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PATHOGENESIS OF TYPE 2 DIABETES AND INSULIN RESISTANCE (M-E PATTI, SECTION EDITOR)

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 and 30 kg/m^2 is defined as overweight, and obesity is defined as BMI over 30 kg/m^2. 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 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 the body's main long-term energy-storing tissue and is a critical source of endocrine signals

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(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 overnutrition. 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 the 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 the 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 (Fig. 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 (Fig. 1a). It can be used to synthesize glycerol-3-phosphate (G3P) from glycolytic metabolites in 3T3-L1 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 PPARγ, 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 [61–63, 64•]. In healthy individuals, DNL in adipocytes is thought to contribute relatively little to overall TAG content (around 20% based on $^{2}H_2O$-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].

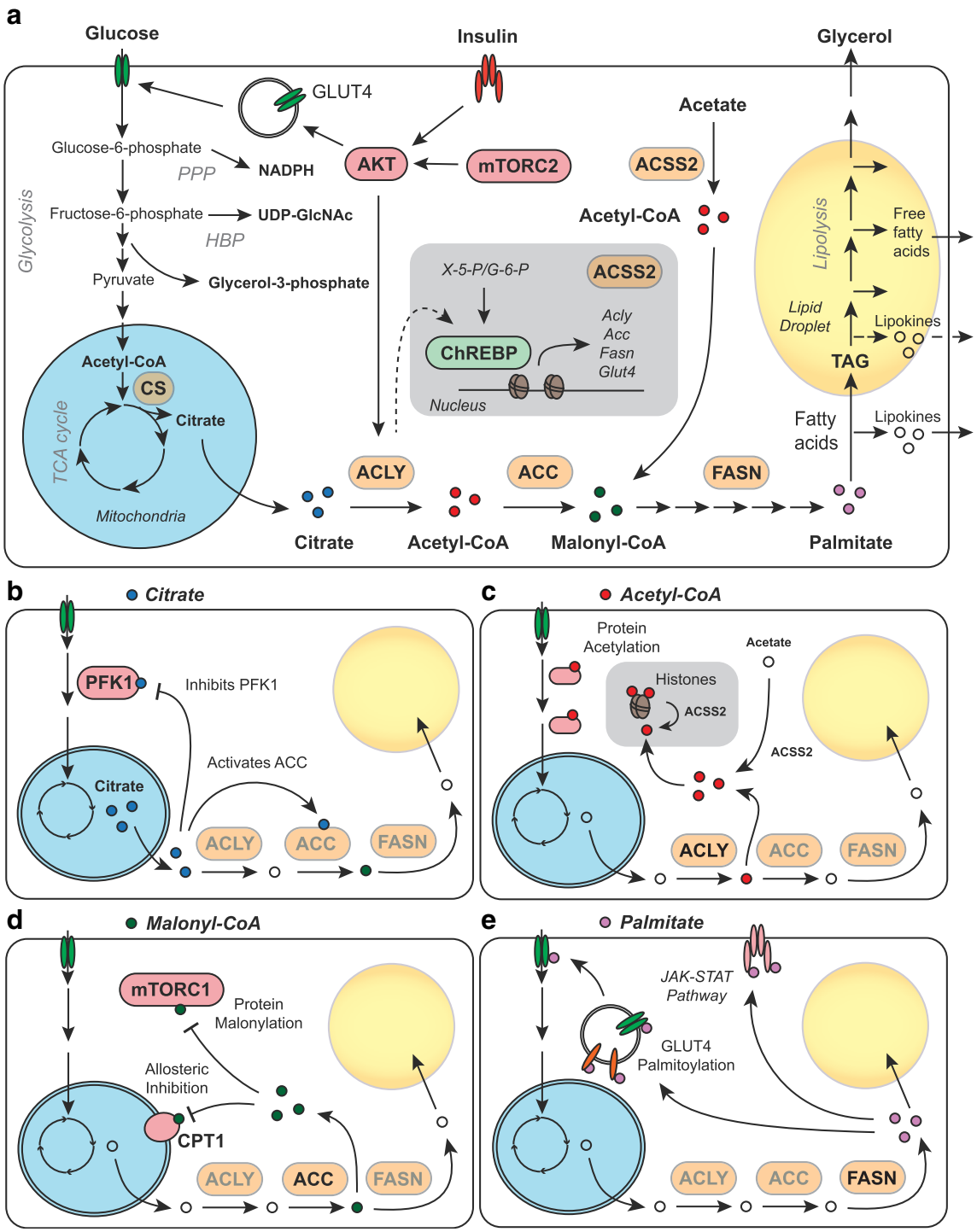
In adipocytes, three key enzymes that mediate glucose-derived de novo lipid synthesis are ATP-citrate lyase (ACLY), acetyl-CoA carboxylase (ACC), and fatty acid synthase (FASN) (Fig. 1a). Metabolized glucose that enters the mitochondria via pyruvate can exit the TCA cycle in the form of citrate, which 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) (Fig. 1a) [69]. Overexpressing GLUT4 in adipose tissue promotes glucose uptake, which increases DNL enzyme expression by stimulating ChREBP activity. In response, ChREBPα stimulates expression of a shorter isoform, called ChREBPβ, 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 the 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 ChREBPβ expression and for expression of the entire DNL pathway (Fig. 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 ChREBPβ 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 (16:1n17), a monounsaturated fatty acid which has been shown to attenuate adipose tissue inflammation and promote

◀ **Fig. 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-phosphoate 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-phophate (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. 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

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

Table 1 Mouse models of key de novo lipogenesis pathway regulators

DNL protein	Modification	Organism/model	Observation	Reference
GLUT4	Loss of function	GLUT4 KO (*aP2-Cre*)	Insulin resistance with preserved AT size.	[71]
	Gain of function	GLUT4 overexpression (*Adiponectin*, AG4OX)	OX mice are obese but have enhanced glucose tolerance.	[72]
ChREBP	Loss of function	ChREBP KO (*Adiponectin-Cre*)	Insulin resistance and increased AT inflammation in KO mice.	[72]
	Gain of function	ChREBP overexpression (*aP2*)	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 (*Adiponectin-Cre*)	Mild insulin resistance (female>male). Gender differences.	[145•, 146•]
ACC	Loss of function	ACC1 KO (*aP2-Cre*)	Decreased lipid accumulation in KO AT under fat-free diet.	[152]
FASN	Loss of function	FASN KO (*Adiponectin-Cre*)	Increased energy expenditure and browning in SAT and resistant to diet-induced obesity in KO mice.	[155]
		FASN inducible KO (*Adiponectin-CreERT2*)	Enhanced glucose tolerance and thermogenic signals in SAT. Increased sympathetic nerve activity in SAT.	[64, 156]

AT, adipose tissue; *DNL*, de novo lipogenesis; *SAT*, subcutaneous adipose tissue

bind to the C-terminus of phosphofructokinase 1 (PFK1) to allosterically inhibit this rate-limiting, unidirectional step in glycolysis (Fig. 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 (Fig. 1a) [95]. Mitochondrial dysfunction has been shown to contribute to metabolic disturbance and obesity [96]. In the 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 subcellular comparts (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 (Fig. 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 sense 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 CIDEC, which is required for forming lipid droplets. Deacetylation of CIDEC by HDAC6 inhibits its activity and destabilizes lipid droplets in adipocytes [119]. The same study showed that HDAC6 is downregulated 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 the 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 (Fig. 1c), is also downregulated 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 (Fig. 1d). Malonyl-CoA levels inversely correlate with insulin resistance in the 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 the 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 "demalonylation" enzyme in the 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 the 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 downregulates 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

Palmitoylation of proteins on cysteine (Spalmitoylation) 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 (Fig. 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 palmityolated in adipocytes [140] (Fig. 1e). JAK-STAT is required for adipocyte differentiation and also modulates insulin sensitivity and 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 toward 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 upregulation 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 ChREBPβ 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 (Fig. 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 PPARγ 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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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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References

Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

1. Hales C, Carroll M, Fryar C, Ogden C. Prevalence of obesity among adults and youth: United States, 2015-2016. NCHS Data Brief. 2017;288.
2. Van Gaal LF, Mertens IL, De Block CE. Mechanisms linking obesity with cardiovascular disease. Nature. 2006;444(7121):875–80.
3. Sullivan PW, Morrato EH, Ghushchyan V, Wyatt HR, Hill JO. Obesity, inactivity, and the prevalence of diabetes and diabetes-related cardiovascular comorbidities in the U.S., 2000-2002. Diabetes Care. 2005;28(7):1599–603.
4. Eckel RH, Kahn SE, Ferrannini E, Goldfine AB, Nathan DM, Schwartz MW, et al. Obesity and type 2 diabetes: what can be unified and what needs to be individualized? J Clin Endocrinol Metab. 2011;96(6):1654–63.
5. Willett WC, Dietz WH, Colditz GA. Guidelines for healthy weight. N Engl J Med. 1999;341(6):427–34.
6. Ashcroft FM, Rorsman P. Diabetes mellitus and the β cell: the last ten years. Cell. 2012;148(6):1160–71.
7. Tabák AG, Herder C, Rathmann W, Brunner EJ, Kivimäki M. Prediabetes: a high-risk state for developing diabetes. Lancet. 2012;379(9833):2279–90.
8. Association AD. 8. Pharmacologic approaches to glycemic treatment: standards of medical care in diabetes—2018. Diabetes Care. 2018 Jan 1;41(Supplement 1):S73–85.
9. Rosen ED, Spiegelman BM. What we talk about when we talk about fat. Cell. 2014;156(1):20–44.
10. Luo L, Liu M. Adipose tissue in control of metabolism. J Endocrinol. 2016;231(3):R77–99.
11. Kusminski CM, Bickel PE, Scherer PE. Targeting adipose tissue in the treatment of obesity-associated diabetes. Nat Rev Drug Discov. 2016;15(9):639–60.
12. Longo M, Zatterale F, Naderi J, Parrillo L, Formisano P, Raciti GA, et al. Adipose tissue dysfunction as determinant of obesity-associated metabolic complications. Int J Mol Sci. 2019;13:20(9).
13. Joffe BI, Panz VR, Raal FJ. From lipodystrophy syndromes to diabetes mellitus. Lancet. 2001;357(9266):1379–81.
14. Kwok KHM, Lam KSL, Xu A. Heterogeneity of white adipose tissue: molecular basis and clinical implications. Exp Mol Med. 2016;48:e215.
15. Sebo ZL, Rodeheffer MS. Assembling the adipose organ: adipocyte lineage segregation and adipogenesis in vivo. Dev Camb Engl. 2019;4:146(7).
16. Sanchez-Gurmaches J, Hung C-M, Guertin DA. Emerging complexities in adipocyte origins and identity. Trends Cell Biol. 2016;26(5):313–26.
17. Chau Y-Y, Bandiera R, Serrels A, Martínez-Estrada OM, Qing W, Lee M, et al. Visceral and subcutaneous fat have different origins and evidence supports a mesothelial source. Nat Cell Biol. 2014;16(4):367–75.
18. Primeau V, Coderre L, Karelis AD, Brochu M, Lavoie M-E, Messier V, et al. Characterizing the profile of obese patients who are metabolically healthy. Int J Obes. 2011;35(7):971–81.
19. Scherer PE. The many secret lives of adipocytes: implications for diabetes. Diabetologia. 2019;62(2):223–32.
20. Ikeda K, Maretich P, Kajimura S. The common and distinct features of brown and beige adipocytes. Trends Endocrinol Metab. 2018;29(3):191–200.
21. Verboven K, Wouters K, Gaens K, Hansen D, Bijnen M, Wetzels S, et al. Abdominal subcutaneous and visceral adipocyte size, lipolysis and inflammation relate to insulin resistance in male obese humans. Sci Rep. 2018;8(1):4677.
22. Tchernof A, Després J-P. Pathophysiology of human visceral obesity: an update. Physiol Rev. 2013;93(1):359–404.
23. Bjørndal B, Burri L, Staalesen V, Skorve J, Berge RK. Different adipose depots: their role in the development of metabolic syndrome and mitochondrial response to hypolipidemic agents. J Obes. 2011;2011:1–15.
24. Drolet R, Richard C, Sniderman AD, Mailloux J, Fortier M, Huot C, et al. Hypertrophy and hyperplasia of abdominal adipose tissues in women. Int J Obes 2005. 2008 Feb;32(2):283–91.
25. Veilleux A, Caron-Jobin M, Noël S, Laberge PY, Tchernof A. Visceral adipocyte hypertrophy is associated with dyslipidemia independent of body composition and fat distribution in women. Diabetes. 2011;60(5):1504–11.
26. Lessard J, Tchernof A. Depot- and obesity-related differences in adipogenesis. Clin Lipidol. 2012;7(5):587–96.
27. Wang QA, Tao C, Gupta RK, Scherer PE. Tracking adipogenesis during white adipose tissue development, expansion and regeneration. Nat Med. 2013;19(10):1338–44.
28. Foster MT, Shi H, Softic S, Kohli R, Seeley RJ, Woods SC. Transplantation of non-visceral fat to the visceral cavity improves glucose tolerance in mice: investigation of hepatic lipids and insulin sensitivity. Diabetologia. 2011;54(11):2890–9.
29. Foster MT, Softic S, Caldwell J, Kohli R, de Kloet AD, Seeley RJ. Subcutaneous adipose tissue transplantation in diet-induced obese mice attenuates metabolic dysregulation while removal exacerbates It. Phys Rep. 2013;1(2).
30. Hocking SL, Chisholm DJ, James DE. Studies of regional adipose transplantation reveal a unique and beneficial interaction between subcutaneous adipose tissue and the intra-abdominal compartment. Diabetologia. 2008;51(5):900–2.
31. Tran TT, Yamamoto Y, Gesta S, Kahn CR. Beneficial effects of subcutaneous fat transplantation on metabolism. Cell Metab. 2008;7(5):410–20.
32. Tulloch-Reid MK, Hanson RL, Sebring NG, Reynolds JC, Premkumar A, Genovese DJ, et al. Both subcutaneous and

visceral adipose tissue correlate highly with insulin resistance in African Americans. Obes Res. 2004;12(8):1352–9.
33. Sattar N, Gill JM. Type 2 diabetes as a disease of ectopic fat? BMC Med [Internet]. 2014 Dec [cited 2019 May 28];12(1). Available from: http://bmcmedicine.biomedcentral.com/articles/10.1186/s12916-014-0123-4
34. Unger RH, Clark GO, Scherer PE, Orci L. Lipid homeostasis, lipotoxicity and the metabolic syndrome. Biochim Biophys Acta BBA - Mol Cell Biol Lipids. 2010;1801(3):209–14.
35. Kwon H, Pessin JE. Adipokines mediate inflammation and insulin resistance. Front Endocrinol [Internet]. 2013 Jun 12 [cited 2019 May 26];4. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3679475/
36. Samuel VT, Shulman GI. Mechanisms for insulin resistance: common threads and missing links. Cell. 2012;148(5):852–71.
37. Huh JY, Park YJ, Ham M, Kim JB. Crosstalk between adipocytes and immune cells in adipose tissue inflammation and metabolic dysregulation in obesity. Mol Cell. 2014;37(5):365–71.
38. Chatzigeorgiou A, Chavakis T. Immune cells and metabolism. Handb Exp Pharmacol. 2016;233:221–49.
39. Xia C, Rao X, Zhong J. Role of T Lymphocytes in type 2 diabetes and diabetes-associated inflammation [Internet]. Journal of Diabetes Research. 2017 [cited 2019 Aug 25]. Available from: https://www.hindawi.com/journals/jdr/2017/6494795/
40. Appari M, Channon KM, McNeill E. Metabolic regulation of adipose tissue macrophage function in obesity and diabetes. Antioxid Redox Signal. 2017;29(3):297–312.
41. Kang YE, Kim JM, Joung KH, Lee JH, You BR, Choi MJ, et al. The roles of adipokines, proinflammatory cytokines, and adipose tissue macrophages in obesity-associated insulin resistance in modest obesity and early metabolic dysfunction. PLoS One. 2016 Apr 21;11(4):e0154003.
42. Olefsky JM, Glass CK. Macrophages, inflammation, and insulin resistance. Annu Rev Physiol. 2010;72:219–46.
43. Deng T, Liu J, Deng Y, Minze L, Xiao X, Wright V, et al. Adipocyte adaptive immunity mediates diet-induced adipose inflammation and insulin resistance by decreasing adipose Treg cells. Nat Commun [Internet]. 2017 Jul 12 [cited 2019 Aug 25];8. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5510177/
44. Tracey ML, Li-Fen L, Cindy L, Shen L, John M, Homero R, et al. T-cell profile in adipose tissue is associated with insulin resistance and systemic Inflammation in humans. Arterioscler Thromb Vasc Biol. 2014;34(12):2637–43.
45. Czech MP, Tencerova M, Pedersen DJ, Aouadi M. Insulin signalling mechanisms for triacylglycerol storage. Diabetologia. 2013;56(5):949–64.
46. Morigny P, Houssier M, Mouisel E, Langin D. Adipocyte lipolysis and insulin resistance. Biochimie. 2016;125:259–66.
47. Guilherme A, Henriques F, Bedard AH, Czech MP. Molecular pathways linking adipose innervation to insulin action in obesity and diabetes mellitus. Nat Rev Endocrinol. 2019;15(4):207–25.
48. Mîinea CP, Sano H, Kane S, Sano E, Fukuda M, Peränen J, et al. AS160, the Akt substrate regulating GLUT4 translocation, has a functional Rab GTPase-activating protein domain. Biochem J. 2005;391(Pt 1):87–93.
49. Brewer PD, Romenskaia I, Kanow MA, Mastick CC. Loss of AS160 Akt substrate causes Glut4 protein to accumulate in compartments that are primed for fusion in basal adipocytes. J Biol Chem. 2011;286(30):26287–97.
50. Ramm G, Larance M, Guilhaus M, James DE. A role for 14-3-3 in insulin-stimulated GLUT4 translocation through its interaction with the RabGAP AS160. J Biol Chem. 2006;281(39):29174–80.
51. Chen S, Wasserman DH, MacKintosh C, Sakamoto K. Mice with AS160/TBC1D4-Thr649Ala knockin mutation are glucose intolerant with reduced insulin sensitivity and altered GLUT4 trafficking. Cell Metab. 2011;13(1):68–79.
52. Gonzalez E, McGraw TE. Insulin signaling diverges into Akt-dependent and -independent signals to regulate the recruitment/docking and the fusion of GLUT4 vesicles to the plasma membrane. Mol Biol Cell. 2006;17(10):4484–93.
53. Krycer JR, Yugi K, Hirayama A, Fazakerley DJ, Quek L-E, Scalzo R, et al. Dynamic metabolomics reveals that insulin primes the adipocyte for glucose metabolism. Cell Rep. 2017;21(12):3536–47.
54. Whelan SA, Dias WB, Thiruneelakantapillai L, Lane MD, Hart GW. Regulation of insulin receptor substrate 1 (IRS-1)/AKT kinase-mediated insulin signaling by O-linked β-N-acetylglucosamine in 3T3-L1 adipocytes. J Biol Chem. 2010;285(8):5204–11.
55. Park SY, Ryu J, Lee W. O-GlcNAc modification on IRS-1 and Akt2 by PUGNAc inhibits their phosphorylation and induces insulin resistance in rat primary adipocytes. Exp Mol Med. 2005;37(3):220–9.
56. Ji S, Park SY, Roth J, Kim HS, Cho JW. O-GlcNAc modification of PPARγ reduces its transcriptional activity. Biochem Biophys Res Commun. 2012;417(4):1158–63.
57. Wells L, Vosseller K, Hart GW. A role for N-acetylglucosamine as a nutrient sensor and mediator of insulin resistance. Cell Mol Life Sci CMLS. 2003;60(2):222–8.
58. Mondoux MA, Love DC, Ghosh SK, Fukushige T, Bond M, Weerasinghe GR, et al. O-linked-N-acetylglucosamine cycling and insulin signaling are required for the glucose stress response in Caenorhabditis elegans. Genetics. 2011;188(2):369–82.
59. Sun C, Shang J, Yao Y, Yin X, Liu M, Liu H, et al. O-GlcNAcylation: a bridge between glucose and cell differentiation. J Cell Mol Med. 2016;20(5):769–81.
60. Wollaston-Hayden EE, Harris RBS, Liu B, Bridger R, Xu Y, Wells L. Global O-GlcNAc levels modulate transcription of the adipocyte secretome during chronic insulin resistance. Front Endocrinol. 2014;5:223.
61. Eissing L, Scherer T, Tödter K, Knippschild U, Greve JW, Buurman WA, et al. De novo lipogenesis in human fat and liver is linked to ChREBP-β and metabolic health. Nat Commun. 2013;4:1528.
62. Herman MA, Peroni OD, Villoria J, Schön MR, Abumrad NA, Blüher M, et al. A novel ChREBP isoform in adipose tissue regulates systemic glucose metabolism. Nature. 2012;484(7394):333–8.
63. Roberts R, Hodson L, Dennis AL, Neville MJ, Humphreys SM, Harnden KE, et al. Markers of de novo lipogenesis in adipose tissue: associations with small adipocytes and insulin sensitivity in humans. Diabetologia. 2009;52(5):882–90.
64.• Guilherme A, Pedersen DJ, Henchey E, Henriques FS, Danai LV, Shen Y, et al. Adipocyte lipid synthesis coupled to neuronal control of thermogenic programming. Mol Metab. 2017;6(8):781–96. **This study suggests that knocking-out FASN in the DNL pathway affects the crosstalk between adipose tissue and nervous system**.
65. Strawford A, Antelo F, Christiansen M, Hellerstein MK. Adipose tissue triglyceride turnover, de novo lipogenesis, and cell proliferation in humans measured with 2H2O. Am J Physiol Endocrinol Metab. 2004;286(4):E577–88.
66. Chiu S, Mulligan K, Schwarz J-M. Dietary carbohydrates and fatty liver disease: de novo lipogenesis. Curr Opin Clin Nutr Metab Care. 2018;21(4):277–82.
67. Sanders FWB, Griffin JL. De novo lipogenesis in the liver in health and disease: more than just a shunting yard for glucose. Biol Rev Camb Philos Soc. 2016;91(2):452–68.

68. Harada N, Oda Z, Hara Y, Fujinami K, Okawa M, Ohbuchi K, et al. Hepatic de novo lipogenesis is present in liver-specific ACC1-deficient mice. Mol Cell Biol. 2007;27(5):1881–8.
69. 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. 2004;101(19):7281–6.
70. Yore MM, Syed I, Moraes-Vieira PM, Zhang T, Herman MA, Homan EA, et al. Discovery of a class of endogenous mammalian lipids with anti-diabetic and anti-inflammatory effects. Cell. 2014;159(2):318–32.
71. Abel ED, Peroni O, Kim JK, Kim YB, Boss O, Hadro E, et al. Adipose-selective targeting of the GLUT4 gene impairs insulin action in muscle and liver. Nature. 2001;409(6821):729–33.
72. Vijayakumar A, Aryal P, Wen J, Syed I, Vazirani RP, Moraes-Vieira PM, et al. Absence of carbohydrate response element binding protein in adipocytes causes systemic insulin resistance and impairs glucose transport. Cell Rep. 2017;21(4):1021–35.
73. Kursawe R, Caprio S, Giannini C, Narayan D, Lin A, D'Adamo E, et al. Decreased transcription of ChREBP-α/β isoforms in abdominal subcutaneous adipose tissue of obese adolescents with prediabetes or early type 2 diabetes: associations with insulin resistance and hyperglycemia. Diabetes. 2013;62(3):837–44.
74. Ortega-Prieto P, Postic C. Carbohydrate sensing through the transcription factor ChREBP. Front Genet. 2019;10:472.
75. Kawaguchi T, Takenoshita M, Kabashima T, Uyeda K. Glucose and cAMP regulate the L-type pyruvate kinase gene by phosphorylation/dephosphorylation of the carbohydrate response element binding protein. Proc Natl Acad Sci. 2001;98(24):13710–5.
76. Kabashima T, Kawaguchi T, Wadzinski BE, Uyeda K. Xylulose 5-phosphate mediates glucose-induced lipogenesis by xylulose 5-phosphate-activated protein phosphatase in rat liver. Proc Natl Acad Sci. 2003;100(9):5107–12.
77. Dentin R, Tomas-Cobos L, Foufelle F, Leopold J, Girard J, Postic C, et al. Glucose 6-phosphate, rather than xylulose 5-phosphate, is required for the activation of ChREBP in response to glucose in the liver. J Hepatol. 2012;56(1):199–209.
78. Bricambert J, Miranda J, Benhamed F, Girard J, Postic C, Dentin R. Salt-inducible kinase 2 links transcriptional coactivator p300 phosphorylation to the prevention of ChREBP-dependent hepatic steatosis in mice. J Clin Invest. 2010;120(12):4316–31.
79. Guinez C, Filhoulaud G, Rayah-Benhamed F, Marmier S, Dubuquoy C, Dentin R, et al. O-GlcNAcylation increases ChREBP protein content and transcriptional activity in the liver. Diabetes. 2011;60(5):1399–413.
80. Tang Y, Wallace M, Sanchez-Gurmaches J, Hsiao W-Y, Li H, Lee PL, et al. Adipose tissue mTORC2 regulates ChREBP-driven de novo lipogenesis and hepatic glucose metabolism. Nat Commun. 2016;7:11365.
81. Hung C-M, Calejman CM, Sanchez-Gurmaches J, Li H, Clish CB, Hettmer S, et al. Rictor/mTORC2 loss in the Myf5 lineage reprograms brown fat metabolism and protects mice against obesity and metabolic disease. Cell Rep. 2014;8(1):256–71.
82. Jung SM, Hung C-M, Hildebrand SR, Sanchez-Gurmaches J, Martinez-Pastor B, Gengatharan JM, et al. Non-canonical mTORC2 signaling regulates brown adipocyte lipid catabolism through SIRT6-FoxO1. Mol Cell. 2019;75(4):807–822.e8.
83. Davies MN, O'Callaghan BL, Towle HC. Glucose activates ChREBP by increasing its rate of nuclear entry and relieving repression of its transcriptional activity. J Biol Chem. 2008;283(35):24029–38.
84. Kawaguchi T, Osatomi K, Yamashita H, Kabashima T, Uyeda K. Mechanism for fatty acid "sparing" effect on glucose-induced transcription: regulation of carbohydrate-responsive element-binding protein by amp-activated protein kinase. J Biol Chem. 2002;277(6):3829–35.
85. Cao H, Gerhold K, Mayers JR, Wiest MM, Watkins SM, Hotamisligil GS. Identification of a lipokine, a lipid hormone linking adipose tissue to systemic metabolism. Cell. 2008;134(6):933–44.
86. Syed I, Lee J, Moraes-Vieira PM, Donaldson CJ, Sontheimer A, Aryal P, et al. Palmitic acid hydroxystearic acids activate GPR40, which is involved in their beneficial effects on glucose homeostasis. Cell Metab. 2018;27(2):419–427.e4.
87. Kuda O, Brezinova M, Rombaldova M, Slavikova B, Posta M, Beier P, et al. Docosahexaenoic acid-derived fatty acid esters of hydroxy fatty acids (FAHFAs) with anti-inflammatory properties. Diabetes. 2016;65(9):2580–90.
88.•• Hammarstedt A, Syed I, Vijayakumar A, Eliasson B, Gogg S, Kahn BB, et al. Adipose tissue dysfunction is associated with low levels of the novel palmitic acid hydroxystearic acids. Sci Rep. 2018;8(1):15757. **The finding in this study suggests that a new class of lipid generated from the DNL pathway is anti-diabetic and correlates with insulin sensitivity in human**.
89. Yang Q, Vijayakumar A, Kahn BB. Metabolites as regulators of insulin sensitivity and metabolism. Nat Rev Mol Cell Biol. 2018;19(10):654.
90. Song Z, Xiaoli AM, Yang F. Regulation and metabolic significance of de novo lipogenesis in adipose tissues. Nutrients. 2018;29:10(10).
91. Randle PJ. Regulatory interactions between lipids and carbohydrates: the glucose fatty acid cycle after 35 years. Diabetes Metab Rev. 1998;14(4):263–83.
92. Usenik A, Legiša M. Evolution of allosteric citrate binding sites on 6-phosphofructo-1-kinase. PLoS ONE [Internet]. 2010 Nov 23 [cited 2019 Apr 16];5(11). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2990764/
93. Kim C-W, Moon Y-A, Park SW, Cheng D, Kwon HJ, Horton JD. Induced polymerization of mammalian acetyl-CoA carboxylase by MIG12 provides a tertiary level of regulation of fatty acid synthesis. Proc Natl Acad Sci U S A. 2010;107(21):9626–31.
94. Wakil SJ, Abu-Elheiga LA. Fatty acid metabolism: target for metabolic syndrome. J Lipid Res. 2009;50(Suppl):S138–43.
95. Williams NC, O'Neill LAJ. A Role for the Krebs Cycle Intermediate citrate in metabolic reprogramming in innate immunity and inflammation. Front Immunol [Internet]. 2018 Feb 5 [cited 2019 Apr 16];9. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5807345/
96. Houmard JA. Intramuscular lipid oxidation and obesity. Am J Phys Regul Integr Comp Phys. 2008;294(4):R1111–6.
97. Ortenblad N, Mogensen M, Petersen I, Højlund K, Levin K, Sahlin K, et al. Reduced insulin-mediated citrate synthase activity in cultured skeletal muscle cells from patients with type 2 diabetes: evidence for an intrinsic oxidative enzyme defect. Biochim Biophys Acta. 2005;1741(1–2):206–14.
98. Alhindi Y, Vaanholt LM, Al-Tarrah M, Gray SR, Speakman JR, Hambly C, et al. Low citrate synthase activity is associated with glucose intolerance and lipotoxicity [Internet]. Journal of Nutrition and Metabolism. 2019 [cited 2019 Jun 9]. Available from: https://www.hindawi.com/journals/jnme/2019/8594825/
99. Christe M, Hirzel E, Lindinger A, Kern B, von Flüe M, Peterli R, et al. Obesity affects mitochondrial citrate synthase in human omental adipose tissue. ISRN Obes. 2013;2013:826027.
100. Yin X, Lanza IR, Swain JM, Sarr MG, Nair KS, Jensen MD. Adipocyte mitochondrial function is reduced in human obesity independent of fat cell size. J Clin Endocrinol Metab. 2014;99(2):E209–16.
101. Shi L, Tu BP. Acetyl-CoA and the regulation of metabolism: mechanisms and consequences. Curr Opin Cell Biol. 2015;33:125–31.

102. Lee JV, Shah SA, Wellen KE. Obesity, cancer, and acetyl-CoA metabolism. Drug Discov Today Dis Mech. 2013;10(1–2):e55–61.
103. Pietrocola F, Galluzzi L, Bravo-San Pedro JM, Madeo F, Kroemer G. Acetyl coenzyme A: a central metabolite and second messenger. Cell Metab. 2015;21(6):805–21.
104. Takahashi H, McCaffery JM, Irizarry RA, Boeke JD. Nucleocytosolic acetyl-coenzyme a synthetase is required for histone acetylation and global transcription. Mol Cell. 2006;23(2):207–17.
105. Galdieri L, Vancura A. Acetyl-CoA carboxylase regulates global histone acetylation. J Biol Chem. 2012;287(28):23865–76.
106. Galdieri L, Chang J, Mehrotra S, Vancura A. Yeast phospholipase C is required for normal acetyl-CoA homeostasis and global histone acetylation. J Biol Chem. 2013;288(39):27986–98.
107. Galdieri L, Zhang T, Rogerson D, Lleshi R, Vancura A. Protein acetylation and acetyl coenzyme a metabolism in budding yeast. Eukaryot Cell. 2014;13(12):1472–83.
108. Wellen KE, Hatzivassiliou G, Sachdeva UM, Bui TV, Cross JR, Thompson CB. ATP-citrate lyase links cellular metabolism to histone acetylation. Science. 2009;324(5930):1076–80.
109. Cai L, Sutter BM, Li B, Tu BP. Acetyl-CoA induces cell growth and proliferation by promoting the acetylation of histones at growth genes. Mol Cell. 2011;42(4):426–37.
110. Lee JV, Carrer A, Shah S, Snyder NW, Wei S, Venneti S, et al. Akt-dependent metabolic reprogramming regulates tumor cell histone acetylation. Cell Metab. 2014;20(2):306–19.
111. Campbell SL, Wellen KE. Metabolic signaling to the nucleus in cancer. Mol Cell. 2018;71(3):398–408.
112. Muka T, Nano J, Voortman T, Braun KVE, Ligthart S, Stranges S, et al. The role of global and regional DNA methylation and histone modifications in glycemic traits and type 2 diabetes: a systematic review. Nutr Metab Cardiovasc Dis NMCD. 2016;26(7):553–66.
113. Wang X, Wang L, Sun Y, Li R, Deng J, Deng J. DNA methylation and histone deacetylation regulating insulin sensitivity due to chronic cold exposure. Cryobiology. 2017;74:36–42.
114. Oliva-Olivera W, Lhamyani S, Coín-Aragüez L, Alcaide-Torres J, Cardona F, El Bekay R, et al. Involvement of acetyl-CoA-producing enzymes in the deterioration of the functional potential of adipose-derived multipotent cells from subjects with metabolic syndrome. Metabolism. 2018;88:12–21.
115. Daitoku H, Sakamaki J, Fukamizu A. Regulation of FoxO transcription factors by acetylation and protein–protein interactions. Biochim Biophys Acta BBA - Mol Cell Res. 2011;1813(11):1954–60.
116. Perrot V, Rechler MM. The coactivator p300 directly acetylates the Forkhead transcription factor Foxo1 and stimulates Foxo1-induced transcription. Mol Endocrinol. 2005;19(9):2283–98.
117. Jing E, Gesta S, Kahn CR. SIRT2 regulates adipocyte differentiation through FoxO1 acetylation/deacetylation. Cell Metab. 2007;6(2):105–14.
118. Gonzalez E, Flier E, Molle D, Accili D, McGraw TE. Hyperinsulinemia leads to uncoupled insulin regulation of the GLUT4 glucose transporter and the FoxO1 transcription factor. Proc Natl Acad Sci. 2011;108(25):10162–7.
119. Qian H, Chen Y, Nian Z, Su L, Yu H, Chen F-J, et al. HDAC6-mediated acetylation of lipid droplet-binding protein CIDEC regulates fat-induced lipid storage. J Clin Invest. 2017;127(4):1353–69.
120. Sivanand S, Viney I, Wellen KE. Spatiotemporal control of acetyl-CoA metabolism in chromatin regulation. Trends Biochem Sci. 2018;43(1):61–74.
121. Sharma S, Taliyan R. Histone deacetylase inhibitors: future therapeutics for insulin resistance and type 2 diabetes. Pharmacol Res. 2016;113:320–6.
122. Ye J. Improving insulin sensitivity with HDAC inhibitor. Diabetes. 2013;62(3):685–7.
123. Xiao C, Giacca A, Lewis GF. Sodium phenylbutyrate, a drug with known capacity to reduce endoplasmic reticulum stress, partially alleviates lipid-induced insulin resistance and beta-cell dysfunction in humans. Diabetes. 2011;60(3):918–24.
124. Carrer A, Parris JLD, Trefely S, Henry RA, Montgomery DC, Torres A, et al. Impact of a high-fat diet on tissue acyl-CoA and histone acetylation levels. J Biol Chem. 2017;292(8):3312–22.
125. Berwick DC, Hers I, Heesom KJ, Moule SK, Tavaré JM. The identification of ATP-citrate lyase as a protein kinase B (Akt) substrate in primary adipocytes. J Biol Chem. 2002;277(37):33895–900.
126. Shulman GI. Cellular mechanisms of insulin resistance. J Clin Invest. 2000;106(2):171–6.
127. Kim Y-B, Kotani K, Ciaraldi TP, Henry RR, Kahn BB. Insulin-stimulated protein kinase C lambda/zeta activity is reduced in skeletal muscle of humans with obesity and type 2 diabetes: reversal with weight reduction. Diabetes. 2003;52(8):1935–42.
128. Hirschey MD, Zhao Y. Metabolic regulation by lysine malonylation, succinylation, and glutarylation. Mol Cell Proteomics MCP. 2015;14(9):2308–15.
129. Choudhary C, Weinert BT, Nishida Y, Verdin E, Mann M. The growing landscape of lysine acetylation links metabolism and cell signalling. Nat Rev Mol Cell Biol. 2014;15(8):536–50.
130. Yu J, Sadhukhan S, Noriega LG, Moullan N, He B, Weiss RS, et al. Metabolic characterization of a *Sirt5* deficient mouse model. Sci Rep. 2013;3:2806.
131. Nishida Y, Rardin MJ, Carrico C, He W, Sahu AK, Gut P, et al. SIRT5 regulates both cytosolic and mitochondrial protein malonylation with glycolysis as a major target. Mol Cell. 2015;59(2):321–32.
132. Wang G, Meyer JG, Cai W, Softic S, Li ME, Verdin E, et al. Regulation of UCP1 and mitochondrial metabolism in brown adipose tissue by reversible succinylation. Mol Cell. 2019;74(4):844–857.e7.
133. Wang G, Meyer JG, Cai W, Li ME, Softic S, Kahn CR. Sirt5 plays a critical role in mitochondrial protein acylation and mitochondrial metabolic homeostasis in brown fat. Diabetes. 2018;67(Supplement 1):274-OR.
134. Bruning U, Morales-Rodriguez F, Kalucka J, Goveia J, Taverna F, Queiroz KCS, et al. Impairment of angiogenesis by fatty acid synthase inhibition involves mTOR malonylation. Cell Metab. 2018;28(6):866–880.e15.
135. Mumby SM. Reversible palmitoylation of signaling proteins. Curr Opin Cell Biol. 1997;9(2):148–54.
136. Iwanaga T, Tsutsumi R, Noritake J, Fukata Y, Fukata M. Dynamic protein palmitoylation in cellular signaling. Prog Lipid Res. 2009;48(3–4):117–27.
137. Hu JS, James G, Olson EN. Protein fatty acylation: a novel mechanism for association of proteins with membranes and its role in transmembrane regulatory pathways. BioFactors Oxf Engl. 1988;1(3):219–26.
138. Resh MD. Fatty acylation of proteins: new insights into membrane targeting of myristoylated and palmitoylated proteins. Biochim Biophys Acta. 1999 Aug 12;1451(1):1–16.
139. Smotrys JE, Linder ME. Palmitoylation of intracellular signaling proteins: regulation and function. Annu Rev Biochem. 2004;73:559–87.
140. Ren W, Jhala US, Du K. Proteomic analysis of protein palmitoylation in adipocytes. Adipocyte. 2013;2(1):17–27.
141. Du K, Murakami S, Sun Y, Kilpatrick CL, Luscher B. DHHC7 palmitoylates glucose transporter 4 (Glut4) and regulates Glut4 membrane translocation. J Biol Chem. 2017;292(7):2979–91.

142. Ren W, Sun Y, Du K. Glut4 palmitoylation at Cys223 plays a critical role in Glut4 membrane trafficking. Biochem Biophys Res Commun. 2015;460(3):709–14.
143. Richard AJ, Stephens JM. Emerging roles of JAK-STAT signaling pathways in adipocytes. Trends Endocrinol Metab. 2011;22(8):325–32.
144. de Jong JMA, Sun W, Pires ND, Frontini A, Balaz M, Jespersen NZ, et al. Human brown adipose tissue is phenocopied by classical brown adipose tissue in physiologically humanized mice. Nat Metab. 2019;1(8):830–43.
145.• Zhao S, Torres A, Henry RA, Trefely S, Wallace M, Lee JV, et al. ATP-citrate lyase controls a glucose-to-acetate metabolic switch. Cell Rep. 2016;17(4):1037–52. **This study suggests altering the acetyl-CoA-generating pathway affects adipogenic gene expression and whole body metabolism in certain conditions**.
146.• Fernandez S, Viola JM, Torres A, Wallace M, Trefely S, Zhao S, et al. Adipocyte ACLY facilitates dietary carbohydrate handling to maintain metabolic homeostasis in females. Cell Rep. 2019;27(9):2772–2784.e6 This study suggests altering the acetyl-CoA-generating pathway affects adipogenic gene expression and whole body metabolism in certain conditions.
147. Schug ZT, Peck B, Jones DT, Zhang Q, Grosskurth S, Alam IS, et al. Acetyl-CoA synthetase 2 promotes acetate utilization and maintains cancer cell growth under metabolic stress. Cancer Cell. 2015 Jan;27(1):57–71.
148. Li X, Yu W, Qian X, Xia Y, Zheng Y, Lee J-H, et al. Nucleus-translocated ACSS2 promotes gene transcription for lysosomal biogenesis and autophagy. Mol Cell. 2017;66(5):684–697.e9.
149. Vysochan A, Sengupta A, Weljie AM, Alwine JC, Yu Y. ACSS2-mediated acetyl-CoA synthesis from acetate is necessary for human cytomegalovirus infection. Proc Natl Acad Sci U S A. 2017;114(8):E1528–35.
150. Huang Z, Zhang M, Plec AA, Estill SJ, Cai L, Repa JJ, et al. ACSS2 promotes systemic fat storage and utilization through selective regulation of genes involved in lipid metabolism. Proc Natl Acad Sci U S A. 2018;115(40):E9499–506.
151. Bulusu V, Tumanov S, Michalopoulou E, van den Broek NJ, MacKay G, Nixon C, et al. Acetate recapturing by nuclear acetyl-CoA synthetase 2 prevents loss of histone acetylation during oxygen and serum limitation. Cell Rep. 2017;18(3):647–58.
152. Mao J, Yang T, Gu Z, Heird WC, Finegold MJ, Lee B, et al. aP2-Cre-mediated inactivation of acetyl-CoA carboxylase 1 causes growth retardation and reduced lipid accumulation in adipose tissues. Proc Natl Acad Sci U S A. 2009;106(41):17576–81.
153. Lee KY, Russell SJ, Ussar S, Boucher J, Vernochet C, Mori MA, et al. Lessons on conditional gene targeting in mouse adipose tissue. Diabetes. 2013;62(3):864–74.
154. Mullican SE, Tomaru T, Gaddis CA, Peed LC, Sundaram A, Lazar MA. A novel adipose-specific gene deletion model demonstrates potential pitfalls of existing methods. Mol Endocrinol Baltim Md. 2013;27(1):127–34.
155. Lodhi IJ, Yin L, Jensen-Urstad APL, Funai K, Coleman T, Baird JH, et al. Inhibiting adipose tissue lipogenesis reprograms thermogenesis and PPARγ activation to decrease diet-induced obesity. Cell Metab. 2012;16(2):189–201.
156. Guilherme A, Pedersen DJ, Henriques F, Bedard AH, Henchey E, Kelly M, et al. Neuronal modulation of brown adipose activity through perturbation of white adipocyte lipogenesis. Mol Metab. 2018;16:116–25.
157. Nuotio-Antar AM, Poungvarin N, Li M, Schupp M, Mohammad M, Gerard S, et al. FABP4-Cre Mediated Expression of Constitutively Active ChREBP Protects Against Obesity, Fatty Liver, and Insulin Resistance. Endocrinology. 2015 Nov;156(11):4020–32.