusler R, Thorp E, Accili D, Horton JD, Tall AR. Regulation of hepatic LDL receptors by mTORC1 and PCSK9 in mice. J Clin Invest 122: 1262–1270, 2012. [PubMed: 22426206]
12. Ajufo E, Rader DJ. New therapeutic approaches for familial hypercholesterolemia. Annu Rev Med 69: 113–131, 2018. [PubMed: 29414257]
13. Alessi DR, Andjelkovic M, Caudwell B, Cron P, Morrice N, Cohen P, Hemmings BA. Mechanism of activation of protein kinase B by insulin and IGF-1. EMBO J 15: 6541–6551, 1996. [PubMed: 8978681]
14. Alexander MC, Palmer JL, Pointer RH, Kowaloff EM, Koumjian LL, Avruch J. Insulin-stimulated phosphorylation of ATP-citrate lyase in isolated hepatocytes. Stoichiometry and relation to the phosphoenzyme intermediate. J Biol Chem 257: 2049–2055, 1982. [PubMed: 7035458]
15. Alphonse PAS, Jones PJH. Revisiting human cholesterol synthesis and absorption: The reciprocity paradigm and its key regulators. Lipids 51: 519–536, 2015. [PubMed: 26620375]
16. Altomonte J, Cong L, Harbaran S, Richter A, Xu J, Meseck M, Dong HH. Foxo1 mediates insulin action on apoC-III and triglyceride metabolism. J Clin Invest 114: 1493–1503, 2004. [PubMed: 15546000]
17. Anthonisen EH, Berven L, Holm S, Nygård M, Nebb HI, Grønning-Wang LM. Nuclear receptor liver X receptor is O-GlcNAc-modified in response to glucose. J Biol Chem 285: 1607–1615, 2010. [PubMed: 19933273]
18. Aoyama T, Peters JM, Iritani N, Nakajima T, Furihata K, Hashimoto T, Gonzalez FJ. Altered constitutive expression of fatty acid-metabolizing enzymes in mice lacking the peroxisome proliferator-activated receptor α (PPARα). J Biol Chem 273: 5678–5684, 1998. [PubMed: 9488698]
19. Araki E, Lipes MA, Patti ME, Brüning JC, Haag B 3rd, Johnson RS, Kahn CR. Alternative pathway of insulin signalling in mice with targeted disruption of the IRS-1 gene. Nature 372: 186–190, 1994. [PubMed: 7526222]

20. Ashton KS, Andrews KL, Bryan MC, Chen J, Chen K, Chen M, Chmait S, Croghan M, Cupples R, Fotsch C, Helmering J, Jordan SR, Kurzeja RJM, Michelsen K, Pennington LD, Poon SF, Sivits G, Van G, Vonderfecht SL, Wahl RC, Zhang J, Lloyd DJ, Hale C, St Jean DJ. Small molecule disruptors of the Glucokinase–Glucokinase regulatory protein interaction: 1. Discovery of a novel tool compound for *in vivo* proof-of-concept. J Med Chem 57: 309–324, 2014. [PubMed: 24405172]
21. Badman MK, Pissios P, Kennedy AR, Koukos G, Flier JS, Maratos-Flier E. Hepatic fibroblast growth factor 21 is regulated by PPARα and is a key mediator of hepatic lipid metabolism in Ketotic States. Cell Metab 5: 426–437, 2007. [PubMed: 17550778]
22. Badman MK, Pissios P, Kennedy AR, Koukos G, Flier JS, Maratos-Flier E. Hepatic fibroblast growth factor 21 is regulated by PPARa and is a key mediator of hepatic lipid metabolism in Ketotic States. Cell Metab 5: 426–437, 2007. [PubMed: 17550778]
23. Banting FG. An address on diabetes and insulin: Being the nobel lecture delivered at Stockholm on September 15th, 1925. Can Med Assoc J 16: 221, 1926. [PubMed: 20315695]
24. Barrows BR, Parks EJ. Contributions of different fatty acid sources to very low-density lipoprotein-triacylglycerol in the fasted and fed States. J Clin Endocrinol Metab 91: 1446–1452, 2006. [PubMed: 16449340]
25. Bebernitz GR, Beaulieu V, Dale BA, Deacon R, Duttaroy A, Gao J, Grondine MS, Gupta RC, Kakmak M, Kavana M, Kirman LC, Liang J, Maniara WM, Munshi S, Nadkarni SS, Schuster HF, Stams T, St Denny I, Taslimi PM, Vash B, Caplan SL. Investigation of functionally liver selective glucokinase activators for the treatment of type 2 diabetes. J Med Chem 52: 6142–6152, 2009. [PubMed: 19746978]
26. Beigneux AP, Kosinski C, Gavino B, Horton JD, Skarnes WC, Young SG. ATP-citrate lyase deficiency in the mouse. J Biol Chem 279: 9557–9564, 2004. [PubMed: 14662765]
27. Bennett MJ, Santani AB. Carnitine palmitoyltransferase 1A deficiency. GeneReviews, Bookshelf ID: NBK1527, 2005.
28. Berwick DC, Hers I, Heesom KJ, Kelly Moule S, Tavaré JM. The identification of ATP-citrate lyase as a protein kinase B (Akt) substrate in primary adipocytes. J Biol Chem 211: 33895–33900, 2002.
29. Biddinger SB, Hernandez-Ono A, Rask-Madsen C, Haas JT, Alemán JO, Suzuki R, Scapa EF, Agarwal C, Carey MC, Stephanopoulos G, Cohen DE, King GL, Ginsberg H, Kahn CR. Hepatic insulin resistance is sufficient to produce dyslipidemia and susceptibility to atherosclerosis. Cell Metab 7: 125–134, 2008. [PubMed: 18249172]
30. Bindesbøll C, Fan Q, Nørgaard RC, MacPherson L, Ruan H-B, Wu J, Pedersen TA, Steffensen KR, Yang X, Matthews J, Mandrup S, Nebb HI, Grønning-Wang LM. Liver X receptor regulates hepatic nuclear O-GlcNAc signaling and carbohydrate responsive element-binding protein activity. J Lipid Res 56: 771–785, 2015. [PubMed: 25724563]
31. Blasiole DA, Davis RA, Attie AD. The physiological and molecular regulation of lipoprotein assembly and secretion. Mol Biosyst 3: 608–619, 2007. [PubMed: 17700861]
32. Boren J, Graham L, Wettesten M, Scott J, White A, Olofsson SO. The assembly and secretion of ApoB 100-containing lipoproteins in Hep G2 cells. ApoB 100 is cotranslationally integrated into lipoproteins. J Biol Chem 267: 9858–9867, 1992. [PubMed: 1315773]
33. Bougn PF, Saudubray JM, Marsac C, Bernard O, Odièvre M, Girard J. Fasting hypoglycemia resulting from hepatic carnitine palmitoyl transferase deficiency. J Pediatr 98: 742–746, 1981. [PubMed: 7014807]
34. Bremer J Carnitine in intermediary metabolism reversible acetylation of carnitine. J Biol Chem 237: 2228–2231, 1962. [PubMed: 13872791]
35. Brouwers MCGJ, Troutt JS, van Greevenbroek MMJ, Ferreira I, Feskens EJ, van der Kallen CJH, Schaper NC, Schalkwijk CG, Konrad RJ, Stehouwer CDA. Plasma proprotein convertase subtilisin kexin type 9 is not altered in subjects with impaired glucose metabolism and type 2 diabetes mellitus, but its relationship with non-HDL cholesterol and apolipoprotein B may be modified by type 2 diabetes mellitus: The CODAM study. Atherosclerosis 217: 263–267, 2011. [PubMed: 21497351]

36. Brown AJ, Sun L, Feramisco JD, Brown MS, Goldstein JL. Cholesterol addition to ER membranes alters conformation of SCAP, the SREBP escort protein that regulates cholesterol metabolism. Mol Cell 10: 237–245, 2002. [PubMed: 12191470]
37. Brown EJ, Albers MW, Shin TB, Ichikawa K, Keith CT, Lane WS, Schreiber SL. A mammalian protein targeted by G1-arresting rapamycin-receptor complex. Nature 369: 756–758, 1994. [PubMed: 8008069]
38. Brown MS, Dana SE, Goldstein JL. Regulation of 3-hydroxy-3-methylglutaryl coenzyme a reductase activity in human fibroblasts by lipoproteins. Proc Natl Acad Sci U S A 70: 2162, 1973. [PubMed: 4352976]
39. Brown MS, Goldstein JL. A receptor-mediated pathway for cholesterol homeostasis (Nobel Lecture). Angew Chem Int Ed 25: 583–602, 1986.
40. Brown MS, Goldstein JL. The SREBP pathway: Regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. Cell 89: 331–340, 1997. [PubMed: 9150132]
41. Brown MS, Goldstein JL. Selective versus total insulin resistance: A pathogenic paradox. Cell Metab 7: 95–96, 2008. [PubMed: 18249166]
42. Browning JD, Baxter J, Satapati S, Burgess SC. The effect of short-term fasting on liver and skeletal muscle lipid, glucose, and energy metabolism in healthy women and men. J Lipid Res 53: 577–586, 2011. [PubMed: 22140269]
43. Brunet A, Bonni A, Zigmond MJ, Lin MZ, Juo P, Hu LS, Anderson MJ, Arden KC, Blenis J, Greenberg ME. Akt promotes cell survival by phosphorylating and inhibiting a forkhead transcription factor. Cell 96: 857–868, 1999. [PubMed: 10102273]
44. Bu SY, Mashek MT, Mashek DG. Suppression of long chain acyl-CoA synthetase 3 decreases hepatic de novo fatty acid synthesis through decreased transcriptional activity. J Biol Chem 284: 30474–30483, 2009. [PubMed: 19737935]
45. Burnett PE, Barrow RK, Cohen NA, Snyder SH, Sabatini DM. RAFT1 phosphorylation of the translational regulators p70 S6 kinase and 4E-BP1. Proc Natl Acad Sci U S A 95: 1432–1437, 1998. [PubMed: 9465032]
46. Cantley LC. The phosphoinositide 3-kinase pathway. Science 296: 1655–1657, 2002. [PubMed: 12040186]
47. Carling D, Zammit VA, Hardie DG. A common bicyclic protein kinase cascade inactivates the regulatory enzymes of fatty acid and cholesterol biosynthesis. FEBS Lett 223: 217–222, 1987. [PubMed: 2889619]
48. Carlson LA, Ballantyne D. Changing relative proportions of apolipoproteins CII and CIII of very low density lipoproteins in hypertriglyceridemia. Atherosclerosis 23: 563–568, 1976. [PubMed: 178329]
49. Chait A, Bierman EL, Albers JJ. Regulatory role of triiodothyronine in the degradation of low density lipoprotein by cultured human skin fibroblasts. J Clin Endocrinol Metab 48: 887–889, 1979. [PubMed: 219014]
50. Chakravarthy MV, Pan Z, Zhu Y, Tordjman K, Schneider JG, Coleman T, Turk J, Semenkovich CF. 'New' hepatic fat activates PPARα to maintain glucose, lipid, and cholesterol homeostasis. Cell Metab 1: 309–322, 2005. [PubMed: 16054078]
51. Chalasani N, Younossi Z, Lavine JE. The diagnosis and management of non-alcoholic fatty liver disease: Practice guideline by the American Gastroenterological Association, American Association for the Study of Liver Diseases, and American College of Gastroenterology. Gastroenterology 143: 503, 2012.
52. Chambers KT, Chen Z, Lai L, Leone TC, Towle HC, Kralli A, Crawford PA, Finck BN. PGC-1β and ChREBP partner to cooperatively regulate hepatic lipogenesis in a glucose concentration-dependent manner. Mol Metab 2: 194–204, 2013. [PubMed: 24049734]
53. Chattopadhyay M, Selinger ES, Ballou LM, Lin RZ. Ablation of PI3K p110-α prevents high-fat diet-induced liver steatosis. Diabetes 60: 1483–1492, 2011. [PubMed: 21464441]
54. Chen HC, Smith SJ, Ladha Z, Jensen DR, Ferreira LD, Pulawa LK, McGuire JG, Pitas RE, Eckel RH, Farese RV Jr. Increased insulin and leptin sensitivity in mice lacking acyl CoA:diacylglycerol acyltransferase 1. J Clin Invest 109: 1049–1055, 2002. [PubMed: 11956242]

55. Chen M, Breslow JL, Li W, Leff T. Transcriptional regulation of the apoC-III gene by insulin in diabetic mice: Correlation with changes in plasma triglyceride levels. J Lipid Res 35: 1918–1924, 1994. [PubMed: 7868970]
56. Chen WS, Xu P-Z, Gottlob K, Chen M-L, Sokol K, Shiyanova T, Roninson I, Weng W, Suzuki R, Tobe K, Kadowaki T, Hay N. Growth retardation and increased apoptosis in mice with homozygous disruption of the akt1 gene. Genes Dev 15: 2203–2208, 2001. [PubMed: 11544177]
57. Cheol SC, Savage DB, Kulkarni A, Yu XX, Liu Z-X, Morino K, Kim S, Distefano A, Samuel VT, Neschen S, Zhang D, Wang A, Zhang X-M, Kahn M, Cline GW, Pandey SK, Geisler JG, Bhanot S, Monia BP, Shulman GI. Suppression of diacylglycerol acyltransferase-2 (DGAT2), but not DGAT1, with antisense oligonucleotides reverses diet-induced hepatic steatosis and insulin resistance. J Biol Chem 282: 22678–22688, 2007. [PubMed: 17526931]
58. Cho H, Mu J, Kim JK, Thorvaldsen JL, Chu Q, Crenshaw EB 3rd, Kaestner KH, Bartolomei MS, Shulman GI, Birnbaum MJ. Insulin resistance and a diabetes mellitus-like syndrome in mice lacking the protein kinase Akt2 (PKBβ). Science (1979) 292: 1728–1731, 2001.
59. Cho H, Thorvaldsen JL, Chu Q, Feng F, Birnbaum MJ. Akt1/PKBα is required for normal growth but dispensable for maintenance of glucose homeostasis in mice. J Biol Chem 276: 38349–38352, 2001. [PubMed: 11533044]
60. Coleman RA, Lewin TM, Muoio DM. Physiological and nutritional regulation of enzymes of triacylglycerol synthesis. Annu Rev Nutr 20: 77–103, 2003.
61. Conlon DM. Role of sortilin in lipid metabolism. Curr Opin Lipidol 30: 198–204, 2019. [PubMed: 30946050]
62. Costet P, Cariou B, Lambert G, Lalanne F, Lardeux B, Jarnoux A-L, Grefhorst A, Staels B, Krempf M. Hepatic PCSK9 expression is regulated by nutritional status via insulin and sterol regulatory element-binding protein 1c. J Biol Chem 281: 6211–6218, 2006. [PubMed: 16407292]
63. Cotter DG, Ercal B, Huang X, Leid JM, d'Avignon DA, Graham MJ, Dietzen DJ, Brunt EM, Patti GJ, Crawford PA. Ketogenesis prevents diet-induced fatty liver injury and hyperglycemia. J Clin Invest 124: 5175–5190, 2014. [PubMed: 25347470]
64. Curry DL. Effects of mannose and fructose on the synthesis and secretion of insulin. Pancreas 4: 2–9, 1989. [PubMed: 2654926]
65. Dai D, Wen F, Zhou S, Su Z, Liu G, Wang M, Zhou J, He F. Association of MTTP gene variants with pediatric NAFLD: A candidate-gene-based analysis of single nucleotide variations in obese children. PLoS One 12: e0185396, 2017. [PubMed: 28953935]
66. Daitoku H, Yamagata K, Matsuzaki H, Hatta M, Fukamizu A. Regulation of PGC-1 promoter activity by protein kinase B and the forkhead transcription factor FKHR. Diabetes 52: 642–649, 2003. [PubMed: 12606503]
67. Dashti N, Williams DL, Alaupovic P. Effects of oleate and insulin on the production rates and cellular mRNA concentrations of apolipoproteins in HepG2 cells. J Lipid Res 30: 1365–1373, 1989. [PubMed: 2689548]
68. Day CP, James OFW. Steatohepatitis: A tale of two 'Hits'? Gastroenterology 114: 842–845, 1998. [PubMed: 9547102]
69. De Ceuninck F, Kargar C, Ilic C, Caliez A, Rolin J-O, Umbdenstock T, Vinson C, Combettes M, de Fanti B, Harley E, Sadlo M, Lefèvre A-L, Broux O, Wierzbicki M, Fourquez J-M, Perron-Sierra F, Kotschy A, Ktorza A. Small molecule glucokinase activators disturb lipid homeostasis and induce fatty liver in rodents: A warning for therapeutic applications in humans. Br J Pharmacol 168: 339–353, 2013. [PubMed: 22925001]
70. Denechaud P-D, Bossard P, Lobaccaro J-MA, Millatt L, Staels B, Girard J, Postic C. ChREBP, but not LXRs, is required for the induction of glucose-regulated genes in mouse liver. J Clin Invest 118: 956–964, 2008. [PubMed: 18292813]
71. Dentin R, Benhamed F, Hainault I, Fauveau V, Foufelle F, Dyck JRB, Girard J, Postic C. Liver-specific inhibition of ChREBP improves hepatic steatosis and insulin resistance in ob/ob mice. Diabetes 55: 2159–2170, 2006. [PubMed: 16873678]
72. Deprince A, Haas JT, Staels B. Dysregulated lipid metabolism links NAFLD to cardiovascular disease. Mol Metab 42: 101092, 2020. DOI: 10.1016/j.molmet.2020.101092. [PubMed: 33010471]

73. Detheux M, Vandercammen A, Schaftingen EVAN. Effectors of the regulatory protein acting on liver glucokinase: A kinetic investigation. Eur J Biochem 200: 553–561, 1991. [PubMed: 1889418]
74. Dibble CC, Manning BD. Signal integration by mTORC1 coordinates nutrient input with biosynthetic output. Nat Cell Biol 15: 555–564, 2013. [PubMed: 23728461]
75. Doege H, Baillie RA, Ortegon AM, Tsang B, Wu Q, Punreddy S, Hirsch D, Watson N, Gimeno RE, Stahl A. Targeted deletion of FATP5 reveals multiple functions in liver metabolism: Alterations in hepatic lipid homeostasis. Gastroenterology 130: 1245–1258, 2006. [PubMed: 16618416]
76. Dong XC, Copps KD, Guo S, Li Y, Kollipara R, DePinho RA, White MF. Inactivation of hepatic foxo1 by insulin signaling is required for adaptive nutrient homeostasis and endocrine growth regulation. Cell Metab 8: 65–76, 2008. DOI: 10.1016/j.cmet.2008.06.006. [PubMed: 18590693]
77. Donnelly KL, Smith CI, Schwarzenberg SJ, Jessurun J, Boldt MD, Parks EJ. Sources of fatty acids stored in liver and secreted via lipoporteins in patients with NAFLD. J Clin Invest 115: 1343–1351, 2005. [PubMed: 15864352]
78. Drel VR, Mashtalir N, Ilnytska O, Shin J, Li F, Lyzogubov VV, Obrosova IG. The leptin-deficient (ob/ob) mouse: A new animal model of peripheral neuropathy of type 2 diabetes and obesity. Diabetes 55: 3335–3343, 2006. [PubMed: 17130477]
79. Easton RM, Cho H, Roovers K, Shineman DW, Mizrahi M, Forman MS, Lee VM-Y, Szabolcs M, de Jong R, Oltersdorf T, Ludwig T, Efstratiadis A, Birnbaum MJ. Role for Akt3/protein kinase B in attainment of normal brain size. Mol Cell Biol 25: 1869–1878, 2005. [PubMed: 15713641]
80. Ehrhardt N, Doche ME, Chen S, Mao HZ, Walsh MT, Bedoya C, Guindi M, Xiong W, Irudayam JI, Iqbal J, Fuchs S, French SW, Hussain MM, Arditi M, Arumugaswami V, Péterfy M. Hepatic Tm6sf2 overexpression affects cellular ApoB-trafficking, plasma lipid levels, hepatic steatosis and atherosclerosis. Hum Mol Genet 26: 2719–2731, 2017. [PubMed: 28449094]
81. Elliott SS, Keim NL, Stern JS, Teff K, Havel PJ. Fructose, weight gain, and the insulin resistance syndrome. Am J Clin Nutr 76: 11–22, 2002.
82. Elshourbagy NA, Near JC, Kmetz PJ, Sathe GM, Southan C, Strickler JE, Gross M, Young JF, Wells TN, Groot PH. Rat ATP citrate-lyase. Molecular cloning and sequence analysis of a full-length cDNA and mRNA abundance as a function of diet, organ, and age. J Biol Chem 265: 1430–1435, 1990. [PubMed: 2295639]
83. Engelman JA, Luo J, Cantley LC. The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. Nat Rev Genet 7: 606–619, 2006. [PubMed: 16847462]
84. Erion DM, Lapworth A, Amor PA, Bai G, Vera NB, Clark RW, Yan Q, Zhu Y, Ross TT, Purkal J, Gorgoglione M, Zhang G, Bonato V, Baker L, Barucci N, D'Aquila T, Robertson A, Aiello RJ, Yan J, Trimmer J, Rolph TP, Pfefferkorn JA. The hepatoselective glucokinase activator PF-04991532 ameliorates hyperglycemia without causing hepatic steatosis in diabetic rats. PLoS One 9: e97139, 2014. [PubMed: 24858947]
85. Faeh D, Minehira K, Schwarz J-M, Periasamy R, Park S, Tappy L. Effect of fructose overfeeding and fish oil administration on hepatic de novo lipogenesis and insulin sensitivity in healthy men. Diabetes 54: 1907–1913, 2005. [PubMed: 15983189]
86. Falcon A, Doege H, Fluitt A, Tsang B, Watson N, Kay MA, Stahl A. FATP2 is a hepatic fatty acid transporter and peroxisomal very long-chainacyl-CoA synthetase. Am J Physiol Endocrinol Metab 299: E384–E393, 2010. [PubMed: 20530735]
87. Farquhar JW, Frank A, Gross RC, Reaven GM. Glucose, insulin, and triglyceride responses to high and low carbohydrate diets in man. J Clin Invest 45: 1648–1656, 1966. [PubMed: 4288715]
88. Fast DG, Vance DE. Nascent VLDL phospholipid composition is altered when phosphatidylcholine biosynthesis is inhibited: Evidence for a novel mechanism that regulates VLDL secretion. Biochim Biophys Acta (BBA)/Lipids Lipid Metab 1258: 159–168, 1995.
89. Finck BN, Kelly DP. PGC-1 coactivators: Inducible regulators of energy metabolism in health and disease. J Clin Invest 116: 615–622, 2006. [PubMed: 16511594]
90. Finocchiaro G, Ito M, Tanaka K. Purification and properties of short chain acyl-CoA, medium chain acyl-CoA, and isovaleryl-CoA dehydrogenases from human liver. J Biol Chem 262: 7982–7989, 1987. [PubMed: 3597357]

91. Fisher FM, Kim MS, Doridot L, Cunniff JC, Parker TS, Levine DM, Hellerstein MK, Hudgins LC, Maratos-Flier E, Herman MA. A critical role for ChREBP-mediated FGF21 secretion in hepatic fructose metabolism. Mol Metab 6: 14–21, 2017. [PubMed: 28123933]
92. Flier JS, Maratos-Flier E. Leptin's physiologic role: Does the emperor of energy balance have no clothes? Cell Metab 26: 24–26, 2017. [PubMed: 28648981]
93. Folli F, Saad MJA, Backer JM, Kahn CR. Insulin stimulation of phosphatidylinositol 3-kinase activity and association with insulin receptor substrate 1 in liver and muscle of the intact rat. J Biol Chem 267: 22171–22177, 1992. [PubMed: 1385396]
94. Foretz M, Guichard C, Ferre P, Foufelle F. Sterol regulatory element binding protein-1c is a major mediator of insulin action on the hepatic expression of glucokinase and lipogenesis- related genes. PNAS 96: 12737–12742, 1999. [PubMed: 10535992]
95. Foretz M, Pacot C, Dugail I, Lemarchand P, Guichard C, Le Lièpvre X, Berthelier-Lubrano C, Spiegelman B, Kim JB, Ferré P, Foufelle F. ADD1/SREBP-1c is required in the activation of hepatic lipogenic gene expression by glucose. Mol Cell Biol 19: 3760–3768, 2015.
96. Fukuda H, Katsurada A, Iritani N. Effects of nutrients and hormones on gene expression of ATP citrate-lyase in rat liver. Eur J Biochem 209: 217–222, 1992. [PubMed: 1396700]
97. Furuta S, Mayazawa S, Hashimoto T. Purification and properties of rat liver acyl-CoA dehydrogenases and electron transfer flavoprotein. J Biochem 90: 1739–1750, 1981. [PubMed: 7334008]
98. Furuta S, Miyazawa S, Osumi T, Hashimoto T, Ui N. Properties of mitochondrial and peroxisomal enoyl-CoA hydratases from rat liver1. J Biochem 88: 1059–1070, 1980. [PubMed: 6778855]
99. Ganley IG, Lam DH, Wang J, Ding X, Chen S, Jiang X. ULK1·ATG13·FIP200 complex mediates mTOR signaling and is essential for autophagy. J Biol Chem 284: 12297–12305, 2009. [PubMed: 19258318]
100. George S, Rochford JJ, Wolfrum C, Gray SL, Schinner S, Wilson JC, Soos MA, Murgatroyd PR, Williams RM, Acerini CL, Dunger DB, Barford D, Umpleby AM, Wareham NJ, Davies HA, Schafer AJ, Stoffel M, O'Rahilly S, Barroso I. A family with severe insulin resistance and diabetes due to a mutation in AKT2. Science (1979) 304: 1325–1328, 2004.
101. Thampy KG, Wakil SJ. Regulation of acetyl-coenzyme A carboxylase. Purification and properties of two forms of acetyl. J Biol Chem 263: 6447–6453, 1988. [PubMed: 2896193]
102. Thampy KG, Wakil SJ. Regulation of acetyl-coenzyme A carboxylase. Effect of fasting and refeeding on the activity, phosphate content, and aggregation state of the enzyme. J Biol Chem 263: 6454–6458, 1988. [PubMed: 2896194]
103. Ghadiminejad I, David Saggekson E. The relationship of rat liver overt carnitine palmitoyltransferase to the mitochondrial malonyl-CoA binding entity and to the latent palmitoyltransferase. Biochem J 270: 787–794, 1990. [PubMed: 2241911]
104. Gil G, Faust JR, Chin DJ, Goldstein JL, Brown MS. Membrane-bound domain of HMG CoA reductase is required for sterol-enhanced degradation of the enzyme. Cell 41: 249–258, 1985. [PubMed: 3995584]
105. Ginsberg HN. Synthesis and secretion of apolipoprotein B from cultured liver cells. Curr Opin Lipidol 6: 275–280, 1995. [PubMed: 8520849]
106. Giorgetti S, Ballotti R, Kowalski-Chauvel A, Tartare S, Van Obberghen E. The insulin and insulin-like growth factor-I receptor substrate IRS-1 associates with and activates phosphatidylinositol 3-kinase in vitro. J Biol Chem 268: 7358–7364, 1993. [PubMed: 8385105]
107. Gluchowski NL, Gabriel KR, Chitraju C, Bronson RT, Mejhert N, Boland S, Wang K, Lai ZW, Farese RV Jr, Walther TC. Hepatocyte deletion of triglyceride-synthesis enzyme acyl CoA: Diacylglycerol acyltransferase 2 reduces steatosis without increasing inflammation or fibrosis in mice. Hepatology 70: 1972–1985, 2019. [PubMed: 31081165]
108. Goldstein JL, Brown MS. Familial hypercholesterolemia: Identification of a defect in the regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity associated with overproduction of cholesterol. Proc Natl Acad Sci U S A 70: 2804–2808, 1973. [PubMed: 4355366]

109. Goldstein JL, Brunschede GY, Brown MS. Inhibition of the proteolytic degradation of low density lipoprotein in human fibroblasts by chloroquine, concanavalin A, and triton WR 1339. J Biol Chem 250: 7854–7862, 1975. [PubMed: 170273]
110. Goldstein JL, Rawson RB, Brown MS. Mutant mammalian cells as tools to delineate the sterol regulatory element-binding protein pathway for feedback regulation of lipid synthesis. Arch Biochem Biophys 397: 139–148, 2002. [PubMed: 11795864]
111. Goldsteins JL, Brown MS. Binding and degradation of low density lipoproteins by cultured human fibroblasts. J Biol Chem 249: 5152–5162, 1974.
112. Gordon DA, Jamil H. Progress towards understanding the role of microsomal triglyceride transfer protein in apolipoprotein-B lipoprotein assembly. Biochim Biophys Acta Mol Cell Biol Lipids 1486: 72–83, 2000.
113. Grefhorst A, Schreurs M, Oosterveer MH, Cortés VA, Havinga R, Herling AW, Reijngoud D-J, Groen AK, Kuipers F. Carbohydrate-response-element-binding protein (ChREBP) and not the liver X receptor α (LXRα) mediates elevated hepatic lipogenic gene expression in a mouse model of glycogen storage disease type 1. Biochem J 432: 249–254, 2010. [PubMed: 20854262]
114. Guo L, Guo Y-Y, Li B-Y, Peng W-Q, Chang X-X, Gao X, Tang Q-Q. Enhanced acetylation of ATP-citrate lyase promotes the progression of nonalcoholic fatty liver disease. J Biol Chem 294: 11805–11816, 2019. [PubMed: 31197036]
115. Gutiérrez-Juárez R, Pocai A, Mulas C, Ono H, Bhanot S, Monia BP, Rossetti L. Critical role of stearoyl-CoA desaturase-1 (SCD1) in the onset of diet-induced hepatic insulin resistance. J Clin Invest 116: 1686–1695, 2006. [PubMed: 16741579]
116. Haas JT, Miao J, Chanda D, Wang Y, Zhao E, Haas ME, Hirschey M, Vaitheesvaran B, Farese RV Jr, Kurland IJ, Graham M, Crooke R, Foufelle F, Biddinger SB. Hepatic insulin signaling is required for obesity-dependent expression of SREBP-1c mRNA but not for feeding-dependent expression. Cell Metab 15: 873–884, 2012. [PubMed: 22682225]
117. Haas ME, Attie AD, Biddinger SB. The regulation of ApoB metabolism by insulin. Trends Endocrinol Metab 24: 391–397, 2013. [PubMed: 23721961]
118. Haeusler RA, Hartil K, Vaitheesvaran B, Arrieta-Cruz I, Knight CM, Cook JR, Kammoun HL, Febbraio MA, Gutierrez-Juarez R, Kurland IJ, Accili D. Integrated control of hepatic lipogenesis versus glucose production requires FoxO transcription factors. Nat Commun 5: 1–8, 2014.
119. Hall CL, Kamin H. The purification and some properties of electron transfer flavoprotein and general fatty acyl coenzyme A dehydrogenase from pig liver mitochondria. J Biol Chem 250: 3476–3486, 1975. [PubMed: 1168197]
120. Han S, Liang C-P, Westerterp M, Senokuchi T, Welch CL, Wang Q, Matsumoto M, Accili D, Tall AR. Hepatic insulin signaling regulates VLDL secretion and atherogenesis in mice. J Clin Invest 119: 1029–1041, 2009. [PubMed: 19273907]
121. Harada N, Oda Z, Hara Y, Fujinami K, Okawa M, Ohbuchi K, Yonemoto M, Ikeda Y, Ohwaki K, Aragane K, Tamai Y, Kusunoki J. Hepatic de novo lipogenesis is present in liver-specific ACC1-deficient mice. Mol Cell Biol 27: 1881–1888, 2007. [PubMed: 17210641]
122. Harrington LS, Findlay GM, Gray A, Tolkacheva T, Wigfield S, Rebholz H, Barnett J, Leslie NR, Cheng S, Shepherd PR, Gout I, Downes CP, Lamb RF. The TSC1-2 tumor suppressor controls insulin–PI3K signaling via regulation of IRS proteins. J Cell Biol 166: 213, 2004. [PubMed: 15249583]
123. He L, Hou X, Kanel G, Zeng N, Galicia V, Wang Y, Yang J, Wu H, Birnbaum MJ, Stiles BL. The critical role of AKT2 in hepatic steatosis induced by PTEN loss. Am J Pathol 176: 2302–2308, 2010. [PubMed: 20348245]
124. Hegarty BD, Bobard A, Hainault I, Ferré P, Bossard P, Foufelle F. Distinct roles of insulin and liver X receptor in the induction and cleavage of sterol regulatory element-binding protein-1c. Proc Natl Acad Sci U S A 102: 791–796, 2005. [PubMed: 15637161]
125. Heinz F, Lamprecht W, Kirsch J. Enzymes of fructose metabolism in human liver. J Clin Invest 47: 1826–1832, 1968. [PubMed: 4385849]
126. Heredia VV, Thomson J, Nettleton D, Sun S. Glucose-induced conformational changes in glucokinase mediate allosteric regulation: Transient kinetic analysis. Biochemistry 45: 7553–7562, 2006. [PubMed: 16768451]

127. Herzig S, Long F, Jhala US, Hedrick S, Quinn R, Bauer A, Rudolph D, Schutz G, Yoon C, Puigserver P, Spiegelman B, Montminy M. CREB regulates hepatic gluconeogenesis through the coactivator PGC-1. Nature 413: 179–183, 2001. [PubMed: 11557984]

128. Himsworth HP. Diabetes mellitus its differentiation into insulin-sensitive and insulin-insensitive types. Lancet 227: 127–130, 1936.

129. Hoppel CL, Tomec R. Carnitine palmityltransferase location of two enzymatic activities in rat liver mitochondria. J Biol Chem 247: 832–841, 1972. [PubMed: 5058225]

130. Horie Y, Suzuki A, Kataoka E, Sasaki T, Hamada K, Sasaki J, Mizuno K, Hasegawa G, Kishimoto H, Iizuka M, Naito M, Enomoto K, Watanabe S, Mak TW, Nakano T. Hepatocyte-specific Pten deficiency results in steatohepatitis and hepatocellular carcinomas. J Clin Invest 113: 1774–1783, 2004. [PubMed: 15199412]

131. Horton JD, Shimomura I, Brown MS, Hammer RE, Goldstein JL, Shimano H. Activation of cholesterol synthesis in preference to fatty acid synthesis in liver and adipose tissue of transgenic mice overproducing sterol regulatory element-binding protein-2. J Clin Invest 101: 2331–2339, 1998. [PubMed: 9616204]

132. Hosaka T, Biggs WH 3rd, Tieu D, Boyer AD, Varki NM, Cavenee WK, Arden KC. Disruption of forkhead transcription factor (FOXO) family members in mice reveals their functional diversification. Proc Natl Acad Sci U S A 101: 2975–2980, 2004. [PubMed: 14978268]

133. Hruby A, Hu FB. The epidemiology of obesity: A big picture. Pharmacoeconomics 33: 673–689, 2015. [PubMed: 25471927]

134. Hsiao PJ, Lee M-Y, Wang Y-T, Jiang H-J, Lin P-C, Yang Y-HC, Kuo K-K. MTTP-297H polymorphism reduced serum cholesterol but increased risk of non-alcoholic fatty liver disease-a cross-sectional study. BMC Med Genet 16: 93, 2015. [PubMed: 26458397]

135. Hsu P, Kang SA, Rameseder J, Zhang Y, Ottina KA, Lim D, Peterson TR, Choi Y, Gray NS, Yaffe MB, Marto JA, Sabatini DM. The mTOR-regulated phosphoproteome reveals a mechanism of mTORC1-mediated inhibition of growth factor signaling. Science (1979) 332: 1317–1322, 2011.

136. Hua X, Nohturfft A, Goldstein JL, Brown MS. Sterol resistance in CHO cells traced to point mutation in SREBP cleavage–activating protein xianxin. Cell 87: 415–426, 1996. [PubMed: 8898195]

137. Iizuka K, Bruick RK, Liang G, Horton JD, Uyeda K. Deficiency of carbohydrate response element-binding protein (ChREBP) reduces lipogenesis as well as glycolysis. Proc Natl Acad Sci U S A 101: 7281–7286, 2004. [PubMed: 15118080]

138. Iizuka K, Miller B, Uyeda K. Deficiency of carbohydrate-activated transcription factor ChREBP prevents obesity and improves plasma glucose control in leptin-deficient (ob/ob) mice. Am J Physiol Endocrinol Metab 291: 358–364, 2006.

139. Inoki K, Zhu T, Guan KL. TSC2 mediates cellular energy response to control cell growth and survival. Cell 115: 577–590, 2003. [PubMed: 14651849]

140. Ito Y, Azrolan N, O'Connell A, Walsh A, Breslow JL. Hypertriglyceridemia as a result of human apo CIII gene expression in transgenic mice. Science (1979) 249: 790–793, 1990.

141. Iynedjian PB. Molecular physiology of mammalian glucokinase. Cell Mol Life Sci 66: 27–42, 2009. [PubMed: 18726182]

142. Iynedjian PB, Jotterand D, Nouspikel T, Asfari M, Pilot PR. Transcriptional induction of glucokinase gene by insulin in cultured liver cells and its repression by the glucagon-cAMP system. J Biol Chem 264: 21824–21829, 1989. [PubMed: 2557341]

143. Jacobs RL, Devlin C, Tabas I, Vance DE. Targeted deletion of hepatic CTP:phosphocholine cytidylyltransferase α in mice decreases plasma high density and very low density lipoproteins. J Biol Chem 279: 47402–47410, 2004. [PubMed: 15331603]

144. Jacobs RL, Lingrell S, Zhao Y, Francis GA, Vance DE. Hepatic CTP:phosphocholine cytidylyltransferase-α is a critical predictor of plasma high density lipoprotein and very low density lipoprotein. J Biol Chem 283: 2147–2155, 2008. [PubMed: 18042552]

145. Jaeschke A, Hartkamp J, Saitoh M, Roworth W, Nobukuni T, Hodges A, Sampson J, Thomas G, Lamb R. Tuberous sclerosis complex tumor suppressor-mediated S6 kinase inhibition by phosphatidylinositide-3-OH kinase is mTOR independent. J Cell Biol 159: 217–224, 2002. [PubMed: 12403809]

146. Jang C, Hui S, Lu W, Cowan AJ, Morscher RJ, Lee G, Liu W, Tesz GJ, Birnbaum MJ, Rabinowitz JD. The small intestine converts dietary fructose into glucose and organic acids. Cell Metab 27: 351–361, 2018. [PubMed: 29414685]

147. Kaestner KH, NtambiS JM, Kelly TJ, Daniel Lane M. Differentiation-induced gene expression in 3T3-L1 preadipocytes: A second differentially expressed gene encoding stearoyl-CoA desaturase. J Biol Chem 264: 14755–14761, 1989. [PubMed: 2570068]

148. Kamagate A, Qu S, Perdomo G, Su D, Kim DH, Slusher S, Meseck M, Dong HH. FoxO1 mediates insulin-dependent regulation of hepatic VLDL production in mice. J Clin Invest 118: 2347–2364, 2008. [PubMed: 18497885]

149. Karim M, Jackson P, Jackowski S. Gene structure, expression and identification of a new CTP:phosphocholine cytidylyltransferase β isoform. Biochim Biophys Acta Mol Cell Biol Lipids 1633: 1–12, 2003.

150. Kathiresan S, Melander O, Guiducci C, Surti A, Burtt NP, Rieder MJ, Cooper GM, Roos C, Voight BF, Havulinna AS, Wahlstrand B, Hedner T, Corella D, Tai ES, Ordovas JM, Berglund G, Vartiainen E, Jousilahti P, Hedblad B, Taskinen M-R, Newton-Cheh C, Salomaa V, Peltonen L, Groop L, Altshuler DM, Orho-Melander M. Six new loci associated with blood low-density lipoprotein cholesterol, high-density lipoprotein cholesterol or triglycerides in humans. Nat Genet 40: 189–197, 2008. [PubMed: 18193044]

151. Katsurada A, Iritani N, Fukuda H, Matsumura Y, Nishimoto N, Noguchi T, Tanaka T. Effects of nutrients and hormones on transcriptional and post-transcriptional regulation of acetyl-CoA carboxylase in rat liver. Eur J Biochem 190: 435–441, 1990. [PubMed: 1973097]

152. Ke R, Xu Q, Li C, Luo L, Huang D. Mechanisms of AMPK in the maintenance of ATP balance during energy metabolism. Cell Biol Int 42: 384–392, 2018. [PubMed: 29205673]

153. Kenerson HL, Subramanian S, McIntyre R, Kazami M, Yeung RS. Livers with constitutive mTORC1 activity resist steatosis independent of feedback suppression of akt. PLoS One 10: 1–17, 2015.

154. Kennedy EP, Weiss SB. The function of cytidine coenzyme in the biosynthesis of phospholipides. J Biol Chem 222: 193–214, 1956. [PubMed: 13366993]

155. Kersten S, Seydoux J, Peters JM, Gonzalez FJ, Desvergne B, Wahli W. Peroxisome proliferator-activated receptor alpha mediates the adaptive response to fasting. J Clin Invest 103: 1489–1498, 1999. [PubMed: 10359558]

156. Kim CW, Addy C, Kusunoki J, Anderson NN, Deja S, Fu X, Burgess SC, Li C, Ruddy M, Chakravarthy M, Previs S, Milstein S, Fitzgerald K, Kelley DE, Horton JD. Acetyl CoA carboxylase inhibition reduces hepatic steatosis but elevates plasma triglycerides in mice and humans: A bedside to bench investigation. Cell Metab 26: 394–406.e6, 2017. [PubMed: 28768177]

157. Kim E, Goraksha-Hicks P, Li L, Neufeld TP, Guan KL. Regulation of TORC1 by rag GTPases in nutrient response. Nat Cell Biol 10: 935–945, 2008. [PubMed: 18604198]

158. Kim JH, Lewin TM, Coleman RA. Expression and characterization of recombinant rat Acyl-CoA synthetases 1, 4, and 5. Selective inhibition by triacsin C and thiazolidinediones. J Biol Chem 276: 24667–24673, 2001. [PubMed: 11319222]

159. Kim K-H. Regulation of mammalian acetyl-coenzyme A carboxylase. Annu Rev Nutr 17: 77–99, 1997. [PubMed: 9240920]

160. Kim MS, Krawczyk SA, Doridot L, Fowler AJ, Wang JX, Trauger SA, Noh H-L, Kang HJ, Meissen JK, Blatnik M, Kim JK, Lai M, Herman MA. ChREBP regulates fructose-induced glucose production independently of insulin signaling. J Clin Invest 126: 4372–4386, 2016. [PubMed: 27669460]

161. Kim T-H, Kim H, Park J-M, Im S-S, Bae J-S, Kim M-Y, Yoon H-G, Cha J-Y, Kim K-S, Ahn Y-H. Interrelationship between liver X receptor α, sterol regulatory element-binding protein-1c, peroxisome proliferator-activated receptor γ, and small heterodimer partner in the transcriptional regulation of glucokinase gene expression in liver. J Biol Chem 284: 15071–15083, 2009. [PubMed: 19366697]

162. Kliewer SA, Forman BM, Blumberg B, Ong ES, Borgmeyer U, Mangelsdorf DJ, Umesono K, Evans RM. Differential expression and activation of a family of murine peroxisome proliferator-activated receptors. Proc Natl Acad Sci U S A 91: 7355, 1994. [PubMed: 8041794]
163. Knight ZA, Gonzalez B, Feldman ME, Zunder ER, Goldenberg DD, Williams O, Loewith R, Stokoe D, Balla A, Toth B, Balla T, Weiss WA, Williams RL, Shokat KM. A pharmacological map of the PI3-K family defines a role for p110α in insulin signaling. Cell 125: 733–747, 2006. [PubMed: 16647110]
164. Knottnerus SJG, Bleeker JC, Wüst RCI, Ferdinandusse S, IJlst L, Wijburg FA, Wanders RJA, Visser G, Houtkooper RH. Disorders of mitochondrial long-chain fatty acid oxidation and the carnitine shuttle. Rev Endocr Metab Disord 19: 93–106, 2018. [PubMed: 29926323]
165. Koo SH, Satoh H, Herzig S, Lee C-H, Hedrick S, Kulkarni R, Evans RM, Olefsky J, Montminy M. PGC-1 promotes insulin resistance in liver through PPAR-α-dependent induction of TRB-3. Nat Med 10: 530–534, 2004. [PubMed: 15107844]
166. Korn BS, Shimomura I, Bashmakov Y, Hammer RE, Horton JD, Goldstein JL, Brown MS. Blunted feedback suppression of SREBP processing by dietary cholesterol in transgenic mice expressing sterol-resistant SCAP(D443N). J Clin Invest 102: 2050–2060, 1998. [PubMed: 9854040]
167. Kozlitina J, Smagris E, Stender S, Nordestgaard BG, Zhou HH, Tybjærg-Hansen A, Vogt TF, Hobbs HH, Cohen JC. Exome-wide association study identifies a TM6SF2 variant that confers susceptibility to nonalcoholic fatty liver disease. Nat Genet 46: 352–356, 2014. [PubMed: 24531328]
168. Krammer J, Digel M, Ehehalt F, Stremmel W, Füllekrug J, Ehehalt R. Overexpression of CD36 and acyl-CoA synthetases FATP2, FATP4 and ACSL1 increases fatty acid uptake in human hepatoma cells. Int J Med Sci 8: 599, 2011. [PubMed: 22022213]
169. Kulkarni RN, Brüning JC, Winnay JN, Postic C, Magnuson MA, Kahn CR. Tissue-specific knockout of the insulin receptor in pancreatic β cells creates an insulin secretory defect similar to that in type 2 diabetes. Cell 96: 329–339, 1999. [PubMed: 10025399]
170. Kunau W-H, Dommes V, Schulzt H. B-oxidation of fatty acids in mitochondria, peroxisomes, and bacteria: A century of continued progress. Prog Lipid Res 34: 267–342, 1995. [PubMed: 8685242]
171. Kurtz DM, Rinaldo P, Rhead WJ, Tian L, Millington DS, Vockley J, Hamm DA, Brix AE, Lindsey JR, Pinkert CA, O'Brien WE, Wood PA. Targeted disruption of mouse long-chain acyl-CoA dehydrogenase gene reveals crucial roles for fatty acid oxidation. Proc Natl Acad Sci U S A 95: 15592–15597, 1998. [PubMed: 9861014]
172. Lagace TA, Curtis DE, Garuti R, McNutt MC, Park SW, Prather HB, Anderson NN, Ho YK, Hammer RE, Horton JD. Secreted PCSK9 decreases the number of LDL receptors in hepatocytes and in livers of parabiotic mice. J Clin Invest 116: 2995–3005, 2006. [PubMed: 17080197]
173. Lambert JE, Parks EJ. Postprandial metabolism of meal triglyceride in humans. Biochim Biophys Acta Mol Cell Biol Lipids 1821: 721–726, 2012.
174. Lambert JE, Ramos-Roman MA, Browning JD, Parks EJ. Increased de novo lipogenesis is a distinct characteristic of individuals with non-alcoholic fatty liver disease. Gastroenterology 146: 726–735, 2014. [PubMed: 24316260]
175. Lamming DW, Ye L, Katajisto P, Goncalves MD, Saitoh M, Stevens DM, Davis JG, Salmon AB, Richardson A, Ahima RS, Guertin DA, Sabatini DM, Baur JA. Rapamycin-induced insulin resistance is mediated by mTORC2 loss and uncoupled from longevity. Science (1979) 335: 1638–1643, 2012.
176. Laplante M, Sabatini DM. mTOR signaling in growth control and disease. Cell 149: 274–293, 2012. [PubMed: 22500797]
177. Lazar MA, Willson TM. Sweet dreams for LXR. Cell Metab 5: 159–161, 2007. [PubMed: 17339022]
178. Le W, Abbas AS, Sprecher H, Vockley J, Schulz H. Long-chain acyl-CoA dehydrogenase is a key enzyme in the mitochondrial L-oxidation of unsaturated fatty acids. Biochim Biophys Acta 1485: 121–128, 2000. [PubMed: 10832093]

179. Leavens KF, Easton RM, Shulman GI, Previs SF, Birnbaum MJ. Akt2 is required for hepatic lipid accumulation in models of insulin resistance. Cell Metab 10: 405–418, 2009. [PubMed: 19883618]
180. Lee K, Kerner J, Hoppel CL. Mitochondrial carnitine palmitoyltransferase 1a (CPT1a) is part of an outer membrane fatty acid transfer complex. J Biol Chem 286: 25655, 2011. [PubMed: 21622568]
181. Lee SS, Pineau T, Drago J, Lee EJ, Owens JW, Kroetz DL, Fernandez-Salguero PM, Westphal H, Gonzalez FJ. Targeted disruption of the alpha isoform of the peroxisome proliferator-activated receptor gene in mice results in abolishment of the pleiotropic effects of peroxisome proliferators. Mol Cell Biol 15: 3012, 1995. [PubMed: 7539101]
182. Leone TC, Lehman JJ, Finck BN, Schaeffer PJ, Wende AR, Boudina S, Courtois M, Wozniak DF, Sambandam N, Bernal-Mizrachi C, Chen Z, Holloszy JO, Medeiros DM, Schmidt RE, Saffitz JE, Abel ED, Semenkovich CF, Kelly DP. PGC-1α deficiency causes multi-system energy metabolic derangements: Muscle dysfunction, abnormal weight control and hepatic steatosis. PLoS Biol 3: 0672–0687, 2005.
183. Lettéron P, Sutton A, Mansouri A, Fromenty B, Pessayre D. Inhibition of microsomal triglyceride transfer protein: Another mechanism for drug-induced steatosis in mice. Hepatology 38: 133–140, 2003. [PubMed: 12829995]
184. Lewin TM, Kim JH, Granger DA, Vance JE, Coleman RA. Acyl-CoA synthetase isoforms 1, 4, and 5 are present in different subcellular membranes in rat liver and can be inhibited independently. J Biol Chem 276: 24674–24679, 2001. [PubMed: 11319232]
185. Lewis GF, Uffelman KD, Szeto LW, Weller B, Steiner G. Interaction between free fatty acids and insulin in the acute control of very low density lipoprotein production in humans. J Clin Invest 95: 158–166, 1995. [PubMed: 7814610]
186. Li J, Matye DJ, Li T. Insulin resistance induces posttranslational hepatic sortilin 1 degradation in mice. J Biol Chem 290: 11526–11536, 2015. [PubMed: 25805502]
187. Li LO, Ellis JM, Paich HA, Wang S, Gong N, Altshuller G, Thresher RJ, Koves TR, Watkins SM, Muoio DM, Cline GW, Shulman GI, Coleman RA. Liver-specific loss of long chain acyl-CoA synthetase-1 decreases triacylglycerol synthesis and beta-oxidation and alters phospholipid fatty acid composition. J Biol Chem 284: 27816–27826, 2009. [PubMed: 19648649]
188. Li LO, Mashek DG, An J, Doughman SD, Newgard CB, Coleman RA. Overexpression of rat long chain acyl-coa synthetase 1 alters fatty acid metabolism in rat primary hepatocytes. J Biol Chem 281: 37246–37255, 2006. [PubMed: 17028193]
189. Li S, Brown MS, Goldstein JL. Bifurcation of insulin signaling pathway in rat liver: mTORC1 required for stimulation of lipogenesis, but not inhibition of gluconeogenesis. Proc Natl Acad Sci U S A 107: 3441–3446, 2010. [PubMed: 20133650]
190. Li WW, Dammerman MM, Smith JD, Metzger S, Breslow JL, Leff T. Common genetic variation in the promoter of the human apo CIII gene abolishes regulation by insulin and may contribute to hypertriglyceridemia. J Clin Invest 96: 2601–2605, 1995. [PubMed: 8675624]
191. Li X, Monks B, Ge Q, Birnbaum MJ. Akt/PKB regulates hepatic metabolism by directly inhibiting PGC-1alpha transcription coactivator. Nature 447: 1012–1016, 2007. [PubMed: 17554339]
192. Li Z, Agellon LB, Allen TM, Umeda M, Jewell L, Mason A, Vance DE. The ratio of phosphatidylcholine to phosphatidylethanolamine influences membrane integrity and steatohepatitis. Cell Metab 3: 321–331, 2006. [PubMed: 16679290]
193. Liang G, Yang J, Horton JD, Hammer RE, Goldstein JL, Brown MS. Diminished hepatic response to fasting/refeeding and liver X receptor agonists in mice with selective deficiency of sterol regulatory element-binding protein-1c. J Biol Chem 277: 9520–9528, 2002. [PubMed: 11782483]
194. Lin J, Yang R, Tarr PT, Wu P-H, Handschin C, Li S, Yang W, Pei L, Uldry M, Tontonoz P, Newgard CB, Spiegelman BM. Hyperlipidemic effects of dietary saturated fats mediated through PGC-1β coactivation of SREBP. Cell 120: 261–273, 2005. [PubMed: 15680331]

195. Lin MC, Gordon D, Wetterau JR. Microsomal triglyceride transfer protein (MTP) regulation in HepG2 cells: Insulin negatively regulates MTP gene expression. J Lipid Res 36: 1073–1081, 1995. [PubMed: 7658155]

196. Linden AG, Li S, Choi HY, Fang F, Fukasawa M, Uyeda K, Hammer RE, Horton JD, Engelking LJ, Liang G. Interplay between ChREBP and SREBP-1c coordinates postprandial glycolysis and lipogenesis in livers of mice. J Lipid Res 59: 475–487, 2018. [PubMed: 29335275]

197. Lu M, Wan M, Leavens KF, Chu Q, Monks BR, Fernandez S, Ahima RS, Ueki K, Kahn CR, Birnbaum MJ. Insulin regulates liver metabolism *in vivo* in the absence of hepatic Akt and Foxo1. Nat Med 18: 388–395, 2012. [PubMed: 22344295]

198. Luukkonen PK, Juuti A, Sammalkorpi H, Penttilä AK, Orešič M, Hyötyläinen T, Arola J, Orho-Melander M, Yki-Järvinen H. MARC1 variant rs2642438 increases hepatic phosphatidylcholines and decreases severity of non-alcoholic fatty liver disease in humans. J Hepatol 73: 725–726, 2020. [PubMed: 32471727]

199. Luukkonen PK, Nick A, Hölttä-Vuori M, Thiele C, Isokuortti E, Lallukka-Brück S, Zhou Y, Hakkarainen A, Lundbom N, Peltonen M, Orho-Melander M, Orešič M, Hyötyläinen T, Hodson L, Ikonen E, Yki-Järvinen H. Human PNPLA3-I148M variant increases hepatic retention of polyunsaturated fatty acids. JCI Insight 4: e127902, 2019. [PubMed: 31434800]

200. Luukkonen PK, Tukiainen T, Juuti A, Sammalkorpi H, Haridas PAN, Niemelä O, Arola J, Orho-Melander M, Hakkarainen A, Kovanen PT, Dwivedi O, Groop L, Hodson L, Gastaldelli A, Hyötyläinen T, Orešič M, Yki-Järvinen H. Hydroxysteroid 17-β dehydrogenase 13 variant increases phospholipids and protects against fibrosis in nonalcoholic fatty liver disease. JCI Insight 5: e132158, 2020. [PubMed: 32161197]

201. Luukkonen PK, Zhou Y, Haridas PAN, Dwivedi OP, Hyötyläinen T, Ali A, Juuti A, Leivonen M, Tukiainen T, Ahonen L, Scott E, Palmer JM, Arola J, Orho-Melander M, Vikman P, Anstee QM, Olkkonen VM, Orešič M, Groop L, Yki-Järvinen H. Impaired hepatic lipid synthesis from polyunsaturated fatty acids in TM6SF2 E167K variant carriers with NAFLD. J Hepatol 67: 128–136, 2017. [PubMed: 28235613]

202. Ma XM, Blenis J. Molecular mechanisms of mTOR-mediated translational control. Nat Rev Mol Cell Biol 10: 307–318, 2009. [PubMed: 19339977]

203. Macri J, Adeli K. Studies on intracellular translocation of apolipoprotein B in a permeabilized HepG2 system. J Biol Chem 272: 7328–7337, 1997. [PubMed: 9054431]

204. Malik VS, Hu FB. Fructose and cardiometabolic health: What the evidence from sugar-sweetened beverages tells us. J Am Coll Cardiol 66:1615–1624, 2015. [PubMed: 26429086]

205. Manning BD, Logsdon MN, Lipovsky AI, Abbott D, Kwiatkowski DJ, Cantley LC. Feedback inhibition of Akt signaling limits the growth of tumors lacking Tsc2. Genes Dev 19: 1773–1778, 2005. [PubMed: 16027169]

206. Manning BD, Tee AR, Logsdon MN, Blenis J, Cantley LC. Identification of the tuberous sclerosis complex-2 tumor suppressor gene product tuberin as a target of the phosphoinositide 3-kinase/Akt pathway. Mol Cell 10: 151–162, 2002. [PubMed: 12150915]

207. Manning BD, Toker A. AKT/PKB signaling: Navigating the network. Cell 169: 381–405, 2017. DOI: 10.1016/j.cell.2017.04.001. [PubMed: 28431241]

208. Mao J, DeMayo FJ, Li H, Abu-Elheiga L, Gu Z, Shaikenov TE, Kordari P, Chirala SS, Heird WC, Wakil SJ. Liver-specific deletion of acetyl-CoA carboxylase 1 reduces hepatic triglyceride accumulation without affecting glucose homeostasis. Proc Natl Acad Sci U S A 103: 8552–8557, 2006. [PubMed: 16717184]

209. Markan KR, Naber MC, Ameka MK, Anderegg MD, Mangelsdorf DJ, Kliewer SA, Mohammadi M, Potthoff MJ. Circulating FGF21 is liver derived and enhances glucose uptake during refeeding and overfeeding. Diabetes 63: 4057–4063, 2014. [PubMed: 25008183]

210. Marrs JC, Anderson SL. Bempedoic acid for the treatment of dyslipidemia. Drugs Context 9: 6–5, 2020.

211. Mashek DG, Bornfeldt KE, Coleman RA, Berger J, Bernlohr DA, Black P, DiRusso CC, Farber SA, Guo W, Hashimoto N, Khodiyar V, Kuypers FA, Maltais LJ, Nebert DW, Renieri A, Schaffer JE, Stahl A, Watkins PA, Vasiliou V, Yamamoto TT. Revised nomenclature for the mammalian

long-chain acyl-CoA synthetase gene family. J Lipid Res 45: 1958–1961, 2004. [PubMed: 15292367]

212. Mashek DG, McKenzie MA, van Horn CG, Coleman RA. Rat long chain acyl-CoA synthetase 5 ncreases fatty acid uptake and partitioning to cellular triacylglycerol in McArdle-RH7777 cells. J Biol Chem281: 945–950, 2006. [PubMed: 16263710]
213. Matschinsky FM, Wilson DF. The central role of glucokinase in glucose homeostasis: A perspective 50 years after demonstrating the presence of the enzyme in islets of langerhans. Front Physiol 10: 148, 2019. [PubMed: 30949058]
214. Matschinsky FM, Zelent B, Doliba N, Li C. Glucokinase activators for diabetes therapy. Diabetes Care 34: S236–S243, 2010.
215. Matsubara Y, Narisawa K, Miyabayashi S, Tada K, Coates PM, Bachmann C, Elsas LJ 2nd, Pollitt RJ, Rhead WJ, Roe CR. Identification of a common mutation in patients with medium-chain acyl-CoA dehydrogenase deficiency. Biochem Biophys Res Commun 171: 498–505, 1990. [PubMed: 2393404]
216. Matsuda M, Korn BS, Hammer RE, Moon YA, Komuro R, Horton JD, Goldstein JL, Brown MS, Shimomura I. SREBP cleavage-activating protein (SCAP) is required for increased lipid synthesis in liver induced by cholesterol deprivation and insulin elevation. Genes Dev 15: 1206–1216, 2001. [PubMed: 11358865]
217. Mauvoisin D, Rocque G, Arfa O, Radenne A, Boissier P, Mounier C. Role of the pi3-kinase/mtor pathway in the regulation of the stearoyl coa desaturase (scd1) gene expression by insulin in liver. J Cell Commun Signal 1: 113–125, 2007. [PubMed: 18481202]
218. McGarry JD, Brown NF. The mitochondrial carnitine palmitoyltransferase system. Eur J Biochem 14: 1–14, 1997.
219. Memon RA, Fuller J, Moser AH, Smith PJ, Grunfeld C, Feingold KR. Regulation of putative fatty acid transporters and acyl-CoA synthetase in liver and adipose tissue in ob/ob mice. Diabetes 48: 121–127, 1999. [PubMed: 9892232]
220. Menon S, Dibble CC, Talbott G, Hoxhaj G, Valvezan AJ, Takahashi H, Cantley LC, Manning BD. Spatial control of the TSC complex integrates insulin and nutrient regulation of mTORC1 at the lysosome. Cell 156: 771–785, 2014. [PubMed: 24529379]
221. Miao J, Haas JT, Manthena P, Wang Y, Zhao E, Vaitheesvaran B, Kurland IJ, Biddinger SB. Hepatic insulin receptor deficiency impairs the SREBP-2 response to feeding and statins. J Lipid Res 55: 659–667, 2014. [PubMed: 24516236]
222. Miao J, Manthena PV, Haas ME, Ling AV, Shin D-J, Graham MJ, Crooke RM, Liu J, Biddinger SB. Role of insulin in the regulation of proprotein convertase subtilisin/kexin type 9. Arterioscler Thromb Vasc Biol 35: 1589–1596, 2015. [PubMed: 26023080]
223. Michael MD, Kulkarni RN, Postic C, Previs SF, Shulman GI, Magnuson MA, Ronald Kahn C. Loss of insulin signaling in hepatocytes leads to severe insulin resistance and progressive hepatic dysfunction. Mol Cell 6: 87–97, 2000. [PubMed: 10949030]
224. Middleton B. The existence of ketoacyl-CoA thiolases of differing properties and intracellular localization in ox liver. Biochem Biophys Res Commun 46: 508–515, 1972. [PubMed: 5057890]
225. Minehira K, Young SG, Villanueva CJ, Yetukuri L, Oresic M, Hellerstein MK, Farese RV Jr, Horton JD, Preitner F, Thorens B, Tappy L. Blocking VLDL secretion causes hepatic steatosis but does not affect peripheral lipid stores or insulin sensitivity in mice. J Lipid Res 49: 2038–2044, 2008. [PubMed: 18515909]
226. Mitro N, Mak PA, Vargas L, Godio C, Hampton E, Molteni V, Kreusch A, Saez E. The nuclear receptor LXR is a glucose sensor. Nature 445:219–223, 2007. [PubMed: 17187055]
227. Miyazawa S, Osumi T, Hashimoto T. The presence of a new 3-oxoacyl-CoA thiolase in rat liver peroxisomes. Eur J Biochem 103: 589–596, 1980. [PubMed: 6766863]
228. Molinaro A, Becattini B, Mazzoli A, Bleve A, Radici L, Maxvall I, Sopasakis VR, Molinaro A, Bäckhed F, Solinas G. Insulin-driven PI3K-AKT signaling in the hepatocyte is mediated by redundant PI3Kα and PI3Kβ activities and is promoted by RAS. Cell Metab 29:1400–1409.e5, 2019. [PubMed: 30982732]

229. Murthy MSR, Pande S v. Malonyl-CoA binding site and the overt carnitine palmitoyltransferase activity reside on the opposite sides of the outer mitochondrial membrane. Biochemistry 84: 378–382, 1987.

230. Musunuru K, Strong A, Frank-Kamenetsky M, Lee NE, Ahfeldt T, Sachs KV, Li X, Li H, Kuperwasser N, Ruda VM, Pirruccello JP, Muchmore B, Prokunina-Olsson L, Hall JL, Schadt EE, Morales CR, Lund-Katz S, Phillips MC, Wong J, Cantley W, Racie T, Ejebe KG, Orho-Melander M, Melander O, Koteliansky V, Fitzgerald K, Krauss RM, Cowan CA, Kathiresan S, Rader DJ. From noncoding variant to phenotype via SORT1 at the 1p13 cholesterol locus. Nature 466: 714–719, 2010. [PubMed: 20686566]

231. Nakae J, Biggs WH 3rd, Kitamura T, Cavenee WK, Wright CVE, Arden KC, Accili D. Regulation of insulin action and pancreatic β-cell function by mutated alleles of the gene encoding forkhead transcription factor Foxo1. Nat Genet 32: 245–253, 2002. [PubMed: 12219087]

232. Nakamura A, Terauchi Y. Present status of clinical deployment of glucokinase activators. J Diabetes Investig 6: 124–132, 2015.

233. Nestel PJ, Schreibman PH, Ahrens EH. Cholesterol metabolism in human obesity. J Clin Invest 52: 2389, 1973. [PubMed: 4729038]

234. Niesen M, Bedi M, Lopez D. Diabetes alters LDL receptor and PCSK9 expression in rat liver. Arch Biochem Biophys 470: 111–115, 2008. [PubMed: 18054320]

235. Ntambi JM, Buhrow SA, Kaestner KH, Christy RJ, Sibley E, Kelly TJ Jr, Lane MD. Differentiation-induced gene expression in 3T3-L1 preadipocytes. Characterization of a differentially expressed gene encoding stearoyl-CoA desaturase. J Biol Chem 263: 17291–17300, 1988. [PubMed: 2903162]

236. O'Doherty RM, Lehman DL, Télémaque-Potts S, Newgard CB. Metabolic impact of glucokinase overexpression in liver: Lowering of blood glucose in fed rats is accompanied by hyperlipidemia. Diabetes 48: 2022–2027, 1999. [PubMed: 10512368]

237. O'Neill HM, Holloway GP, Steinberg GR. AMPK regulation of fatty acid metabolism and mitochondrial biogenesis: Implications for obesity. Mol Cell Endocrinol 366: 135–151, 2013. [PubMed: 22750049]

238. O'Neill HM, Lally JS, Galic S, Thomas M, Azizi PD, Fullerton MD, Smith BK, Pulinilkunnil T, Chen Z, Samaan MC, Jorgensen SB, Dyck JRB, Holloway GP, Hawke TJ, van Denderen BJ, Kemp BE, Steinberg GR. AMPK phosphorylation of ACC2 is required for skeletal muscle fatty acid oxidation and insulin sensitivity in mice. Diabetologia 57: 1693–1702, 2014. [PubMed: 24913514]

239. Olefsky JM, Farquhar JW, Reaven GM. Reappraisal of the role of insulin in hypertriglyceridemia. Am J Med 57: 551–560, 1974. [PubMed: 4372881]

240. Olson DP, Pulinilkunnil T, Cline GW, Shulman GI, Lowell BB. Gene knockout of Acc2 has little effect on body weight, fat mass, or food intake. Proc Natl Acad Sci U S A 107(16): 7598–7603, 2010. DOI: 10.1073/pnas.0913492107. [PubMed: 20368432]

241. Oncley JL, Gurd FRN, Melin M. Preparation and properties of serum and plasma proteins. XXV. Composition and properties of human serum β-lipoprotein1,2,3. J Am Chem Soc 72: 458–464, 2002.

242. Ono H, Shimano H, Katagiri H, Yahagi N, Sakoda H, Onishi Y, Anai M, Ogihara T, Fujishiro M, Viana AYI, Fukushima Y, Abe M, Shojima N, Kikuchi M, Yamada N, Oka Y, Asano T. Hepatic Akt activation induces marked hypoglycemia, hepatomegaly, and hypertriglyceridemia with sterol regulatory element binding protein involvement. Diabetes 52: 2905–2913, 2003. [PubMed: 14633850]

243. Orho-melander M, Melander O, Guiducci C, Perez-Martinez P, Corella D, Roos C, Tewhey R, Rieder MJ, Hall J, Abecasis G, Tai ES, Welch C, Arnett DK, Lyssenko V, Lindholm E, Saxena R, de Bakker PIW, Burtt N, Voight BF, Hirschhorn JN, Tucker KL, Hedner T, Tuomi T, Isomaa B, Eriksson K-F, Taskinen M-R, Wahlstrand B, Hughes TE, Parnell LD, Lai C-Q, Berglund G, Peltonen L, Vartiainen E, Jousilahti P, Havulinna AS, Salomaa V, Nilsson P, Groop L, Altshuler D, Ordovas JM, Kathiresan S. Common missense variant in the glucokinase regulatory protein gene is associated with increased plasma triglyceride and C-reactive protein but lower fasting glucose concentrations. Diabetes 57: 3112–3121, 2008. [PubMed: 18678614]

244. Osumi T, Hashimoto T. Purification and properties of mitochondrial and peroxisomal 3-hydroxyacyl-CoA dehydrogenase from rat liver. Arch Biochem Biophys 203: 372–383, 1980. [PubMed: 6773478]

245. Owen JL, Zhang Y, Bae S-H, Farooqi MS, Liang G, Hammer RE, Goldstein JL, Brown MS. Insulin stimulation of SREBP-1c processing in transgenic rat hepatocytes requires p70 S6-kinase. Proc Natl Acad Sci U S A 109: 16184–16189, 2012. [PubMed: 22927400]

246. Pande SV, Brivet M, Slama A, Demaugre F, Aufrant C, Saudubray JM. Carnitine-acylcarnitine translocase deficiency with severe hypoglycemia and auriculo ventricular block translocase assay in permeabilized fibroblasts. J Clin Invest 91: 1247–1252, 1993. [PubMed: 8450053]

247. Park SH, Gammon SR, Knippers JD, Paulsen SR, Rubink DS, Winder WW. Phosphorylation-activity relationships of AMPK and acetyl-CoA carboxylase in muscle. J Appl Physiol (1985) 92: 2475–2482, 2002. [PubMed: 12015362]

248. Parks EJ, Skokan LE, Timlin MT, Dingfelder CS. Dietary sugars stimulate fatty acid synthesis in adults. J Nutr 138: 1039–1046, 2008. [PubMed: 18492831]

249. Patsch W, Franz S, Schonfeld G. Role of insulin in lipoprotein secretion by cultured rat hepatocytes. J Clin Invest 71: 1161–1174, 1983. [PubMed: 6343434]

250. Patti ME, Sun XJ, Bruening JC, Araki E, Lipes MA, White MF, Kahn CR. 4PS/insulin receptor substrate (IRS)-2 is the alternative substrate of the insulin receptor in IRS-1-deficient mice. J Biol Chem 270:24670–24673, 1995. [PubMed: 7559579]

251. Pautsch A, Stadler N, Löhle A, Rist W, Berg A, Glocker L, Nar H, Reinert D, Lenter M, Heckel A, Schnapp G, Kauschke SG. Crystal structure of glucokinase regulatory protein. Biochemistry 52: 3523–3531, 2013. [PubMed: 23621087]

252. Peter A, Stefan N, Cegan A, Walenta M, Wagner S, Königsrainer A, Königsrainer I, Machicao F, Schick F, Häring H-U, Schleicher E. Hepatic glucokinase expression is associated with lipogenesis and fatty liver in humans. J Clin Endocrinol Metab 96: 1126–1130, 2011.

253. Peterson RT, Beal PA, Comb MJ, Schreiber SL. FKBP12-rapamycin-associated protein (FRAP) autophosphorylates at serine 2481 under translationally repressive conditions. J Biol Chem 275: 7416–7423, 2000. [PubMed: 10702316]

254. Pihlajamäki J, Gylling H, Miettinen TA, Laakso M. Insulin resistance is associated with increased cholesterol synthesis and decreased cholesterol absorption in normoglycemic men. J Lipid Res 45: 507–512, 2004. [PubMed: 14657199]

255. Pinkosky SL, Filippov S, Srivastava RAK, Hanselman JC, Bradshaw CD, Hurley TR, Cramer CT, Spahr MA, Brant AF, Houghton JL, Baker C, Naples M, Adeli K, Newton RS. AMP-activated protein kinase and ATP-citrate lyase are two distinct molecular targets for ETC-1002, a novel small molecule regulator of lipid and carbohydrate metabolism. J Lipid Res 54: 134–151, 2013. [PubMed: 23118444]

256. Pinkosky SL, Newton Rs, Day EA, Ford RJ, Lhotak S, Austin RC, Birch CM, Smith BK, Filippov S, Groot PHE, Steinberg GR, Lalwani ND. Liver-specific ATP-citrate lyase inhibition by bempedoic acid decreases LDL-C and attenuates atherosclerosis. Nat Commun 7: 13457, 2016. [PubMed: 27892461]

257. Pirola CJ, Garaycoechea M, Flichman D, Arrese M, Martino JS, Gazzi C, Castaño GO, Sookoian S. Splice variant rs72613567 prevents worst histologic outcomes in patients with nonalcoholic fatty liver disease. J Lipid Res 60: 176–185, 2019. [PubMed: 30323112]

258. Porstmann T, Santos CR, Griffiths B, Cully M, Wu M, Leevers S, Griffiths JR, Chung Y-L, Schulze A. SREBP activity is regulated by mTORC1 and contributes to Akt-dependent cell growth. Cell Metab 8: 224–236, 2008. [PubMed: 18762023]

259. Potapova IA, El-Maghrabi MR, Doronin SV, Benjamin WB. Phosphorylation of recombinant human ATP:citrate lyase by cAMP-dependent protein kinase abolishes homotropic allosteric regulation of the enzyme by citrate and increases the enzyme activity. Allosteric activation of atp:citrate lyase by phosphorylated sug. Biochemistry 39: 1169–1179, 2000. [PubMed: 10653665]

260. Powell LM, Wallis SC, Pease RJ, Edwards YH, Knott TJ, Scott J. A novel form of tissue-specific RNA processing produces apolipoprotein-B48 in intestine. Cell 50: 831–840, 1987. [PubMed: 3621347]

261. Puigserver P, Rhee J, Donovan J, Walkey CJ, Yoon JC, Oriente F, Kitamura Y, Altomonte J, Dong H, Accili D, Spiegelman BM. Insulin-regulated hepatic gluconeogenesis through FOXO1–PGC-1α interaction. Nature 423: 550–555, 2003. [PubMed: 12754525]
262. Qiang L, Banks AS, Accili D. Uncoupling of acetylation from phosphorylation regulates FoxO1 function independent of its subcellular localization. J Biol Chem 285: 27396–27401, 2010. [PubMed: 20519497]
263. Quarfordt SH, Michalopoulos G, Schirmer B. The effect of human C apolipoproteins on the in vitro hepatic metabolism of triglyceride emulsions in the rat. J Biol Chem 257: 14642–14647, 1982. [PubMed: 7174660]
264. Quinn WJ, Wan M, Shewale SV, Gelfer R, Rader DJ, Birnbaum MJ, Titchenell PM. mTORC1 stimulates phosphatidylcholine synthesis to promote triglyceride secretion. J Clin Invest 127: 4207–4215, 2017. [PubMed: 29035283]
265. Rader DJ. Effect of insulin resistance, dyslipidemia, and intraabdominal adiposity on the development of cardiovascular disease and diabetes mellitus. Am J Med 120: 12–18, 2007. [PubMed: 17208071]
266. Repa JJ, Liang G, Ou J, Bashmakov Y, Lobaccaro JM, Shimomura I, Shan B, Brown MS, Goldstein JL, Mangelsdorf DJ. Regulation of mouse sterol regulatory element-binding protein-1c gene (SREBP-1c) by oxysterol receptors, LXRa and LXRß. Genes Dev 14: 2819–2830, 2000. DOI: 10.1101/gad.844900.four. [PubMed: 11090130]
267. Rogala KB, Gu X, Kedir JF, Abu-Remaileh M, Bianchi LF, Bottino AMS, Dueholm R, Niehaus A, Overwijn D, Fils A-CP, Zhou SX, Leary D, Laqtom NN, Brignole EJ, Sabatini DM. Structural basis for the docking of mTORC1 on the lysosomal surface. Science (1979) 1: eaay0166, 2019.
268. Rong S, Cortés VA, Rashid S, Anderson NN, McDonald JG, Liang G, Moon Y-A, Hammer RE, Horton JD. Expression of SREBP-1c requires SREBP-2-mediated generation of a sterol ligand for LXR in livers of mice. Elife 6: 1–17, 2017.
269. Root HF. Insulin resistance and bronze diabetes. N Engl J Med 201: 201–206, 1929.
270. Roth U, Curth K, Unterman TG, Kietzmann T. The transcription factors HIF-1 and HNF-4 and the coactivator p300 are involved in insulin-regulated glucokinase gene expression via the phosphatidylinositol 3-kinase/protein kinase B pathway. J Biol Chem 279: 2623–2631, 2004. [PubMed: 14612449]
271. Ruderman NB, Kapeller R, White MF, Cantley LC. Activation of phosphatidylinositol 3-kinase by insulin. Proc Natl Acad Sci U S A 87: 1411–1415, 1990. [PubMed: 2154747]
272. Russel-Jones D, Lim K, Girousse A, Brown RJ, Kory N, Robbins A, Xue Y, Sleigh A, Cochran E, Adams C, Borman AD, Russel-Jones D, Gorden P, Semple RK, Saudek V, O'Rahilly S, Walther TC, Barroso I, Savage DB. Mutations disrupting the Kennedy phosphatidylcholine pathway in humans with congenital lipodystrophy and fatty liver disease. Proc Natl Acad Sci U S A 111: 8901–8906, 2014. [PubMed: 24889630]
273. Ruzicka FJ, Beinert H. A new iron-sulfur flavoprotein of the respiratory chain. A component of the fatty acid beta oxidation pathway. J Biol Chem 252: 8440–8445, 1977. [PubMed: 925004]
274. Saavedra YGL, Dufour R, Davignon J, Baass A. PCSK9 R46L, lower LDL, and cardiovascular disease risk in familial hypercholesterolemia a cross-sectional cohort study. Arterioscler Thromb Vasc Biol 34: 2700–2705, 2014. [PubMed: 25278291]
275. Sabatini DM, Erdjument-Bromage H, Lui M, Tempst P, Snyder SH. RAFT1: A mammalian protein that binds to FKBP12 in a rapamycin-dependent fashion and is homologous to yeast TORs. Cell 78: 35–43, 1994. [PubMed: 7518356]
276. Sabers CJ, Martin MM, Brunn GJ, Williams JM, Dumont FJ, Wiederrecht G, Abraham RT. Isolation of a protein target of FKBP12-rapamycin complex in mammalian cells. J Biol Chem 270: 815–822, 1995. [PubMed: 7822316]
277. Sahng WP, Moon YA, Horton JD. Post-transcriptional regulation of low density lipoprotein receptor protein by proprotein convertase subtilisin/kexin type 9a in mouse liver. J Biol Chem 279: 50630–50638, 2004. [PubMed: 15385538]
278. Sakai J, Nohturfft A, Goldstein JL, Brown MS. Cleavage of sterol regulatory element-binding proteins (SREBPs) at site-1 requires interaction with SREBP cleavage-activating protein. J Biol Chem 273: 5785–5793, 2002.

279. Sancak Y, Peterson TR, Shaul YD, Lindquist RA, Thoreen CC, Bar-Peled L, Sabatini DM. The rag GTPases bind raptor and mediate amino acid signaling to mTORC1. Science (1979) 320: 1496–1501, 2008.

280. Sanyal AJ, Campbell-Sargent C, Mirshahi F, Rizzo WB, Contos MJ, Sterling RK, Luketic VA, Shiffman ML, Clore JN. Nonalcoholic steatohepatitis: Association of insulin resistance and mitochondrial abnormalities. Gastroenterology 120: 1183–1192, 2001. [PubMed: 11266382]

281. Sarbassov DD, Ali SM, Sabatini DM. Growing roles for the mTOR pathway. Curr Opin Cell Biol 17: 596–603, 2005. [PubMed: 16226444]

282. Sarbassov DD, Guertin DA, Ali SM, Sabatini DM. Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. Science (1979) 307: 1098–1101, 2005.

283. Sarbassov DD, Ali SM, Sengupta S, Sheen J-H, Hsu PP, Bagley AF, Markhard AL, Sabatini DM. Prolonged rapamycin treatment inhibits mTORC2 assembly and Akt/PKB. Mol Cell 22(2): 159–168, 2006. [PubMed: 16603397]

284. Sato R, Okamoto A, Inoue J, Miyamoto W, Sakai Y, Emoto N, Shimano H, Maeda M. Transcriptional regulation of the ATP citrate-lyase gene by sterol regulatory element-binding proteins. J Biol Chem 275: 12497–12502, 2000. [PubMed: 10777536]

285. Schaffer JE, Lodish HF. Expression cloning and characterization of a novel adipocyte long chain fatty acid transport protein. Cell 79: 427–436, 1994. [PubMed: 7954810]

286. Schneider CV, Schneider KM, Conlon DM, Park J, Vujkovic M, Zandvakili I, Ko Y-A, Trautwein C, Center R, Carr RM, Strnad P, Thaiss CA, Rader DJ. A genome-first approach to mortality and metabolic phenotypes in MTARC1 homozygotes. Med 2:851–863.e3, 2021. DOI: 10.1016/j.medj.2021.04.011. [PubMed: 34258604]

287. Schoonjans K, Watanabe M, Suzuki H, Mahfoudi A, Krey G, Wahli W, Grimaldi P, Staels B, Yamamoto T, Auwerx J. Induction of the acylcoenzyme A synthetase gene by fibrates and fatty acids is mediated by a peroxisome proliferator response element in the C promoter. J Biol Chem 270: 19269–19276, 1995. [PubMed: 7642600]

288. Semple RK, Sleigh A, Murgatroyd PR, Adams CA, Bluck L, Jackson S, Vottero A, Kanabar D, Charlton-Menys V, Durrington P, Soos MA, Carpenter TA, Lomas DJ, Cochran EK, Gorden P, O'Rahilly S, Savage DB. Postreceptor insulin resistance contributes to human dyslipidemia and hepatic steatosis. J Clin Invest 119: 315–322, 2009. [PubMed: 19164855]

289. Sengupta S, Peterson TR, Laplante M, Oh S, Sabatini DM. mTORC1 controls fasting-induced ketogenesis and its modulation by ageing. Nature 468: 1100–1104, 2010. [PubMed: 21179166]

290. Shah OJ, Wang Z, Hunter T. Inappropriate activation of the TSC/Rheb/mTOR/S6K cassette induces IRS1/2 depletion, insulin resistance, and cell survival deficiencies. Curr Biol 14: 1650–1656, 2004. [PubMed: 15380067]

291. Sharp D, Blinderman L, Combs KA, Kienzle B, Ricci B, Wager-Smith K, Gil CM, Turck CW, Boumas M-E, Rader DJ, Aggerbeck LP, Gregg RE, Gordon DA, Wetterau JR. Cloning and gene defects in microsomal triglyceride transfer protein associated with abetalipoproteinaemia. Nature 365: 65–69, 1993. [PubMed: 8361539]

292. Shimano H, Horton JD, Shimomura I, Hammer RE, Brown MS, Goldstein JL. Isoform 1c of sterol regulatory element binding protein is less active than isoform 1a in livers of transgenic mice and in cultured cells. J Clin Invest 99: 846–854, 1997. [PubMed: 9062341]

293. Shimomura I, Bashmakov Y, Ikemoto S, Horton JD, Brown MS, Goldstein JL. Insulin selectively increases SREBP-1c mRNA in the livers of rats with streptozotocin-induced diabetes. Proc Natl Acad Sci U S A 96: 13656–13661, 2002.

294. Simard JR, Kamp F, Hamilton JA. Measuring the adsorption of fatty acids to phospholipid vesicles by multiple fluorescence probes. Biophys J 94: 4493–4503, 2008. [PubMed: 18296488]

295. Simonen PP, Gylling HK, Miettinen TA. Diabetes contributes to cholesterol metabolism regardless of obesity. Diabetes Care 25: 1511–1515, 2002. [PubMed: 12196419]

296. Simons N, Dekker JM, van Greevenbroek MMJ, Nijpels G, 't Hart LM, van der Kallen CJH, Schalkwijk CG, Schaper NC, Stehouwer CDA, Brouwers MCGJ. A common gene variant in glucokinase regulatory protein interacts with glucose metabolism on diabetic dyslipidemia: The combined CODAM and hoorn studies. Diabetes Care 39: 1811–1817, 2016. [PubMed: 27660121]

297. Smith GI, Shankaran M, Yoshino M, Schweitzer GG, Chondronikola M, Beals JW, Okunade AL, Patterson BW, Nyangau E, Field T, Sirlin CB, Talukdar S, Hellerstein MK, Klein S. Insulin resistance drives hepatic de novo lipogenesis in nonalcoholic fatty liver disease. J Clin Invest 130: 1453–1460, 2020. [PubMed: 31805015]
298. Softic S, Cohen DE, Kahn CR. Role of dietary fructose and hepatic de novo lipogenesis in fatty liver disease. Dig Dis Sci 61: 1282–1293, 2016. [PubMed: 26856717]
299. Soo Choi C, Savage DB, Abu-Elheiga L, Liu Z-X, Kim S, Kulkarni A, Distefano A, Hwang Y-J, Reznick RM, Codella R, Zhang D, Cline GW, Wakil SJ, Shulman GI. Continuous fat oxidation in acetyl-CoA carboxylase 2 knockout mice increases total energy expenditure, reduces fat mass, and improves insulin sensitivity. Proc Natl Acad Sci U S A 104: 16480–16485, 2007. DOI: 10.1073/pnas.0706794104. [PubMed: 17923673]
300. Sopasakis VR, Liu P, Suzuki R, Kondo T, Winnay J, Tran TT, Asano T, Smyth G, Sajan MP, Farese RV, Kahn CR, Zhao JJ. Specific roles of the p110α isoform of phosphatidylinsositol 3-kinase in hepatic insulin signaling and metabolic regulation. Cell Metab 11: 220–230, 2010. [PubMed: 20197055]
301. Souri M, Aoyama T, Yamaguchi S, Hashimoto T. Relationship between structure and substrate-chain-length specificity of mitochondrial very-long-chain acyl-coenzyme A dehydrogenase. Eur J Biochem 257: 592–598, 1998. [PubMed: 9839948]
302. Sparks JD, Chamberlain J, O'Dell C, Khatun I, Hussain MM, Sparks CE. Acute suppression of apo B secretion by insulin occurs independently of MTP. Biochem Biophys Res Commun 406: 252–256, 2011. [PubMed: 21316344]
303. Sparks JD, Sparks CE. Insulin modulation of hepatic synthesis and secretion of apolipoprotein B by rat hepatocytes. J Biol Chem 265: 8854–8862, 1990. [PubMed: 2187873]
304. Srere PA. The citrate enzymes: Their structures, mechanisms, and biological functions. Curr Top Cell Regul 5: 229–283, 1972. [PubMed: 4587829]
305. Stanhope KL, Schwarz JM, Keim NL, Griffen SC, Bremer AA, Graham JL, Hatcher B, Cox CL, Dyachenko A, Zhang W, McGahan jP, Seibert A, Krauss RM, Chiu S, Schaefer EJ, Ai M, Otokozawa S, Nakajima K, Nakano T, Beysen C, Hellerstein MK, Berglund L, Havel PJ. Consuming fructose-sweetened, not glucose- sweetened, beverages increases visceral adiposity and lipids and decreases insulin sensitivity in overweight/obese humans. J Clin Invest 119: 1322–1334, 2009. [PubMed: 19381015]
306. Stanley CA, Hale DE, Berry GT, Deleeuw S, Boxer J, Bonnefont JP. Brief report: A deficiency of carnitine-acylcarnitine translocase in the inner mitochondrial membrane. N Engl J Med 327: 19–23, 1992. [PubMed: 1598097]
307. Steenkamp DJ. Preferential cross-linking of the small subunit of the electron-transfer flavoprotein to general acyl-CoA dehydrogenase. Biochem J 243: 519–524, 1987. [PubMed: 3115254]
308. Steenkamp DJ. Cross-linking of the electron-transfer flavoprotein to electron-transfer flavoprotein-ubiquinone oxidoreductase with heterobifunctional reagents. Biochem J 255: 869–876, 1988. [PubMed: 3145738]
309. Steiner G, Haynes FJ, Yoshino G, Vranic M. Hyperinsulinemia and *in vivo* very-low-density lipoprotein-triglyceride kinetics. Am J Physiol Endocrinol Metab 246: E187–E192, 1984.
310. Sterols O, Brown MS, Dana SE, Goldsteins JL. Cholesterol ester formation in cultured human fibroblasts. Stimulation by oxygenated sterols. J Biol Chem 250: 4025–4027, 1975. [PubMed: 1126942]
311. Stone SJ, Levin MC, Farese RV Jr. Membrane topology and identification of key functional amino acid residues of murine acyl-CoA:diacylglycerol acyltransferase-2. J Biol Chem 281: 40273–40282, 2006. [PubMed: 17035227]
312. Stone SJ, Myers HM, Watkins SM, Brown BE, Feingold KR, Elias PM, Farese RV Jr. Lipopenia and skin barrier abnormalities in DGAT2-deficient mice. J Biol Chem 279: 11767–11776, 2004. [PubMed: 14668353]
313. Stremmel W, Strohmeyer G, Borchard F, Kochwat S, Berktf PD. Isolation and partial characterization of a fatty acid binding protein in rat liver plasma membranes (hepatocyte/hepatic uptake). Proc Natl Acad Sci U S A 82: 4–8, 1985. [PubMed: 3881757]

314. Sullivan AC, Triscari J, Hamilton JG, Miller ON, Wheatley VR. Effect of (−)-hydroxycitrate upon the accumulation of lipid in the rat: I lipogenesis. Lipids 9: 121–128, 1974. [PubMed: 4815799]
315. Sun XJ, Wang LM, Zhang Y, Yenush L, Myers MG Jr, Glasheen E, Lane WS, Pierce jH, White MF. Role of IRS-2 in insulin and cytokine signalling. Nature 377: 173–177, 1995. [PubMed: 7675087]
316. Swigoňová Z, Mohsen AW, Vockley J. Acyl-CoA dehydrogenases: Dynamic history of protein family evolution. J Mol Evol 69: 176–193, 2009. [PubMed: 19639238]
317. Syed-Abdul MM, Parks EJ, Gaballah AH, Bingham K, Hammoud GM, Kemble G, Buckley D, McCulloch W, Manrique-Acevedo C. Fatty acid synthase inhibitor TVB-2640 reduces hepatic de novo lipogenesis in males with metabolic abnormalities. Hepatology 72: 103–118, 2020. [PubMed: 31630414]
318. Taniguchi CM, Emanuelli B, Kahn CR. Critical nodes in signaling pathways: Insights into insulin action. Nat Rev Mol Cell Biol 7: 85–96, 2006. [PubMed: 16493415]
319. Taniguchi CM, Tran TT, Kondo T, Luo J, Ueki K, Cantley LC, Kahn CR. Phosphoinositide 3-kinase regulatory subunit p85α suppresses insulin action via positive regulation of PTEN. Proc Natl Acad Sci U S A 103: 12093–12097, 2006. [PubMed: 16880400]
320. Tao R, Wang C, Stöhr O, Qiu W, Hu Y, Miao J, Dong XC, Leng S, Stefater M, Stylopoulos N, Lin L, Copps KD, White MF. Inactivating hepatic follistatin alleviates hyperglycemia. Nat Med 24: 1058–1069, 2018. [PubMed: 29867232]
321. Tappy L, Le K-A. Metabolic effects of fructose and the worldwide increase in obesity. Physiol Rev 90: 23–46, 2010. [PubMed: 20086073]
322. Tian J, Goldstein JL, Brown MS. Insulin induction of SREBP-1c in rodent liver requires LXRα-c/EBPβ complex. Proc Natl Acad Sci U S A 113: 8182–8187, 2016. [PubMed: 27382175]
323. Titchenell PM, Chu Q, Monks BR, Birnbaum MJ. Hepatic insulin signalling is dispensable for suppression of glucose output by insulin *in vivo*. Nat Commun 6: 1–9, 2015.
324. Titchenell PM, Lazar MA, Birnbaum MJ. Unraveling the regulation of hepatic metabolism by insulin. Trends Endocrinol Metab 28: 497–505, 2017 [PubMed: 28416361]
325. Titchenell PM, Quinn WJ, Lu M, Chu Q, Lu W, Li C, Chen H, Monks BR, Chen J, Rabinowitz JD, Birnbaum MJ. Direct hepatocyte insulin signaling is required for lipogenesis but is dispensable for the suppression of glucose production. Cell Metab 23: 1154–1166, 2016. [PubMed: 27238637]
326. Topping DL, Mayes PA. The immediate effects of insulin and fructose on the metabolism of the perfused liver. Changes in lipoprotein secretion, fatty acid oxidation and esterification, lipogenesis and carbohydrate metabolism. Biochem J 126: 295–311, 1972. [PubMed: 5071176]
327. Tremblay F, Marette A. Amino acid and insulin signaling via the mTOR/p70 S6 kinase pathway. J Biol Chem 276: 38052–38060, 2001. [PubMed: 11498541]
328. Trépo E, Romeo S, Zucman-Rossi J, Nahon P. PNPLA3 gene in liver diseases. J Hepatol 65: 399–412, 2016. [PubMed: 27038645]
329. Uehara K, Sostre-Colón J, Gavin M, Santoleri D, Leonard K-A, Jacobs RL, Titchenell PM. Activation of liver mTORC1 protects against NASH via dual regulation of VLDL-TAG secretion and de novo lipogenesis. Cell Mol Gastroenterol Hepatol 13: 1625–1647, 2022. [PubMed: 35240344]
330. V Li M, Chang B, Imamura M, Poungvarin N, Chan L. Glucose-dependent transcriptional regulation by an evolutionarily conserved glucose-sensing module. Diabetes 55: 1179–1189, 2006. [PubMed: 16644671]
331. van Horn CG, Caviglia JM, Li LO, Wang S, Granger DA, Coleman RA. Characterization of recombinant long-chain rat acyl-CoA syncomthetase isoforms 3 and 6: Identification of a novel variant of isoform 6. Biochemistry 44: 1635–1642, 2005. [PubMed: 15683247]
332. Van Schaftingen E, Detheux M, Veiga Da Cunha V. Short-term control of glucokinase activity: Role of a regulatory protein. FASEB J 8: 414–419, 1994. [PubMed: 8168691]
333. Vandercammen A, van Schaftingen E. The mechanism by which rat liver glucokinase is inhibited by the regulatory protein. Eur J Biochem 191:483–489, 1990. [PubMed: 2384095]

334. Veiga-da-cunha M, Van Schaftingen E. Identification of fructose 6-phosphate-and fructose 1-phosphate- binding residues in the regulatory protein of glucokinase. J Biol Chem 277: 8466–8473, 2002. [PubMed: 11756407]
335. Vella A, Freeman JLR, Dunn I, Keller K, Buse JB, Valcarce C. Targeting hepatic glucokinase to treat diabetes with TTP399, a hepatoselective glucokinase activator. Sci Transl Med 11: eaau3441, 2019. [PubMed: 30651321]
336. Vézina C, Kudelski A. Rapamycin (AY-22,989), a new antifungal antibiotic. I. Taxonomy of the producing streptomycete and isolation of the active principle. J Antibiot (Tokyo) 28: 721–726, 1975. [PubMed: 1102508]
337. Volpe JJ, Vagelos PR. Mechanisms and regulation of biosynthesis of saturated fatty acids. Physiol Rev 56:339–417, 1976. [PubMed: 6981]
338. Wan M, Leavens KF, Saleh D, Easton RM, Guertin DA, Peterson TR, Kaestner KH, Sabatini DM, Birnbaum MJ. Postprandial hepatic lipid metabolism requires signaling through Akt2 independent of the transcription factors FoxA2, FoxO1, and SREBP1c. Cell Metab 14: 516–527, 2011. [PubMed: 21982711]
339. Wang D, Sul HS. Insulin stimulation of the fatty acid synthase promoter is mediated by the phosphatidylinositol 3-kinase pathway: Involvement of protein kinase B/Akt. J Biol Chem 273: 25420–25426, 1998. [PubMed: 9738010]
340. Wang Q, Jiang L, Wang J, Li S, Yu Y, You J, Zeng R, Gao X, Rui L, Li W, Liu Y. Abrogation of hepatic ATP-citrate lyase protects against fatty liver and ameliorates hyperglycemia in leptin receptor-deficient mice. Hepatology 49: 1166–1175, 2009. [PubMed: 19177596]
341. White MF. Insulin signaling in health and disease. Science (1979) 302: 1710–1711, 2003.
342. Williamson JR, Scholz R, Browning ET. Control mechanisms of gluconeogenesis and ketogenesis. J Biol Chem 244: 4617–4627, 1969. [PubMed: 5808508]
343. Willy PJ, Umesono K, Ong ES, Evans RM, Heyman RA, Mangelsdorf DJ. LXR, a nuclear receptor that defines a distinct retinoid response pathway. Genes Dev 9: 1033–1045, 1995. [PubMed: 7744246]
344. Windler E, Gavel RJ. Inhibitory effects of C apolipoproteinsfromrats and humans on the uptake of triglyceride-rich lipoproteins and their remnants by the perfused rat liver. J Lipid Res 26: 556–565, 1985. [PubMed: 4020294]
345. Withers DJ, Gutierrez JS, Towery H, Burks DJ, Ren JM, Previs S, Zhang Y, Bernal D, Pons S, Shulman GI, Bonner-Weir S, White MF. Disruption of IRS-2 causes type 2 diabetes in mice. Nature 391: 900–904, 1998. [PubMed: 9495343]
346. Witters LA, Kemp BE. Insulin activation of acetyl-CoA carboxylase accompanied by inhibition of the 5'-AMP-activated protein kinase. J Biol Chem 267: 2864–2867, 1992. [PubMed: 1346611]
347. Yabe D, Brown MS, Goldstein JL. Insig-2, a second endoplasmic reticulum protein that binds SCAP and blocks export of sterol regulatory element-binding proteins. Proc Natl Acad Sci U S A 99: 12753–12758, 2002. [PubMed: 12242332]
348. Yabe D, Komuro R, Liang G, Goldstein JL, Brown MS. Liver-specific mRNA for insig-2 down-regulated by insulin: Implications for fatty acid synthesis. Proc Natl Acad Sci U S A 100: 3155–3160, 2003. [PubMed: 12624180]
349. Yahagi N, Shimano H, Hasty AH, Matsuzaka T, Ide T, Yoshikawa T, Amemiya-Kudo M, Tomita S, Okazaki H, Tamura Y, Iizuka Y, Ohashi K, Osuga J-I, Harada K, Gotoda T, Nagai R, Ishibashi S, Yamada N. Absence of sterol regulatory element-binding protein-1 (SREBP-1) ameliorates fatty livers but not obesity or insulin resistance in Lepob/Lepob mice. J Biol Chem 277: 19353–19357, 2002. [PubMed: 11923308]
350. Yang H, Jiang X, Li B, Yang HJ, Miller M, Yang A, Dhar A, Pavletich NP. Mechanisms of mTORC1 activation by RHEB and inhibition by PRAS40. Nature 552: 368–373, 2017. [PubMed: 29236692]
351. Yang J, Cron P, Good VM, Thompson V, Hemmings BA, Barford D. Crystal structure of an activated Akt/protein kinase B ternary complex with GSK3-peptide and AMP-PNP. Nat Struct Biol 9: 940–944, 2002. [PubMed: 12434148]

352. Yang ZZ, Tschopp O, Hemmings-Mieszczak M, Feng J, Brodbeck D, Perentes E, Hemmings BA. Protein kinase Bα/Akt1 regulates placental development and fetal growth. J Biol Chem 278: 32124–32131, 2003. [PubMed: 12783884]

353. Yates DW, Garland PB. Carnitine palmitoyltransferase activities (EC 2.3.1.-) of rat liver mitochondria. Biochem J 119: 547–552, 1970. [PubMed: 5500315]

354. Yecies JL, Zhang HH, Menon S, Liu S, Yecies D, Lipovsky AI, Gorgun C, Kwiatkowski DJ, Hotamisligil GS, Lee C-H, Manning BD. Akt stimulates hepatic SREBP1c and lipogenesis through parallel mTORC1-dependent and independent pathways. Cell Metab 14: 21–32, 2011. [PubMed: 21723501]

355. Yellaturu CR, Deng X, Park EA, Raghow R, Elam mB. Insulin enhances the biogenesis of nuclear sterol regulatory element-binding protein (SREBP)-1c by posttranscriptionaldown-regulation of insig-2A and its dissociation from SREBP cleavage-activating protein (SCAP)·SREBP-1c complex. J Biol Chem 284: 31726–31734, 2009. [PubMed: 19759400]

356. Yoon jC, Puigserver P, Chen G, Donovan J, Wu Z, Rhee J, Adelmant G, Stafford J, Kahn CR, Granner DK, Newgard CB, Spiegelman BM. Control of hepatic gluconeogenesis through the transcriptional coaotivator PGC-1. Nature 413: 131–138, 2001. [PubMed: 11557972]

357. Yu XX, Murray SF, Pandey SK, Booten SL, Bao D, Song X-Z, Kelly S, Chen S, McKay R, Monia BP, Bhanot S. Antisense oligonucleotide reduction of DGAT2 expression improves hepatic steatosis and hyperlipidemia in obese mice. Hepatology 42: 362–371, 2005. [PubMed: 16001399]

358. Zammit VA, Lankester DJ, Brown AM, Park BS. Insulin stimulates triacylglycerol secretion by perfused livers from fed rats but inhibits it in livers from fasted or insulin-deficient rats. Eur J Biochem 263: 859–864, 1999. [PubMed: 10469151]

359. Zhang J, Zhang W, Zou D, Chen G, Wan T, Zhang M, Cao X. Cloning and functional characterization of ACAD-9, a novel member of human acyl-CoA dehydrogenase family. Biochem Biophys Res Commun 297: 1033–1042, 2002. [PubMed: 12359260]

360. Zhao S, Jang C, Liu J, Uehara K, Gilbert M, Izzo L, Zeng X, Trefely S, Fernandez S, Carrer A, Miller KD, Schug ZT, Snyder NW, Gade TP, Titchenell PM, Rabinowitz JD, Wellen KE. Dietary fructose feeds hepatic lipogenesis via microbiota-derived acetate. Nature 579: 586–591, 2020. [PubMed: 32214246]

361. Zhao S, Torres AM, Henry RA, Trefely S, Wallace M, Lee JV, Carrer A, Sengupta A, Campbell SL, Kuo Y-M, Frey AJ, Meurs N, Viola JM, Blair IA, Weljie AM, Metallo CM, Snyder Nw, Andrews AJ, Wellen KE. ATP-citrate lyase controls a glucose-to-acetate metabolic switch. Cell Rep 17: 1037–1052, 2016. [PubMed: 27760311]

Didactic Synopsis

Major teaching points

- Insulin signals through a complex pathway involving phosphoinositide 3-kinase (PI3K) and AKT to mediate both liver and systemic metabolic homeostasis.
- The liver responds to insulin and carbohydrates by promoting lipogenesis through changes in gene expression and posttranslational mechanisms.
- Insulin inhibits hepatic fatty acid oxidation through complex regulation of several mitochondrial enzymes.
- Cholesterol synthesis is upregulated in response to insulin action in a homeostatic balance with degradation of lipoprotein receptors.
- Fatty acids are taken up by the liver to be packaged as triglycerides, which are secreted into circulation and delivered to peripheral tissues upon insulin stimulation.
- In insulin-resistant states, the liver continues to promote lipid synthesis but fails to suppress glucose production.

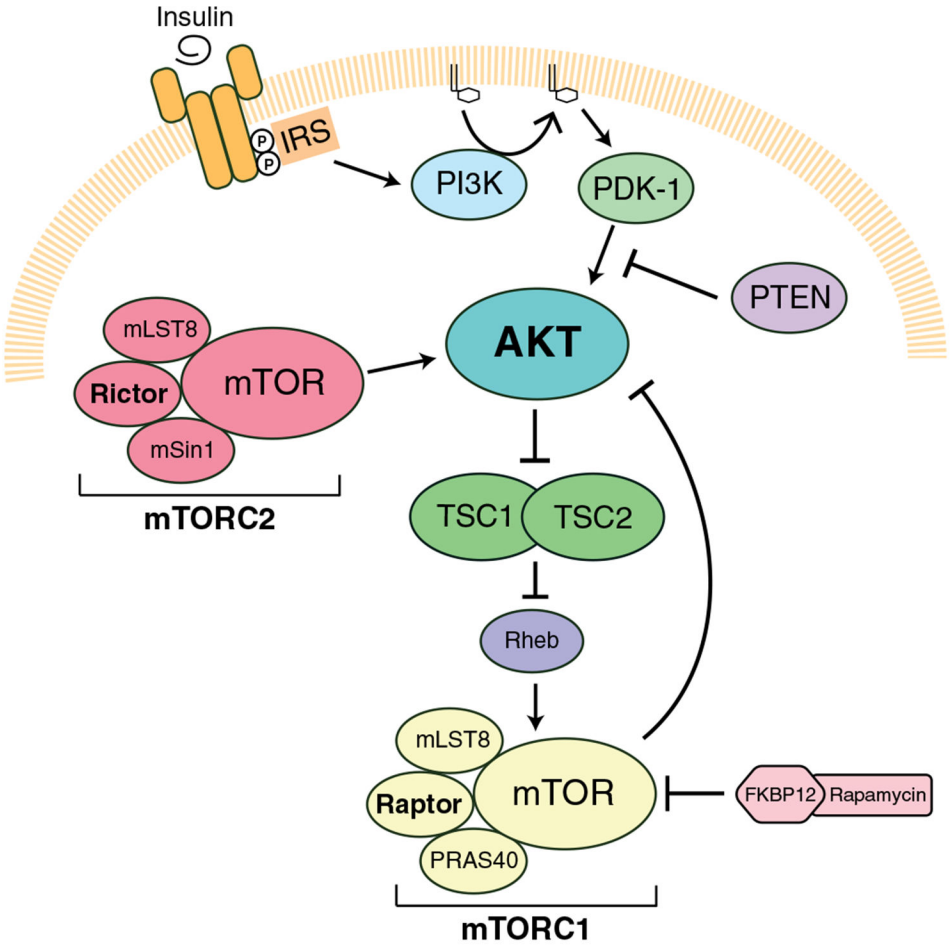

Figure 1.

Hepatic insulin signaling cascade via mTOR. Insulin is released into circulation from beta cells in the pancreas and bind to the insulin receptor (IR). Upon binding, IR autophosphorylates itself, resulting in recruitment and activation of insulin receptor substrates (IRS). IRS proteins then recruit phosphatidylinositol-3-kinase (PI3K), which phosphorylates phosphatidylinositol 4,5-biphosphate (PIP2) to generate phosphatidylinositol-3,4,5-triphosphate (PIP3). Generation of PIP3 recruits and activates PDK-1 which phosphorylates Akt at Threonine-308, a step that can be inhibited by PTEN. Mechanistic target of rapamycin complex 2 (mTORC2) is also able to phosphorylates Akt at Serine-473. Akt phosphorylates and inhibits tuberous sclerosis complex (TSC), which allows Rheb-dependent activation of mechanistic target of rapamycin complex 1 (mTORC1). mTORC1 and mTORC2 are distinguished by complex components Raptor and Rictor, respectively. mTORC1 can be inhibited by the rapamycin-FKBP12 complex.

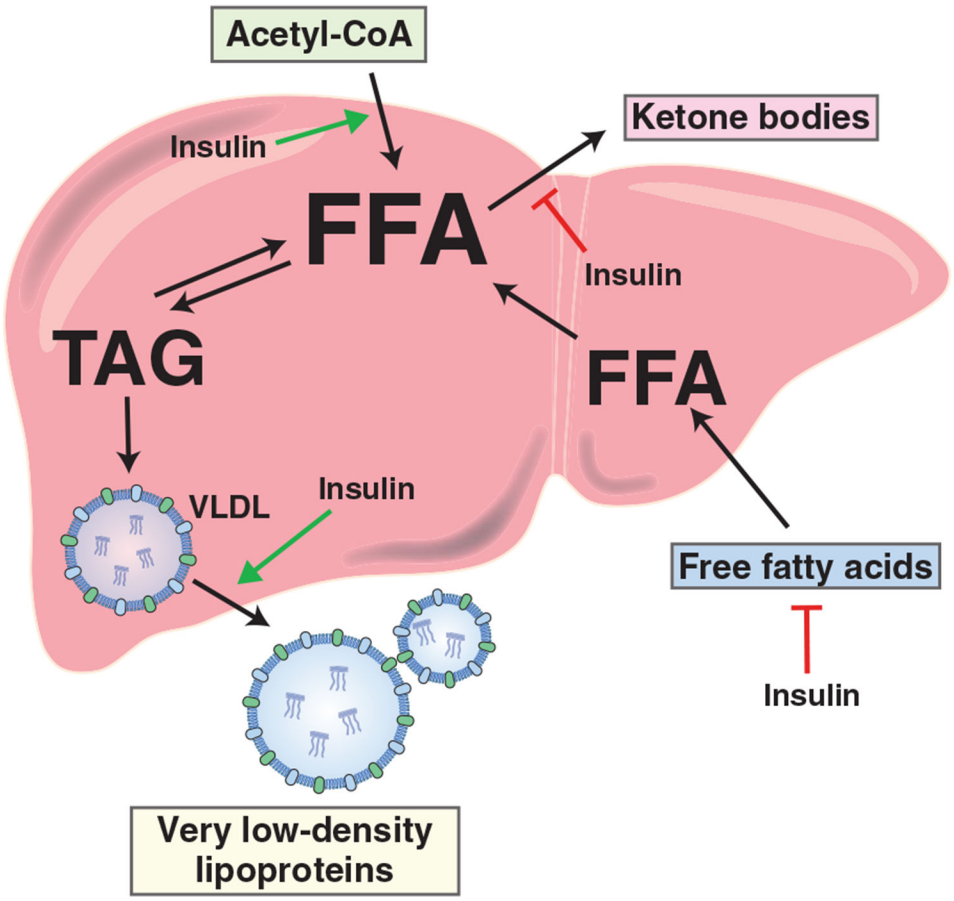

Figure 2.

Movement of lipids in the liver. Free fatty acids (FFA) are released from peripheral tissues and taken up by the liver. FFA can then undergo re-esterification to triacylglycerides (TAG), where they are then packaged in very low-density lipoproteins (VLDL) and secreted into circulation. FFA can also be broken down via beta-oxidation into ketone bodies, which can then be released into circulation. FFA are also synthesized from acetyl-coA via *de novo* lipogenesis.

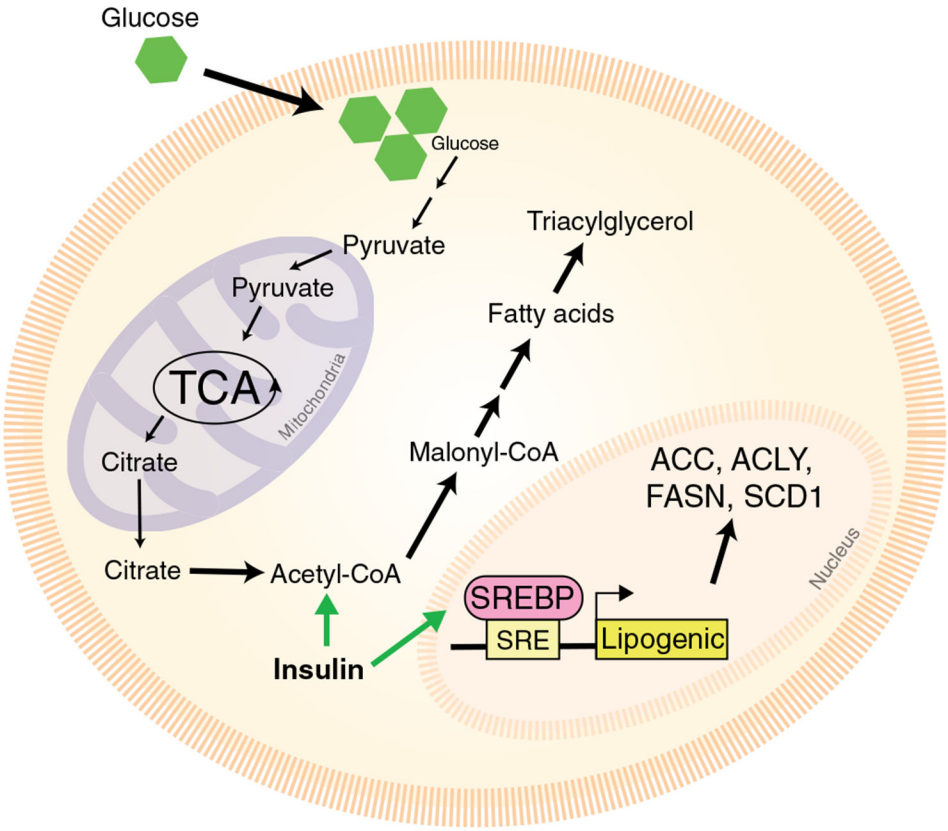

Figure 3.

Mechanisms of fatty acid synthesis. Glucose, or other carbohydrates, are taken up by the cell, then undergo glycolysis to generate pyruvate. Pyruvate enters the mitochondria and the citric acid cycle (TCA). Citrate is able to leave the mitochondria and cytoplasmic citrate is converted to acetyl-coA via ATP citrate lyase (ACLY). Acetyl-coA is then used as a building block for fatty acid synthesis or *de novo* lipogenesis (DNL), culminating in the production of triacylglycerides. Enzymes involved in DNL are transcribed in the nucleus upon SREBP transcription factor binding to sterol response elements (SRE) in the genome: acetyl coenzyme A carboxylase (ACC), fatty acid synthase (FASN), and stearoyl coenzyme A desaturase 1 (SCD1).

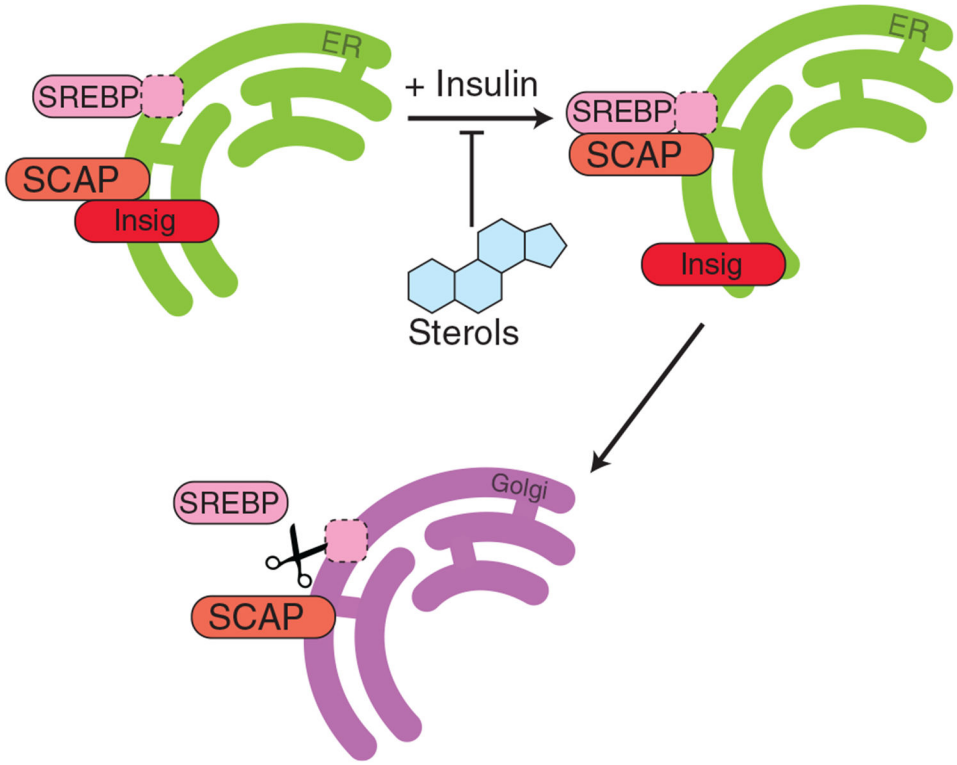

Figure 4.

Sterol regulatory element binding proteins (SREBP) activation at the endoplasmic reticulum (ER), insig proteins bind SREB cleavage-activating protein (SCAP) preventing SCAP binding to SREBPs. In the absence of insig proteins, SCAP binds SREBP, allowing for translocation from the ER to the golgi membrane. Once at the golgi, proteases in the golgi cleave the membrane-bound domain of SREBPs, allowing for its dissociation and translocation to the nucleus.

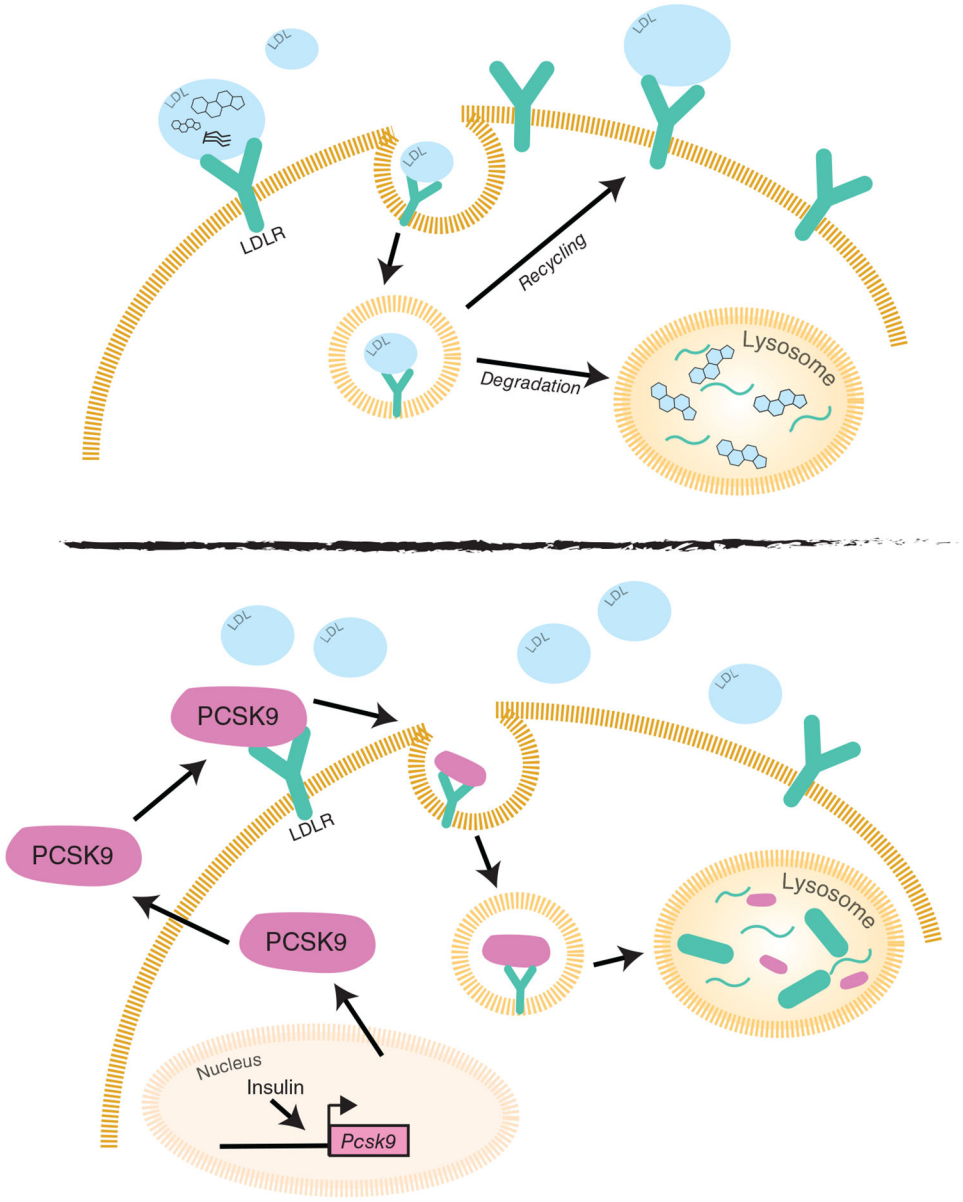

Figure 5.

Low-density lipoprotein (LDL) and low-density lipoprotein receptor (LDLR) degradation LDL in the serum, containing cholesterol and fats, bind to the hepatic LDLR, which undergoes endocytosis. The resulting endosome then gets sent to the lysosome for cholesterol degradation and LDLR is recycled back to the membrane. However, upon insulin stimulation, PCSK9 transcription is induced. PCSK9 protein is then secreted into circulation, binds to the LDLR, inhibiting uptake of LDL-cholesterol. PCSK9-LDL undergoes endocytosis, where the LDLR is sent to the lysosome for degradation, resulting in reduced uptake of serum LDL.