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De Novo Lipogenesis as a Source of Second Messengers in Adipocytes

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Abstract

Purpose of review: Obesity is a major risk factor for Type 2 Diabetes. Although adipose tissue allows storage of excess calories in periods of overnutrition, in obesity, adipose tissue metabolism becomes dysregulated and can promote metabolic diseases. This review discusses recent advances in understandings how adipocyte metabolism impacts metabolic homeostasis.

Recent findings: The ability of adipocytes to synthesize lipids from glucose is a marker of metabolic fitness, e.g. low *de novo* lipogenesis (DNL) in adipocytes correlates with insulin resistance in obesity. Adipocyte DNL may promote synthesis of special “insulin sensitizing” signaling lipids that act hormonally. However, each metabolic intermediate in the DNL pathway (i.e. citrate, acetyl-CoA, malonyl-CoA, and palmitate) also has second messenger functions. Mounting evidence suggests these signaling functions may also be important for maintaining healthy adipocytes.

Summary: While adipocyte DNL contributes to lipid storage, lipid precursors may have additional second messenger functions critical for maintaining adipocyte health, and thus systemic metabolic homeostasis.

Keywords

obesity; insulin resistance; citrate; acetyl-CoA; malonyl-CoA; palmitate

Introduction

Overweight and obesity affect much of the US population and its global prevalence is increasing (1). Body mass index (BMI) between 25–30 kg/m² is defined as overweight, and obesity is defined as BMI over 30 kg/m². One-third of adults in the US are considered obese, or approximately 93.3 million people affected as of 2015–2016 (1). Obesity is a major risk factor for Type 2 Diabetes (T2D), cardiovascular diseases and several cancer types (2). The

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Conflict of Interest

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onset of T2D, in particular, parallels with obesity in all ethnic groups (3,4), and more than 80% of T2D cases can be attributed to obesity (5).

T2D is characterized by hyperglycemia, which is caused by blunted insulin sensitivity of metabolic tissues in combination with inappropriate insulin secretion. Impaired insulin sensitivity decreases glucose uptake and utilization in muscle cells and adipocytes. In early disease stages, this drives pancreatic beta cells to secrete more insulin in response to elevated glucose levels. Persistent hyperinsulinemia can lead to beta cell dysfunction, causing decreased insulin secretion in late-stage disease (6). In prediabetes (even before the onset of hyperglycemia), isolated insulin resistance and hyperinsulinemia are present (7).

Lifestyle change and therapeutic intervention in early stages are the preferred approach to T2D. However, the current treatments are unsatisfactory, evidenced by the high rates of patients with suboptimal glucose control (8), indicating substantial unmet need for new therapeutics. To this end, understanding the molecular pathogenesis of insulin resistance is critical. The goal of this review is to discuss recent advances in the biology of insulin sensitivity and less appreciated aspects of adipose tissue's role in contributing to metabolic homeostasis.

Adipose tissue basics

Center stage in the obesity epidemic is body fat, or adipose tissue. Having healthy adipose tissue is essential for metabolic health. It functions as body's main long-term energy storing tissue and is a critical source of endocrine signals (called adipokines) that regulate systemic metabolic homeostasis (9,10). Adipose tissue has become the culprit of the obesity and T2D epidemics even though a main function of adipose tissue expansion is to protect against the metabolic consequences of over-nutrition. Nevertheless, excessive adipose tissue can lead to its dysregulation, driving insulin resistance and prediabetes (11,12). Conversely, having too little or dysfunctional adipose tissue, such as in lipodystrophy, can also cause insulin resistance and metabolic disease (13). Thus, understanding how adipose tissue responds to and regulates metabolism is essential for understanding and treating T2D.

Adipose tissue morphology and function vary based on its anatomical location (14,15). Generally speaking, there are two distinct classes of adipose tissue: brown adipose tissue (BAT) and white adipose tissue (WAT). BAT contains multilocular lipid droplets and generates heat. WAT is generally considered energy storing and can be further subcategorized as subcutaneous adipose tissue (SAT) or visceral adipose tissue (VAT) based on its location. However, this is even an oversimplification as different SAT and VAT depots can have different metabolic properties and developmental origins (16,17). SAT, which resides under the skin, is often considered a "healthy" adipose tissue as studies show that some obese individuals with higher amounts of SAT have normal insulin sensitivity and less tissue inflammation (18,19). Moreover, a portion of white adipocytes in SAT can adopt brown adipocyte-like characteristics, becoming what is called brite or beige adipocytes, in response to cold stimulation, exercise, and other stresses (20). This so-called "browning" potential might also make SAT more metabolically beneficial. In contrast, excess VAT, which is adjacent to internal organs, is often metabolically unhealthy. Clinical studies reveal

that expanding VAT (as seen in central obesity) positively correlates with the development of insulin resistance (21,22).

SAT and VAT also differ in their mechanism of growth (23). Some human studies suggest that SAT preadipocytes have greater adipogenic capacity and differentiate into new adipocytes, thereby expanding tissue size by increasing cell number, especially in women (21,24–26). VAT, on the other hand, expands by increasing individual adipocyte size, or by hypertrophy (26). Curiously, a mouse study marking newly-synthesized adipocytes shows that, upon high-fat diet feeding, VAT formed new adipocytes from precursors, whereas SAT expanded mainly by hypertrophy (27). Thus, how adipose tissues expand may vary depending upon the stimulus, gender, or species. Interestingly, transplantation of donor SAT to either visceral or subcutaneous sites in recipient mice improves glucose tolerance and insulin sensitivity (28–31). This suggests that there are also intrinsic properties of each depot that are independent of anatomical location. Other studies argue that increasing individual adipocyte size in either or both depots correlates with insulin resistance (32); in this scenario, expanding SAT may also promote insulin resistance. The contribution of different fat depots to energy storage, adipokine production, and overall insulin sensitivity is complex and thorough research still needs to be done.

Mechanisms of adipose tissue insulin resistance

The ability of an adipocyte to balance anabolic and catabolic lipid metabolism is essential for overall metabolic health. In the post-prandial state, insulin stimulates the transport of circulating glucose and lipids into adipocytes and suppresses lipolysis. During fasting, anabolic pathways abate while glucagon stimulates lipolysis. Lipolysis occurs when stored lipids in the form of triacylglycerol (TAG) are hydrolyzed into free fatty acids (FFAs) and glycerol. These FFAs are released into circulation to provide fuel for other tissues while glycerol is used by the liver for gluconeogenesis or TAG synthesis. In insulin resistance, these tightly regulated metabolic processes fail to respond normally to increased circulating insulin following a meal. In adipocytes, this translates into insulin's inability to stimulate glucose and lipid uptake and suppress lipolysis. When adipocytes cannot normally store lipids, these lipids can "spillover" and accumulate in other tissues such as liver and skeletal muscle, causing toxicity (19,33,34). Obesity also impairs the synthesis and/or secretion of important adipokines, such as adiponectin, which normally promotes insulin sensitivity in other tissues (35).

Understanding how diet, lifestyle, and genetics influence the carbohydrate and lipid handling capacity of adipocytes and their endocrine functions is critical to advancing new therapies. Yet despite many major advances, a key question still under intense investigation is how exactly adipose tissue dysfunction promotes insulin resistance in obesity. Both reduced glucose uptake and increased lipolysis, have been shown to drive systemic insulin resistance (36). Obesity is also associated with adipose tissue inflammation, which can promote insulin resistance by interfering with insulin signaling (37–39). Both innate and adaptive immune functions, mediated by macrophages and T cells respectively, are dysregulated in obesity, which leads to proinflammatory cytokine secretion (40–44). Many recent reviews discuss the roles of lipolysis, reduced adipogenesis, inflammation, and

hyperinsulinemia in insulin resistance (45–47). Below, we will focus on key aspects of glucose uptake and *de novo* lipid synthesis.

Glucose uptake and utilization by adipocytes

In the fed state, insulin promotes glucose uptake into adipocytes by triggering the translocation of the glucose transporter, GLUT4, to the plasma membrane (Figure 1A). This is regulated at least in part by insulin-stimulated AKT-dependent inhibitory phosphorylation of the AS160 Rab-GTPase, which allows Rab protein to remain GTP-bound and facilitates translocation of GLUT4-containing vesicles to the plasma membrane (48–51). AKT and AS160-independent pathways also reportedly contribute to GLUT4 translocation (52). Within adipocytes, glucose has several potential fates (Figure 1A). It can be used to synthesize glycerol-3-phosphate (G3P) from glycolytic metabolites in 3T3L1 adipocytes (53). G3P is required to make glycerol for the esterification of FFAs, which is an essential step in TAG synthesis. Glucose can also enter the hexosamine biosynthetic pathway (HBP), a branch of glycolysis, in which O-linked N-acetylglucosamine (O-GlcNAc) is generated. O-GlcNAc can be used for post-translational modification of proteins by a process called O-GlcNAcylation. Key regulators of insulin signaling including IRS-1 and AKT can be O-GlcNAcylated (54,55), as can PPAR γ , the master transcription factor in adipocyte differentiation (56). Glycosylation is thought to affect protein activity and insulin sensitivity in adipocytes (57–60). Glucose metabolites also contribute to the pentose phosphate pathway (PPP) in adipocytes, which generates NADPH needed for lipogenesis (53). Fatty acids are also synthesized *de novo* from glucose-derived carbons via a pathway called *de novo* lipogenesis (DNL, discussed below). Thus, metabolite flux through these pathways dynamically reflects glucose availability and utilization by adipocytes, which is coupled to hormonal signaling.

De novo lipogenesis in adipocytes

The DNL pathway is positively correlated with insulin sensitivity in human obesity. For example, human and mouse models of obesity have both a downregulated DNL pathway and reduced GLUT4 gene expression in adipose tissue (61–64). In healthy individuals, DNL in adipocytes is thought to contribute relatively little to overall TAG content (around 20% based on $^2\text{H}_2\text{O}$ -tracing study in human); most TAG is derived from the uptake and esterification of exogenous lipids from circulation (65). However, only subcutaneous depots have been carefully examined and differences in DNL between depots at different anatomical sites are not well defined. Notably, the positive correlation between DNL and insulin sensitivity is specific to adipocytes. For example, in the liver, increased DNL drives nonalcoholic fatty liver disease (NAFLD) and insulin resistance (61,66,67)

In adipocytes, three key enzymes that mediate glucose-derived *de novo* lipid synthesis are ATP-citrate lyase (ACLY), acetyl-CoA carboxylase (ACC) and fatty acid synthase (FASN) (Figure 1A). Metabolized glucose that enters the mitochondria via pyruvate can exit the TCA cycle in the form of citrate, which is exported to the cytosol. Once in the cytosol, citrate is cleaved into acetyl-CoA and oxaloacetate by ACLY. Cytosolic acetyl-CoA serves as the 2-carbon building block for subsequent lipogenesis. ACC, especially the cytosolic isoform ACC1 (68), then converts acetyl-CoA to malonyl-CoA. FASN, through a multi-step

reaction, then assembles malonyl-CoA units into the first lipogenic end product, the 16-carbon palmitate. Palmitate can then be processed into different lipid species via the action of elongases and desaturases.

ACLY, ACC, and FASN are transcriptionally regulated by a glucose metabolite-sensing transcription factor called carbohydrate-responsive element-binding protein (ChREBP), and by sterol regulatory element-binding protein (SREBP) (Figure 1A)(69). Overexpressing GLUT4 in adipose tissue promotes glucose uptake, which increases DNL enzyme expression by stimulating ChREBP activity. In response, ChREBP_a stimulates expression of a shorter isoform, called ChREBP_b, which is transcribed from an alternative start site, and has greater transcriptional activity (70).

Deleting GLUT4 in adipose tissue, on the other hand, causes systemic insulin resistance despite preserved adipose tissue mass (71). Similarly, adipocyte-specific ChREBP KO mice develop insulin resistance upon chow diet feeding, which is exacerbated by high fat diet (HFD). Interestingly, mice lacking adipose ChREBP also have impaired glucose uptake (72), suggesting ChREBP may function both upstream and downstream of glucose uptake in a feed-forward loop. The key findings of these animal models are summarized in Table 1; for the most part, these data are consistent with observations in obese humans that low GLUT4 and low ChREBP activity, particularly in SAT, correlate with insulin resistance (62,73).

Exactly how ChREBP is regulated remains an open question, but it appears to involve multiple processes (74). It is reportedly activated by glycolytic metabolites such as xylulose-5-phosphate (75,76) and possibly glucose-6-phosphate (77). ChREBP can also be post-translationally modified by acetylation. In mouse liver, acetylation of ChREBP causes nuclear translocation and activation of DNL (78). Another modification of ChREBP is O-GlcNAcylation, which seems to be important for its protein stability and transcriptional activity in liver (79). Thus, one mechanism for ChREBP regulation may be via direct sensing of glucose-derived metabolites.

Signaling pathways may also regulate ChREBP. For example, the mechanistic target of rapamycin complexes 2 (mTORC2), which phosphorylates and activates AKT, is required in adipose tissue for ChREBP_b expression and for expression of the entire DNL pathway (Figure 1A) (80). Moreover, mice lacking mTORC2 in adipose tissue exhibit insulin resistance (80–82). How mTORC2 and AKT regulate ChREBP expression is still not well understood; however, loss of adipose tissue mTORC2 phenotypically shows partial overlap with the effects of HFD on ChREBP_b and DNL pathway expression (80), potentially suggesting a common mechanism. ChREBP can also be inhibited by phosphorylation mediated by protein kinase A (PKA) and AMP-activated protein kinase (AMPK) (83,84). Understanding ChREBP regulation remains an important area of investigation.

DNL promotes insulin sensitivity

What is the connection between DNL and insulin sensitivity? One possibility is that adipocytes synthesize special signaling lipids that have systemic insulin sensitizing functions. One such “lipokine” that has been reported is palmitoleate (16:1n7), a monounsaturated fatty acid which has been shown to attenuate adipose tissue inflammation

and promote insulin sensitivity in mice (85). Another is a novel class of lipids called branched fatty acid esters of hydroxyl fatty acids (FAHFAs). FAHFAs have also been shown to sensitize the insulin response and have anti-inflammatory function (70,86,87). Among these lipids, palmitic acid esters of hydroxyl-stearic acids (PAHSAs) are enriched specifically in adipose tissue, and their level decreases in humans with adipose tissue hypertrophy and insulin resistance (70,88). GLUT4 overexpression in mouse adipose tissue correlates with elevated serum PAHSA levels (70). Moreover, PAHSA supplementation in mice with insulin resistance can improve insulin sensitivity (70). These studies suggest one potential mechanism by which DNL can influence insulin sensitivity, and this exciting area of research has been recently reviewed elsewhere (89,90).

A less appreciated aspect of DNL is that each metabolic intermediate in the pathway (i.e. citrate, acetyl-CoA, malonyl-CoA, and palmitate) has important second messenger functions that might also be critical for adipocyte health. Alterations in their availability or flux could impact signaling activities, metabolic pathways, or lipid storage, and this could have deleterious consequences on adipocyte metabolism. Below, we review these functions and comment on their potential contributions to adipocyte health.

Citrate

The Randle cycle describes the process by which glucose and fatty acid oxidation compete, and how increased fatty acid oxidation can inhibit glucose uptake in muscles and adipocytes (91). Subsequent research showed that citrate is the key metabolite in this process. In the mitochondria, citrate is a TCA cycle intermediate derived from the condensation of acetyl-CoA and oxaloacetate. When nutrients and energy are plentiful, mitochondrial citrate can be shuttled into the cytosol by the citrate carrier for use in lipid synthesis. However, as described in the Randle cycle, cytoplasmic citrate can also bind to the C-terminus of phosphofructokinase 1 (PFK1) to allosterically inhibit this rate-limiting, unidirectional step in glycolysis (Figure 1B) (92). This can also cause accumulation of the upstream metabolite glucose-6-phosphate, which in turn inhibits hexokinase 2 (HK2) and impairs glucose uptake. Additionally, citrate is thought to activate ACC by promoting multiple ACC monomers to associate into a higher-order polymer, which may increase activity of the DNL pathway (93,94). However, the amount of citrate required for ACC polymerization is much higher than the normal concentration in cells (93) and thus whether it has physiological relevance needs to be elucidated. Nevertheless, since cytosolic citrate is the substrate for ACLY, the first enzyme in the DNL pathway, it is reasonable to speculate that decreased DNL might affect cytoplasmic citrate levels.

Notably, the mitochondrial enzyme citrate synthase (CS) condenses oxaloacetate and acetyl-CoA into citrate in the first step of the TCA cycle (Figure 1A)(95). Mitochondrial dysfunction has been shown to contribute to metabolic disturbance and obesity (96). In skeletal muscle, decreased CS activity or protein level correlates with lipotoxicity and insulin resistance demonstrating a link between CS activity and insulin resistance (97,98). A clinical study analyzing gene expression in omental adipose tissue from obese patients also found that obesity correlated with both decreased CS protein level and enzymatic activity

(99,100). These findings suggest citrate and CS might play a role in regulating glucose metabolism, but in vivo studies are required to understand the mechanisms.

Acetyl-CoA

The amount of acetyl-CoA present in different sub-cellular compartments (i.e. cytoplasm, nucleus, and mitochondria) serves as a key dynamic indicator of cellular energy levels (101,102). Acetyl-CoA in the cytoplasm and nucleus is generated by the cleavage of mitochondrial-exported citrate by ACLY in a reaction that additionally generates oxaloacetate. In addition to providing the initial two-carbon unit for de novo lipid synthesis, nuclear-cytoplasmic acetyl-CoA is the precursor for acetylation of lysine residues on histones and non-histone proteins, such as transcription factors and metabolic enzymes (Figure 1C) (103). Thus, acetyl-CoA availability can link cellular nutrient status to gene expression and metabolic regulation. The effect of cellular acetyl-CoA level on histone acetylation profiles has been shown in budding yeast, in which decreased intracellular acetyl-CoA levels results in downregulation of global histone acetylation and transcription (104–107). In the context of mammalian cells, such as stem cells and cancer cells, altering acetyl-CoA level affects metabolism, proliferation, and differentiation by modulating histone acetylation (102,108–111). These studies lead to a hypothesis that compartmentalization of acetyl-CoA is linked to nutrient availability. For example, in the fed state, the nuclear-cytoplasmic acetyl-CoA generated from glycolysis and the mitochondrial export of citrate can alter histone acetylation in addition to driving lipid synthesis (104,108). On the other hand, in the fasted state mitochondrial acetyl-CoA is generated from the oxidation of pyruvate, lipids, and amino acids and is used for ATP synthesis (101). Many studies support a link between histone acetylation and insulin sensitivity (112,113), and there is also a link between acetyl-CoA level and adipocyte function (114). Thus, acetyl-CoA dynamics are likely critical for regulating adipocyte gene expression and maintaining metabolic flexibility.

Acetylation of proteins other than histones may also contribute to insulin sensitivity. For example, forkhead box 1 (FoxO1) is one of the transcription factors which senses nutrient status and its activity is regulated by acetylation in addition to its classic regulation by insulin-stimulated phosphorylation (115). Acetylation, like phosphorylation, promotes FoxO1 accumulation in the cytoplasm thereby preventing FoxO1-driven catabolic activity (82,116–118). Another example is CIDEA, which is required for forming lipid droplets. Deacetylation of CIDEA by HDAC6 inhibits its activity and destabilizes lipid droplets in adipocytes (119). The same study showed that HDAC6 is down-regulated in adipocytes from obese mice and adipocyte-specific HDAC6 KO mice have increased lipid accumulation and reduced insulin sensitivity (119). Thus, acetylation of non-histone proteins may also be related to nutrient status and can modulate metabolism to fit environmental demands.

Protein acetylation is modulated by lysine acetyltransferases (KATs) and deacetylases (DACs), which are sensitive to intracellular acetyl-CoA level (120). It has been proposed that increased acetyl-CoA level promotes protein acetylation by activating KATs (103,110,115). Many studies suggest that altering KAT or DAC activity may affect insulin sensitivity by regulating protein and/or histone acetylation in liver and adipose tissue. Several histone deacetylase inhibitors (or HDAC inhibitors) have been shown to improve

insulin sensitivity in mouse models (121,122). In clinical trials, only a few HDAC inhibitors such as sodium phenylbutyrate and valproate, improved insulin resistance in obese patients and this correlates with insulin level, respectively (121–123). However, the results from other types of HDAC inhibitors tested are not consistent and more testing is clearly needed.

The levels of acetyl-CoA-generating enzymes correlate with body adiposity and insulin sensitivity. For example, a recent mouse study suggests that HFD may reduce acetyl-CoA level and the acetyl-CoA/CoA ratio in adipose tissue, and this correlates with reduced ACLY expression and histone acetylation (124). In obese patients with abnormal glucose and lipid profiles, ACLY level is also decreased in adipocyte precursors isolated from these individuals (114). Notably, another enzyme called Acyl-CoA synthetase short chain family member 2 (ACSS2), which generates acetyl-CoA from an acetate (Figure 1C), is also down-regulated in these isolated precursors (114). ACLY may also be a particularly important link between hormonal signaling through the mTORC2/AKT pathway and metabolic regulation as ACLY has multiple phosphorylation sites, and at least one site (Serine 455) is directly phosphorylated by AKT to stimulate its activity (125). The links between nuclear-cytoplasmic acetyl-CoA production, upstream hormonal signals, and downstream lipid synthesis and gene expression, makes it likely that acetyl-CoA's second messenger functions are critical for maintaining healthy adipocytes.

Malonyl-CoA

Glucose-derived malonyl-CoA is generated from acetyl-CoA carboxylation mainly by ACC1 in adipocytes. In addition to being a substrate for FASN, malonyl-CoA can inhibit fatty acid oxidation by allosterically inhibiting CPT1, the rate-limiting step for fatty acid uptake into the mitochondria (Figure 1D). Malonyl-CoA levels inversely correlate with insulin resistance in skeletal muscle; increasing Malonyl-CoA causes elevated lipid products such as triglyceride, diacylglycerol, and long-chain fatty acyl-CoA, which is suggested to attenuate glucose transport localization and impairs glucose uptake (126,127).

Malonyl-CoA can also be used to modify proteins by malonylation, much like acetylation (128,129). Protein malonylation on lysine residues has been most widely studied in liver. A recent proteomic study in both *db/db* (T2D model) and *ob/ob* mice shows that lysine malonylation was enriched on 268 proteins, many of which regulate glucose and lipid metabolic pathways. Other recent work suggests the deacetylase family member, Sirtuin 5 (SIRT5), is the major “de-malonylation” enzyme in liver (130,131). A study using affinity enrichment proteomics examined the mitochondrial SIRT5-dependent lysine malonylome (131). Comparing the liver malonylome from WT and SIRT5 KO mice suggests many glycolytic pathway proteins can be malonylated (131), implicating a role in metabolic regulation. In BAT, malonylation is higher relative to liver or WAT (132), and malonylation as well as succinylation are decreased on mitochondrial proteins in BATs from genetically obese *db/db* mice (132,133). BAT-specific SIRT5 KO mice, similar to liver-specific KO, have enriched malonylation and succinylation of mitochondrial proteins. How malonylation of these proteins regulates glucose and lipid metabolism, and how it contributes to insulin sensitivity in a tissue-specific manner require further investigation.

A recent report suggests the mTOR kinase is malonylated in cultured endothelial cells. In FASN knockdown endothelial cells, malonylation of mTOR at K1218 is enriched. Functional studies suggest mTOR malonylation specifically down-regulates mTORC1 activity resulting in reduced protein synthesis, although whether mTORC2 function is also affected was not completely resolved. In endothelial cells, mTOR malonylation correlates with decreased angiogenesis, and inhibiting the malonyl-CoA producing enzyme ACC in FASN-deficient cells rescues mTORC1 activity (134). Thus, the contribution of protein malonylation to white adipose tissue insulin sensitivity warrants investigation.

Palmitate

Protein S-palmitoylation is reported as the most common and reversible type of post-translational S-acylation (135,136). When a 16-carbon palmitic acid is attached to a protein, it increases its hydrophobicity which aids in association with cell membranes (137,138). Protein-membrane interactions are essential for compartmentalization, protein-protein interactions and cell signaling (139). Recent studies suggest that many proteins are palmitoylated in adipocytes. One study conducted thiopropyl captivation (TPC) of S-acylated proteins followed by mass spectrometry (MS) in both 3T3-L1 cells and adipose tissues and isolated more than 800 putative palmitoylated proteins, more than half of which are related to lipid metabolism (140). Interestingly, palmitoylated proteins appear to play a role in GLUT4 vesicle membrane trafficking (Figure 1E). These include cargo proteins (GLUT4 and IRAP), sorting Golgi proteins (Sortilin) and trafficking, docking and fusion proteins (i.e. Munc18c, AS160). High-fat feeding also increases palmitoylation of GLUT4 and IRAP (140).

Specifically, palmitoylation of GLUT4 at Cys223 is mediated by DHHC domain-containing protein 7 (DHHC7; also known as ZDHHC7)(141,142), and DHHC7 KO mice develop insulin resistance and glucose intolerance (141). In vitro analysis attributes this to a defect in GLUT4 translocation, suggesting palmitoylation promotes GLUT4 trafficking (141). DHHC7 itself is also regulated by palmitoylation which responds to insulin stimulation via an unclear mechanism (141). However, the animal model examined was a whole-body knockout and a detailed physiological role for palmitoylation specifically in adipose tissue needs further study. Several proteins in the JAK-STAT (Janus Kinase-signal Transducer and Activator of Transcription) pathway are also palmitoylated in adipocytes (140) (Figure 1E). JAK-STAT is required for adipocyte differentiation and also modulates insulin sensitivity, glucose and lipid metabolism in mature adipocytes (143). However, whether these and other palmitoylation events have key adipocyte functions is not yet clear.

Genetic studies of DNL enzymes

One way to examine the function of DNL in adipose tissue is to make tissue specific knockout and transgenic (overexpressing) mice. Some of these models are listed in Table 1. While these studies are invaluable steps towards examining in vivo functionality, it is also important to recognize the caveats of such studies when interpreting the data. It is often said that mice are not humans, which is true, but modifying living conditions—such as raising mice at thermoneutrality—may lessen potential differences (144). Many mouse models are also congenital and compensatory effects can occur over time. Completely knocking-out a

gene can be much different than more physiological changes such as decreased mRNA expression, or inhibition of a gene product's activity. Finally, the choice of Cre driver used for conditional deletion is important. Currently, the most widely accepted tool for WAT studies is the adiponectin-Cre driver, though it is also not without its imperfections.

The phenotype of adipocyte-specific ACLY KO mice was recently reported. Interestingly, complete loss of ACLY results in elevated acetyl-CoA levels in both SAT and VAT, which appears to result from compensatory up-regulation of ACSS2 (145). Yet despite these tissues having higher total acetyl-CoA levels, histone acetylation is reduced, suggesting that the source and/or compartmentalization of acetyl-CoA important for its second messenger functions (145). Notably, deleting ACLY also inhibits ChREBP expression both in vitro and in vivo (146). This provides further evidence of a feed forward loop in which ACLY may be both upstream of ChREBP regulation, and downstream of ChREBP transcriptional activity (Figure 1A). The mechanism requires further investigation. ACLY KO mice also exhibit interesting gender-specific differences. For example, male fat-specific ACLY KO mice have only mild systemic insulin resistance with no difference in adipose tissue mass (145), while female fat-specific ACLY KO mice develop insulin resistance on chow, which is even worse when fed a high-sucrose diet (146). Why such gender differences exist is not known but could be related to hormonal patterns or differences in ACSS2 expression.

Although genetic studies suggest ACSS2 can compensate for ACLY loss, the normal role of ACSS2 in adipose tissue is not yet known. In cancer cells, ACSS2 can provide a significant portion of the carbon for fatty acid and phospholipid synthesis under stress, such as during hypoxic and/or nutrient-deprived conditions (147,148). Under these conditions, ACSS2 appears to selectively regulate genes involved in lipid metabolism including ACC and FASN (149,150). Whole body ACSS2-null mice are protected from HFD-induced weight gain and liver steatosis, and this correlates with smaller adipocytes and decreased whole body lipid content (150); tissue specific ACSS2 functions have not yet been reported. The signaling upstream of ACSS2 and how it regulates lipogenesis are also unclear, although ACSS2 appears to function both in the cytoplasm and the nucleus, the latter reportedly to “recapture” acetyl-CoA from the acetate generated by deacetylation reactions (148,151).

Adipose tissue-specific ACC1 KO have been generated using the *aP2*-Cre driver, which was previously the standard driver used for adipocyte-specific deletion. This study showed less lipid accumulation in adipose tissues upon HFD feeding as well as effects on bone formation (152). However, it is difficult to make specific conclusions about adipocytes in this model because the *aP2-Cre* has significant non-adipose tissue targeting effects (153,154) and thus, further investigation is required to understand the adipocyte-specific roles of ACC in vivo.

Interestingly, mice with fat FASN deletion are more insulin sensitive and appear to have increased energy expenditure driven by SAT browning (155). These mice are also resistant to diet-induced obesity (155). Early studies indicate that this may be related in part to a decrease in PPAR γ transcriptional activity and/or adipogenesis. Recent studies using a tamoxifen inducible adiponectin-Cre driver to ablate FASN in mature mice additionally finds that induced FASN ablation in white and brown fat also improves glucose tolerance and causes SAT browning. Moreover, this study shows that induced FASN-knockout triggers

sympathetic nerve expansion into adipose tissues and increases neuronal stimulation of the thermogenic program (64,156). A follow-up study further suggests FASN loss in WAT may also signal to BAT to increase thermogenesis because deleting FASN only in BAT (i.e. with UCP1-Cre) did not trigger sympathetic innervation of BAT (64,156). The signal triggering this phenotype remains elusive, and understanding how FASN deletion affects the levels, fluxes, and second messenger functions of upstream metabolic intermediates and downstream products, will be important for understanding this interesting phenotype. Nevertheless, it supports the emerging idea that adipose tissues are intricately wired to other tissues through innervation and that metabolic signals within adipocytes can communicate to distal tissues through neuronal networks.

Conclusions

Adipocyte dysfunction in obesity can drive systemic insulin resistance. But exactly how adipocytes become dysfunctional, and how various adipocyte pathways contribute to insulin sensitivity, remains poorly understood. Here, we discuss the *de novo* lipogenesis pathway because mounting evidence suggest that in addition to its role in lipid storage, DNL metabolites and special de novo synthesized lipid species have important roles in insulin sensitivity. However, it is likely the case that multiple mechanisms acting at different disease stages or in different fat depots, coordinately contribute to insulin resistance in obesity. Ongoing work into understanding how adipocyte metabolism connects with metabolic homeostasis may provide new avenues for therapeutic development against insulin resistance and type 2 diabetes.

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Figure 1. Glucose Metabolism and De Novo Lipogenesis (DNL) in Adipocytes.

(A) Model depicting major metabolic routes of glucose metabolites in adipocytes. In the fed state, insulin stimulates GLUT4 translocation to the plasma membrane and facilitates glucose uptake. Metabolites of glucose generated during glycolysis can enter the pentose phosphate pathway (PPP), which generates NADPH for lipid synthesis; the hexosamine biosynthetic pathway (HBP), which generates UDP-N-acetylglucosamine (UDP-GlcNAc) and promotes O-GlcNAcylation of proteins; and the glycerol-3-phosphate pathway, which is required to make the glycerol backbone for assembling triacylglycerides (TAGs). Glucose carbons that enter into the mitochondrial TCA cycle as pyruvate are converted to citrate by citrate synthase (CS). Citrate can remain in the TCA cycle, or in times of nutrient abundance, be exported into the cytoplasm and enter the DNL pathway. The major DNL

enzymes are ATP citrate lyase (ACLY), which generates acetyl-CoA; acetyl-CoA carboxylase (ACC), which generates malonyl-CoA; and fatty acid synthase (FASN), which synthesizes palmitate. Palmitate is the primary end product of DNL, which can be further processed by desaturases and elongases. DNL enzyme and Glut4 transcription are regulated by ChREBP. Other glycolytic-derived metabolites, such as xylulose-5-phosphate (X-5-P) and glucose-6-phosphate (G-6-P), are thought to directly stimulate ChREBP activity. Acetyl-CoA can also be produced from acetate by ACSS2 although ACSS2's function in adipocytes is not well understood. Note that a large fraction of the lipid stored in TAGs is derived from circulating lipids taken up into the adipocytes, which is discussed in the text but excluded from the figure for simplicity. **(B-E)** Metabolic intermediates in the DNL pathway also have important second messenger functions, some of which are indicated in each panel for the indicated metabolites, **(B)** citrate, **(C)** acetyl-CoA, **(D)** malonyl-CoA, and **(E)** palmitate. Disruption in the utilization of these metabolites as second messengers by altering their synthesis, degradation, or flux towards different pathways, could also lead to adipocyte dysfunction in obesity.

Table 1:Mouse models of key *de novo* lipogenesis pathway regulators

DNL protein	Modification	Organism/Model	Observation	Reference
GLUT4	Loss of function	GLUT4 KO (<i>aP2-Cre</i>)	Insulin resistance with preserved AT size.	(71)
	Gain of function	GLUT4 overexpression (<i>Adiponectin</i> , AG4OX)	OX mice are obese but have enhanced glucose tolerance.	(72)
ChREBP	Loss of function	ChREBP KO (<i>Adiponectin-Cre</i>)	Insulin resistance and increased AT inflammation in KO mice.	(72)
	Gain of function	ChREBP overexpression (<i>aP2</i>)	Improved insulin sensitivity and glucose tolerance in OX mice under HFD fed.	(157)
		AG4OX crossbred with adipocyte-specific ChREBP KO	AG4OX restores glucose and insulin sensitivity in AT-ChREBP KO mice.	(62)
ACLY	Loss of function	ACLY KO (<i>Adiponectin-Cre</i>)	Mild insulin resistance (Female>Male). Gender differences.	(145,146)
ACC	Loss of function	ACC1 KO (<i>aP2-Cre</i>)	Decreased lipid accumulation in KO AT under fat-free diet.	(152)
FASN	Loss of function	FASN KO (<i>Adiponectin-Cre</i>)	Increased energy expenditure and browning in SAT and resistant to diet-induced obesity in KO mice.	(155)
		FASN inducible KO (<i>Adiponectin-CreERT2</i>)	Enhanced glucose tolerance and thermogenic signals in SAT. Increased sympathetic nerve activity in SAT.	(64,156)

Abbreviations: AT: adipose tissue; DNL: de novo lipogenesis; SAT: subcutaneous adipose tissue