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REVIEW ARTICLE

Insulin signaling to hepatic lipid metabolism in health and disease

Karla F. Leavens and Morris J. Birnbaum

Department of Medicine, Institute for Diabetes, Obesity and Metabolism, University of Pennsylvania School of Medicine, Philadelphia, PA, USA

Abstract

The increasing prevalence of overnutrition and reduced activity has led to a worldwide epidemic of obesity. In many cases, this is associated with insulin resistance, an inability of the hormone to direct its physiological actions appropriately. A number of disease states accompany insulin resistance such as type 2 diabetes mellitus, the metabolic syndrome, and non-alcoholic fatty liver disease. Though the pathways by which insulin controls hepatic glucose output have been of intense study in recent years, considerably less attention has been devoted to how lipid metabolism is regulated. Thus, both the proximal signaling pathways as well as the more distal targets of insulin remain uncertain. In this review, we consider the signaling pathways by which insulin controls the synthesis and accumulation of lipids in the mammalian liver and, in particular, how this might lead to abnormal triglyceride deposition in liver during insulin-resistant states.

Keywords: Lipids, liver, steatosis, triglyceride, SREBP1c, Akt

Introduction

Though a continuous supply of nutrients is required for our survival, their excess has lead to a group of related diseases, representing some of the leading causes of death in the developed world. Cardiovascular disease and other disorders of over-nutrition dominate the health care systems of high- and middle-income countries, accounting for over 20% of mortality (WHO, 2008). Meanwhile, the incidence of these diseases is increasing in low-income countries, even while diseases associated with malnutrition are still prevalent. Physiologic processes evolved to maximize nutrient storage in a world where sustenance was scarce are now resulting in obesity, type 2 diabetes mellitus (T2DM), and their sequelae in the face of surplus.

Insulin signaling serves to coordinate nutrient allocation and storage upon food ingestion. Nutrient influx after a meal stimulates secretion of insulin from the β-cells of the pancreas, promoting nutrient uptake in peripheral metabolic tissues and suppressing glucose production by the liver (reviewed in Saltiel, 2001). This process is tightly regulated so that nutrient intake and storage are perfectly balanced. Insulin promotes the uptake of dietary glucose into muscle and adipose by stimulating the translocation of the glucose transporter, Glut4, to the cell surface. The uptake of dietary lipids by peripheral tissues is also regulated by insulin, which stimulates the production and release of lipoprotein lipase (LPL) by endothelial cells. LPL hydrolyzes triglycerides into fatty acids, which are permeable to the cell membrane and can be taken up into adipocytes to be re-esterified and stored or into muscle cells to be oxidized for energy (reviewed in Ginsberg et al., 2005). Simultaneously, in order to prevent release of those stored fatty acids back into the circulation, insulin blocks lipolysis in adipocytes. Just as insulin promotes the storage of nutrients by its effects in the periphery, the liver is also a major site of insulin's action on metabolism, though to what extent this represents a direct action of the hormone on hepatocytes remains controversial (Bergman, 2000; Pocai et al., 2005). Insulin blocks hepatic glucose production, which is high in the postabsorptive state, by inhibiting gluconeogenesis and glycogenolysis

Address for Correspondence: Morris J. Birnbaum, 415 Curie Blvd., 322 Clinical Research Building, Philadelphia, PA 19104, USA. Tel: 215-898-5001, Fax: 215-573-9138. E-mail: birnbaum@mail.med.upenn.edu

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(Saltiel, 2001). Additionally, insulin signaling switches the liver from oxidation to synthesis of fatty acids from excess dietary nutrients absorbed during prandial and postprandial conditions. Thus, hepatic insulin signaling is essential to the maintenance of energy homeostasis through its regulation of glucose and lipid metabolism.

Correspondingly, in T2DM, which is characterized by "insulin resistance", i.e. the inability to appropriately direct its physiological responses, insulin's control of hepatic metabolism becomes impaired. During times of nutritional abundance, insulin signals in a normal liver to halt catabolism and to stimulate anabolism, resulting in the conversion of substrate into fatty acids and triglyceride for local storage as well as export. However, when this point of regulation goes awry due to the insulin resistance of T2DM, protective processes become maladaptive. Hepatic insulin resistance can simultaneously result in the dysregulation of both anabolism, leading to the excessive output of glucose, and, catabolism driving reduced oxidation and augmented accumulation of lipid in the liver, resulting in hepatic steatosis. Though much of the treatment of type 2 diabetic patients has focused on the control of serum glucose levels, it has been long recognized that lipid abnormalities are equally intrinsic to the dysregulation of insulin signaling in T2DM (McGarry, 1992). Though hyperglycemia can result in substantial morbidity in T2DM, cardiovascular disease remains the leading cause of death in these patients, and the processes, especially those in the liver, that underlie these lipid abnormalities contributes to substantial mortality associated with this disease. These issues are further heightened by the results of the ACCORD study, in which attempts at tight control of glucose did not lead to an improvement in mortality (Boyko, 2010; Ismail-Beigi et al., 2010). Thus, an understanding of hepatic insulin signaling and its regulation of metabolism are important to the development of therapies to combat the most common pathologies facing the world.

Insulin's control of hepatic lipid metabolism

Insulin promotes de novo lipogenesis while inhibiting fatty acid oxidation and very low density lipoprotein export

Following food intake, insulin instructs the liver to switch from fatty acid oxidation to fatty acid synthesis. When carbohydrate flux into the liver is high after a meal, insulin promotes the synthesis of fatty acids from excess substrates, called *de novo* lipogenesis. These fatty acids, along with dietary fatty acids, are esterified into triglycerides and eventually exported in very low density lipoproteins (VLDLs) for storage and utilization by peripheral tissues. Insulin regulates *de novo* lipogenesis through the increase of the genes encoding lipogenic enzymes, as described below in more detail. As fatty acid synthesis proceeds, accumulation of malonyl-coenzyme A (CoA), a precursor of fatty acids synthesis, directly inhibits carnitine palmitoyltransferase-1 (CPT1), a transporter that shuttles fatty acids into the mitochondria and the rate-limiting step of fatty acid β-oxidation. Simultaneously, insulin turns off β-oxidation of fatty acids though its inhibition of peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α), a master regulator of fatty acid oxidation (reviewed in Lin et al., 2005).

While insulin stimulates lipogenesis and thus increases lipid substrates for VLDL particles, it also acutely decreases VLDL secretion (reviewed in Sparks and Sparks, 1994). VLDL assembly requires the presence of both of its substrates, hepatic lipids and apolipoprotein B (apoB), and insulin likely functions by reducing the latter. Insulin inhibits the release of VLDL in perfused rat liver and *in vivo* in rats injected with glucose to stimulate insulin production (Sparks et al., 1989; Chirieac et al., 2000). Correspondingly, mice lacking the hepatic expression of the insulin receptor have increased apoB levels in their serum (Biddinger et al., 2008). Most regulation of apoB is thought to occur post-translationally, as increased fatty acid delivery to the liver by infusion in mice causes an increase in apoB secretion but not an increase in apoB mRNA levels (Zhang et al., 2004b). Insulin may work to suppress VLDL secretion directly by increasing degradation of apoB: in primary rat hepatocytes, the inhibition of apoB secretion by insulin correlates with an increase in intracellular degradation of apoB (Sparks and Sparks, 1994). Though the precise mechanism is unclear, insulin's effect on apoB degradation may be mediated through phosphatidylinositol-3-kinase (PI3K) signaling as inhibition of PI3K by wortmannin results in increased VLDL production and secretion *in vivo* (Chirieac et al., 2006).

Hepatic steatosis

While hepatic steatosis, the accumulation of excess lipid in the liver, is usually benign, in some individuals it can progress to inflammatory steatohepatitis, fibrosis, and cirrhosis (Anderson and Borlak, 2008). This entire spectrum is termed non-alcoholic fatty liver disease (NAFLD), and is likely present in 14–24% of the general population (reviewed in Browning and Horton, 2004). However, when obese individuals are considered, the prevalence rises to between 57 and 74% and to 85% in the morbidly obese (Angulo and Lindor, 2002; Fabbrini et al., 2010). Thus, as the prevalence of obesity and T2DM increase, so will the number of people with hepatic steatosis and other NAFLDs. While it is unclear what mediates the progression of hepatic steatosis to a more serious disease, increasing prevalence of NAFLD will result in increasing numbers of patients with end-stage liver disease: NAFLD is already a dominant indication for liver transplant (Browning and Horton, 2004; Anderson and Borlak, 2008).

The underlying pathogenesis of hepatic steatosis remains undefined, though it is clearly associated with states of hepatic insulin resistance. One important contributing factor is likely to be the increased concentration of fatty acids for esterification into triglycerides available to insulin-resistant livers (reviewed in (Ginsberg et al.,

2005). During insulin resistance, insulin fails to fully block lipolysis in adipose tissue, resulting in increased hydrolysis of triglycerides and circulating non-esterified fatty acid (NEFA) levels for the liver to take up from the serum (Adeli et al., 2001). In regard to new lipid synthesis in insulin-resistant states, there is a fundamental paradox: insulin promotes *de novo* lipogenesis in a normal liver and thus, in the presence of insulin resistance, hepatic lipid synthesis should be impaired. Unexpectedly, insulin's promotion of lipogenesis is maintained and even enhanced by hyperinsulinemia occurring in T2DM (see below). Furthermore, hyperglycemia may drive pathological lipid accumulation in T2DM as the excess glucose provides increased substrate and further drive for *de novo* lipogenesis. Additionally, fatty acid oxidation is decreased in insulin-resistant livers, though whether this is due to inhibition by *de novo* lipogenesis or secondary to mitochondrial overload and oxidative stress is unclear (Angulo and Lindor, 2002; Browning and Horton, 2004; Parekh and Anania, 2007). However, ketogenesis is decreased by 40% in Zucker obese rats and malonyl-CoA, a precursor of lipogenesis and inhibitor of fatty acids oxidation, is increased (Azain et al., 1985). Additionally, Zucker diabetic fatty (ZDF) rats exhibit decreased fatty acid oxidation and ketogenesis as demonstrated by tracer measurements of mitochondrial fluxes (Satapati et al., 2008).

Dyslipidemia

In addition to hepatic steatosis, type 2 diabetic patients have characteristic, atherogenic serum lipid abnormalities, called diabetic dyslipidemia; this is a triad of increased levels of triglycerides in VLDL, high or normal levels of LDL cholesterol, and low levels of high density lipoprotein (HDL) cholesterol (Taskinen, 2003). The presence of insulin resistance and diabetic dyslipidemia is one of the major risk factors in the development of atherosclerosis and other cardiovascular disease as well as two important criteria for the diagnosis of metabolic syndrome (Isomaa et al., 2001; Reilly and Rader, 2003; Lusis et al., 2008). While high serum cholesterol levels have been recognized as a major risk factor for cardiovascular disease and LDL has been the major target of lipid-lowering therapy, hypertriglyceridemia is emerging as another major risk factor. Though these particles are not thought to be directly atherogenic, increased VLDL levels can lead to the generation of small, dense LDL through the action of cholesterol ester transfer protein (CEPT) (Ginsberg et al., 2005). These small, dense cholesterol ester-depleted LDL particles are highly atherogenic, and low levels of HDL cholesterol can also result from this CEPT-mediated process. Thus, elevated serum triglycerides are considered by some to be an independent risk factor for heart disease, though the pathogenesis remains unclear.

Hypertriglyceridemia is thought to be caused in the insulin-resistant state by VLDL overproduction in the liver (Taskinen, 2003). There are three major sources of triglyceride for hepatic production of VLDL, all of which have been shown to be increased in insulin-resistant states: fatty acid flux from adipose to the liver, hepatic uptake of VLDL remnants, and *de novo* lipogenesis (reviewed in Ginsberg et al., 2005). Studies using a fructose-fed hamster model of insulin resistance and dyslipidemia suggested that VLDL overproduction appears to result from decreased apoB degradation normally induced by insulin (Taghibiglou et al., 2000). When hepatic insulin signaling is improved pharmacologically in this model, apoB degradation increases, and VLDL secretion decreases (Carpentier et al., 2002). However, which process actually drives hypertriglyceridemia present in T2DM remains unclear: leptin-deficient obese *ob/ob* mice have massively increased rates of *de novo* lipogenesis but do not exhibit increased VLDL triglyceride or apoB levels (Li et al., 1997; Wiegman et al., 2003). Regardless, insulin still fails to inhibit effectively VLDL secretion in *ob/ob* livers under hyperinsulinemic euglycemic clamp conditions, and this may be the point of dysregulation that contributes to hypertriglyceridemia in this model (Wiegman et al., 2003). Triglyceride secretion in perfused liver from Zucker obese rats is five times greater than that from lean rats, and their secreted VLDL particles have increased apoB content (Azain et al., 1985). Therefore, the precise pathogenesis underlying VLDL oversecretion in T2DM remains unclear.

Selective insulin resistance: insulin signaling is simultaneously defective to glucose metabolism and enhanced to lipid metabolism

In an insulin-resistant type 2 diabetic, when insulin no longer regulates carbohydrate and lipid metabolism, hyperglycemia, hepatic steatosis and dyslipidemia ensue. Hepatic insulin resistance has been classically defined as an inability of insulin to suppress hepatic glucose output. Since insulin signaling normally induces *de novo* lipogenesis in the liver, this pathway should be impaired as well in states of insulin resistance. However, the existence of hepatic steatosis argues against this assumption and, unlike insulin signaling to glucose homeostasis, insulin's promotion of lipogenesis is preserved, driving the synthesis and accumulation of triglyceride in the liver. One theory explaining this phenomenon has been termed "selective insulin resistance", in which the insulin signaling pathways inhibiting glucose metabolism are impaired while those stimulating lipid metabolism are preserved, resulting in the devastating co-existence of hyperglycemia and hypertriglyceridemia in T2DM (Brown and Goldstein, 2008). As pointed out by Brown and Goldstein, mice lacking the insulin receptor in the liver, so-called "pure hepatic insulin-resistant" mice, ironically exhibit a less severe condition consisting of hyperglycemia but hypotriglyceridemia (Brown and Goldstein, 2008). Therefore, according to this model, there is a bifurcation of insulin signaling below the level of the insulin receptor

into two distinct pathways: one that suppresses glucose metabolism and is lost in insulin-resistant states and one that stimulates lipid metabolism and is retained in insulin-resistant states. One interesting implication of this is that development of the full T2DM phenotype, which includes steatosis, actually depends on continued signaling by insulin. It has been proposed that glucose metabolism is controlled distally by Forkhead box O1 (FoxO1) and lipid metabolism by sterol regulatory element-binding protein-1c (SREBP1c), as discussed in more detail below (Li et al., 2010).

De novo lipogenesis: a site of dysregulation in insulin resistance

De novo lipogenesis is inappropriately elevated in livers of patients with hepatic steatosis

Rates of hepatic *de novo* lipogenesis are fairly low in lean individuals, as measured by contribution of newly synthesized fatty acids to VLDL. Under postabsorptive conditions in humans, only approximately 4% of triglycerides secreted into the serum come from *de novo* lipogenesis, while 50% can be attributed to NEFA re-esterification (Diraison and Beylot, 1998). *De novo* synthesis of fatty acids increases following food intake, rising from approximately 5% of fatty acids in VLDL to approximately up to 23% postprandially at the peak, occurring just over 4 h after meals (Timlin and Parks, 2005). Diets rich in carbohydrates can further stimulate *de novo* lipogenesis in lean individuals, and have been reported to result in a 10-fold increase of fatty acid synthesis (Schwarz et al., 1995). However, even under these conditions, *de novo* lipogenesis only accounts for the synthesis of 5 g of fatty acids per day (Schwarz et al., 1995).

Though *de novo* lipogenesis may be low in lean individuals, it contributes significantly to hepatic steatosis in obese and insulin-resistant patients. Individuals with existing hepatic steatosis should have very low rates of *de novo* lipogenesis as there are already ample stores of triglycerides in their livers. However, lipogenesis is inappropriately elevated in the livers of patients with hepatic steatosis, accounting for approximately 25–30% of the total hepatic triglyceride content and a three-fold increased contribution to VLDL triglyceride secretion (Forcheron et al., 2002; Diraison et al., 2002; Diraison et al., 2003; Donnelly et al., 2005). Lean individuals exhibit a cycle in the rates of hepatic *de novo* lipogenesis, corresponding to food intake and elevations in serum insulin levels (Timlin and Parks, 2005). However, Donnelly et al. reported that in individuals with NAFLD, *de novo* lipogenesis is elevated even during postabsorptive states and exhibits no diurnal variation (Donnelly et al., 2005). Even following the consumption of a low-carbohydrate high-fat diet, which decreases lipogenesis in lean individuals, hyperinsulinemic, obese individuals have three- to five-fold higher rates of lipogenesis than lean or obese but normoinsulinemic individuals (Hillgartner et al., 1995; Schwarz et al., 2003). Paradoxically elevated fatty acid synthesis has been also found in rodent models of NAFLD: triglycerides secreted from the perfused livers of Zucker obese rats contain five-fold higher levels of *de novo* synthesized fatty acids compared with lean rats (Azain et al., 1985). Additionally, high rates of *de novo* lipogenesis in ZDF rats are not suppressed by high-fat feeding as observed in lean rats (Lee et al., 2000).

Mechanism and control of fatty acid synthesis

Acetyl-CoA is formed in the mitochondria from pyruvate by pyruvate dehydrogenase and can enter the citric acid cycle for eventual oxidation and generation of energy. However, following a meal, more acetyl-CoA is produced in the liver than needs to be oxidized for energy production: this excess acetyl-CoA is converted into fatty acids by *de novo* lipogenesis. The primary source of substrate for fatty acid synthesis in the liver is glucose, but fructose, galactose, lactate, pyruvate and amino acids also contribute to the pool of acetyl-CoA (reviewed in Hillgartner et al., 1995). Acetyl-CoA must be exported from the mitochondria as citrate and converted back into acetyl-CoA by adenosine triphosphate citrate lyase (ACL), an enzyme activated by insulin. In the cytosol, acetyl-CoA carboxylase (ACC) converts acetyl-CoA into malonyl-CoA, the source of carbon for fatty acid synthesis; this step is also the major site of regulation of *de novo* lipogenesis. Citrate activates ACC allosterically while simultaneously inhibiting flux through glycolysis; ACC can also be inhibited by the end product of fatty acid synthesis, palmitoyl-CoA or by cyclic adenosine monophosphate (AMP)-dependent phosphorylation induced by glucagon or by AMP-activated protein kinase (Hillgartner et al., 1995). The two-carbon units provided by malonyl-CoA are joined and reduced sequentially by fatty acid synthase (FAS), eventually forming the saturated 16-carbon fatty acid palmitate. Palmitate is the major product of *de novo* lipogenesis, but also serves as a substrate for elongases and desaturases, such as stearoyl-CoA desaturase-1 (SCD1), for the generation of longer chain and unsaturated fatty acids. In the liver, most fatty acids are joined to a glycerol backbone by acyltransferases to generate triglycerides for storage. Accumulating malonyl-CoA shuts off β-oxidation by inhibiting CPT1 so that futile cycling of fatty acid synthesis and breakdown does not occur.

The transcription of multiple lipogenic enzymes, including FAS, SCD1, ACC, ACL, glycerol phosphate acyltransferase (GPAT), glucokinase (GCK), and liver pyruvate kinase (L-PK), are increased in response to feeding (reviewed in Postic and Girard, 2008a). Inhibition or deletion of many of these target genes themselves, including *SCD1*, *ACC*, and *GPAT* can prevent animals from developing hepatic steatosis due to leptin-deficiency or diet-induced obesity (DIO) by either decreasing lipogenesis or increasing flux of fatty acids into β-oxidation (Cohen et al., 2002; Ntambi et al., 2002; Jiang et al., 2005; Neschen et al., 2005; Mao et al., 2006; Gutiérrez-Juárez et al., 2006; Savage et al., 2006; Harada et al., 2007; Miyazaki et al., 2007). The influences of both insulin and glucose on

lipogenic gene expression are controlled by two distinct transcription factors: insulin via SREBP1c and glucose via carbohydrate response element-binding protein (ChREBP) (Koo et al., 2001; Dentin et al., 2004). These two factors coordinately stimulate lipogenic gene expression in response to both the nutrient influx and increased insulin secretion that occur during feeding.

The control of lipogenic gene expression by two transcription factors: SREBP1c and ChREBP

Much research has been directed toward determining the transcriptional control of *de novo* lipogenesis by insulin and glucose via SREBP1c and ChREBP, respectively (Koo et al., 2001; Postic et al., 2007; Postic and Girard, 2008b). Though each transcription factor is required for the maximal accumulation of hepatic triglyceride during insulin resistance in mice, the relative roles of these two transcription factors under more physiological conditions and in the development of hepatic steatosis remain unclear (Yahagi et al., 2002; Dentin et al., 2006; Postic and Girard, 2008b).

Sterol regulatory element-binding protein-1c

The SREBP family members are synthesized as precursor molecules in the endoplasmic reticulum and proteolytically cleaved in the Golgi complex to release the active amino-terminal transcript factors that translocate to the nucleus (reviewed in Horton, 2002). There are two SREBP1 isoforms generated by alternative splicing: SREBP1a is more active than SREBP1c in cultured cells and stimulates genes for both triglyceride and cholesterol synthesis, while SREBP1c only activates triglyceride synthetic genes (Shimano et al., 1996; Shimano et al., 1997a). Transgenic mice overexpressing the active nuclear transcription factor fragment of SREBP1a (nSREBP1a) have greater fatty livers, increased hepatic cholesterol and triglyceride levels, and increased lipogenic gene expression compared with mice overexpressing nSREBP1c (Shimano et al., 1996; Shimano et al., 1997a; Shimomura et al., 1997). However, though SREBP1a is more active and the more highly expressed variant in cultured cell lines, SREBP1c is expressed at nine-fold higher levels in the liver (Shimomura et al., 1997). The other SREBP isoform, SREBP2 preferentially activates genes of cholesterol synthesis, and transgenic nSREBP2 mice have hepatic steatosis due to massive cholesterol accumulation without a significant increase in hepatic triglyceride content (Horton et al., 1998b).

Insulin increases *SREBP1c* mRNA levels, resulting in a parallel increase of lipogenic target genes, and, conversely, *SREBP1c* expression is low in rats with insulin-deficiency caused by streptozotocin treatment (Foretz et al., 1999; Shimomura et al., 1999b). nSREBP1c protein and lipogenic mRNAs are low in fasted animals, but refeeding with a high-carbohydrate low-fat diet results in a four-fold increase in both compared with pre-fasted fed conditions, whereas nSREBP2 protein and cholesterol synthetic genes only return to non-fasted levels (Horton et al., 1998a). Transgenic mice overexpressing the active nuclear transcription factor fragment of SREBP1c (nSREBP1c) in liver have hepatic steatosis, a four-fold increase in fatty acid synthesis and increased lipogenic gene expression, which remains high even under fasting conditions (Shimano et al., 1997a; Horton et al., 1998a; Shimomura et al., 1998; Shimomura et al., 1999a). Adenoviral overexpression of nSREBP1c in streptozotocin-induced diabetic mice also induces lipogenic gene expression and increases hepatic triglyceride content (Bécard et al., 2001).

The majority of *SREBP1*$^{-/-}$ mice die prior to birth, but those that do survive have decreased lipogenic gene expression during refeeding or prolonged high-carbohydrate feeding (Shimano et al., 1997b; Shimano et al., 1999). *SREBP1c*$^{-/-}$ mice do not experience embryonic lethality like the *SREBP1*$^{-/-}$ mice, but exhibit a similar reduction in lipogenic gene expression and serum triglycerides under both fasted and refed conditions (Liang et al., 2002). Both models also have decreased serum triglycerides at 12 h after refeeding, but only *SREBP1c*$^{-/-}$ mice have a significant reduction in hepatic triglycerides, and only after high-carbohydrate refeeding. Interestingly, cholesterol biosynthetic genes are increased in both mouse models, likely due to the compensatory upregulation of SREBP2 that occurs with loss of SREBP1 or SREBP1c (Liang et al., 2002; Shimano et al., 1999). Leptin-deficient obese *ob/ob* mice have increased mRNA and nuclear protein levels of SREBP1c and increased expression of its lipogenic targets, which correlates with the increased rates of *de novo* lipogenesis and hepatic triglyceride content of these animals (Shimomura et al., 1999a). Hepatic steatosis and lipogenic gene expression are markedly reduced in *ob/ob* mice lacking SREBP1 without a reduction in obesity or insulin resistance (Yahagi et al., 2002).

Though *SREBP1c* expression is important with regards to lipogenesis and hepatic steatosis, the post-translational regulation of SREBP1c is also critical. Following high-carbohydrate feeding, nSREBP1c protein increases to a much greater extent than either *SREBP1* mRNA or full-length protein, suggesting direct regulation of processing (Horton et al., 1998a). A mouse model of lipodystrophy exhibits increased hepatic lipogenesis, lipogenic gene expression, and nSREBP1c protein in liver without exhibiting an increase in *SREBP1c* mRNA (Shimomura et al., 1999a). Liver-specific deletion of either SREBP cleavage-activating protein (SCAP) or Site-1 protease, two proteins required for the processing of precursor SREBP1c into active nSREBP1c, results in decreased hepatic lipid synthesis and decreased hepatic and serum triglyceride levels (Matsuda et al., 2001; Yang et al., 2001). Liver-specific deletion of SCAP also results in decreased expression of SREBP1c targets (Kuriyama et al., 2005). As these proteins are also required for the processing of SREBP1a and SREBP2, hepatic cholesterol biosynthesis and content, and serum cholesterol is decreased as well in these mice.

Carbohydrate response element-binding protein

Though SREBP1c is an important regulator of lipogenic gene expression, it is clearly not the sole influence. Liver-specific deletion of SCAP in mice eliminates most of the active nSREBP1c protein, yet some lipogenic gene expression does remain (Matsuda et al., 2001). Certain lipogenic genes (*FAS* and *ACC*) show less of a reduction than others (*GPAT*) in *SREBP1*$^{-/-}$ and *SREBP1c*$^{-/-}$ mice following high-carbohydrate refeeding (Shimano et al., 1999; Liang et al., 2002). In addition, the maximal upregulation of many lipogenic genes in primary hepatocytes requires glucose as well as insulin stimulation, and the glycolytic gene, *L-PK*, responds only to increases in glucose and not SREBP1c activation (Koo and Towle, 2000; Kawaguchi et al., 2001; Koo et al., 2001; Yamashita et al., 2001; Stoeckman and Towle, 2002). These observations are largely explained by the presence of the transcription factor ChREBP, which responds to increased intracellular glucose levels and, in concert with the stimulation of SREBP1c by insulin, drives the expression of lipogenic genes (Kawaguchi et al., 2001; Koo et al., 2001; Yamashita et al., 2001; Dentin et al., 2004). *ChREBP*$^{-/-}$ mice have decreased basal and high-carbohydrate diet-stimulated hepatic expression of *FAS*, *ACL*, *ACC* and *L-PK*, the latter being a specific target of ChREBP, as well as decreased lipogenesis and hepatic triglycerides on a high-carbohydrate diet (Iizuka et al., 2004). These mice have normal hepatic triglyceride levels on chow diet as both lipid oxidation and synthesis are decreased, but exhibit decreased fat stores, and an inability to store and metabolize fatty acids, resulting in increased hepatic glycogen and dependence on carbohydrate metabolism (Iizuka et al., 2004; Burgess et al., 2008). As with *SREBP1c*, *ChREBP* is increased in the livers of *ob/ob* mice, and lipogenic gene expression and hepatic steatosis is reduced in *ob/ob* mice lacking ChREBP either globally or specifically in the liver. However, unlike *SREBP1*$^{-/-}$ mice on an *ob/ob* background, ChREBP deficiency improves the insulin resistance and glucose intolerance of *ob/ob* mice, revealing different roles for these transcription factors beyond the stimulation of lipogenic gene expression (Yahagi et al., 2002; Dentin et al., 2006; Iizuka et al., 2006).

Overview of the insulin/PI3K signaling pathway

Insulin signals to cellular processes through at least two major branches: PI3K and mitogen-activated protein kinase (MAPK). Much of insulin's effects on metabolism have been attributed to signaling through PI3K, while MAPK is thought to mediate insulin's effects on growth and differentiation; the latter will not be discussed further in this review. Insulin signaling is initiated when insulin binds to its receptor present on the membrane surface of the cell (Figure 1) (reviewed in Taniguchi et al., 2006a). The insulin receptor is a receptor tyrosine kinase, which, upon binding of insulin, is autophosphorylated and activated. Once activated, the receptor can phosphorylate tyrosine residues on the insulin receptor substrate (IRS) molecules. IRS proteins can bind molecules with SH2 domains like PI3K,a lipid kinase, and, by bringing them close to the membrane, activate them. PI3K phosphorylates the membrane lipid phosphatidylinositol 4,5-bisphosphate (PIP_2) converting it into 3,4,5-trisphosphate (PIP_3); PIP_3 can be reverted back to PIP_2 by the phosphatase and tensin homolog (PTEN), a phosphatase which opposes the actions of PI3K. PIP_3 binds and localizes the 3-phosphoinositide-dependent protein kinase-1 (PDK1) and its targets, Akt and atypical protein kinase C (aPKC) to the cell membrane. These two target kinases are phosphorylated and activated by PDK1, eventually leading to many of insulin's effects on glucose, lipid, and protein metabolism (Taniguchi et al., 2006a).

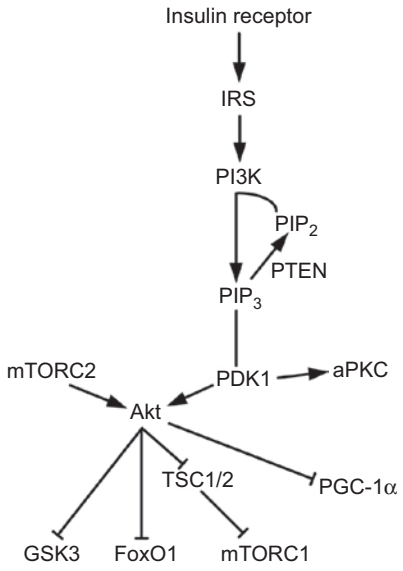

Figure 1. The insulin/phosphatidylinositol-3-kinase (PI3K) signaling pathway. Insulin signaling is initiated by the binding of insulin to its receptor, a receptor tyrosine kinase, resulting in autophosphorylation and activation. The receptor then phosphorylates tyrosine residues on the insulin receptor substrate (IRS) scaffold proteins. IRS proteins bind the lipid kinase PI3K through its SH2 domain and activate it by localization to the membrane. PI3K phosphorylates the membrane lipid phosphatidylinositol 4,5-bisphosphate (PIP_2) converting it into 3,4,5-trisphosphate (PIP_3), an action that can be reversed by the phosphatase PTEN. PIP_3 binds and localizes the 3-phosphoinositide-dependent protein kinase-1 (PDK1) to the cell membrane, along with PDK1's targets, Akt and atypical protein kinase C (aPKC), which can then be phosphorylated and activated. Akt also requires phosphorylation at its carboxy terminal residue by mammalian target of rapamycin complex-2 (mTORC2) for full activation. Once fully activated, Akt can phosphorylate several downstream signaling targets. Akt activates mTOR complex-1 (mTORC1) through its inhibition of the mTORC1 inhibitors tuberin/tuberous sclerosis complex-2 (TSC2) and tuberous sclerosis complex-1 (TSC1). Akt inhibits glycogen synthase kinase-3 (GSK3), FoxO1, and peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α). It must be noted that insulin signaling and Akt are not the only modulators of these distal targets: they also receive input from other signaling cascades.

There are two known activating phosphorylations that the serine-threonine kinase Akt can undergo: PDK1 phosphorylates threonine-308 within the kinase domain and mammalian target of rapamycin complex-2 (mTORC2) and DNA-dependent protein kinase phosphorylate serine-473 within the regulatory domain (Alessi et al., 1997; Stephens et al., 1998; Hresko and Mueckler, 2005; Sarbassov et al., 2005; Bozulic and Hemmings, 2009). Phosphorylation at both of these sites is required for the full activation of Akt (Hanada et al., 2004). Akt exerts its effects through the activation of mTOR complex-1 (mTORC1), the inhibition of glycogen synthase kinase-3 (GSK3), *FoxO1*, and PGC-1α, and other downstream pathways (Figure 1). Akt is central to the hepatic actions of insulin on glucose output: the prevalent model is that activated Akt phosphorylates and inhibits the transcriptional regulators *FoxO1*, PGC-1α, salt-inducible kinase 2 (Sik2), CBP and others, thereby terminating expression of the rate-controlling enzymes of gluconeogenesis (Dentin et al., 2007; Li et al., 2007; Gross et al., 2008; He et al., 2009). Additionally, Akt phosphorylates and inhibits GSK3, releasing its inhibition on glycogen synthase, with the net effect of stimulating the production of glycogen (Cross et al., 1995; Roach, 2002). Akt also positively regulates mTOR signaling and protein synthesis by phosphorylating and inhibiting tuberin/tuberous sclerosis complex-2, which in conjunction with tuberous sclerosis complex-1 negatively regulates mTORC1 (Foster and Fingar, 2010). These most distal insulin signaling targets also receive input from other signaling cascades.

Insight into insulin's control of lipid metabolism from insulin signaling mutants

Insulin receptor

While the signal transduction pathways through which insulin controls lipid metabolism, more specifically *de novo* lipogenesis, have not been fully defined, the study of rodents with mutations in the components of the insulin signaling cascade has contributed meaningful information. Mice lacking the insulin receptor in liver [liver-specific insulin receptor knockout (LIRKO) mice] have no hepatic insulin signaling and as a result exhibit severe glucose intolerance due to an inability to suppress hepatic glucose output (Michael et al., 2000). Though they are able to maintain normal hepatic triglyceride levels on a chow diet, these mice have decreased *SREBP1c, SCD1, FAS, GCK,* and *L-PK* expression as well as dramatically decreased nSREBP1c protein levels (Michael et al., 2000; Biddinger et al., 2008). Suppressed lipogenic gene expression coupled with increased secretion and decreased clearance of VLDL results in triglyceride-deficient cholesterol-rich VLDL particles. Though serum triglyceride levels are low and serum cholesterol levels are normal in chow-fed LIRKO mice, they do have a significantly higher propensity for developing severe atherosclerosis on an atherogenic diet, likely related to their abnormal lipoprotein profile (Biddinger et al., 2008). Similarly, mice with severely impaired insulin signaling (L1 mice) on a *Ldlr*$^{-/-}$ background exhibit decreased serum triglyceride levels along with lower lipogenic gene expression when fed an atherogenic diet; however, these mice have reduced VLDL and serum cholesterol levels and are actually protected from the development of atherosclerosis unlike the LIRKO mice (Han et al., 2009). The differing results observed in these two models are likely due to residual hepatic insulin signaling in the L1 mice as opposed to the complete absence of hepatic insulin signaling in the LIRKO mice. The dyslipidemia and decreased lipogenic gene expression are partially reversed by expression of constitutively-active Akt in both models and by expression of dominant-negative GSK3 in the latter, but it is unclear if this was a physiological action of these proteins or a result of overexpression in the context of complete or almost complete insulin receptor deficiency (Biddinger et al., 2008; Han et al., 2009). It deserves emphasis that even though complete loss of insulin signaling in mouse liver results in dyslipidemia and atherosclerosis, the nature of lipid abnormalities differs strikingly from those in humans with insulin resistance.

Insulin receptor substrate

Once activated, the insulin receptor phosphorylates IRS proteins, and it has been proposed that the bifurcation of selective insulin signaling in the liver may occur at the level of these molecules, though this idea is controversial. Germline disruption of either *IRS1* or *IRS2* results in insulin resistance or overt diabetes, respectively, though the systemic phenotype potentially confounds interpretation of the role of each protein in hepatic lipid metabolism (Araki et al., 1994; Tamemoto et al., 1994; Withers et al., 1998). *IRS1*$^{-/-}$ mice have hypertriglyceridemia likely due to loss of IRS1 in adipose tissue and *IRS2*$^{-/-}$ mice have increased *SREBP1c, ACL,* and *FAS* expression and increased hepatic triglyceride content likely caused by leptin resistance (Abe et al., 1998; Tobe et al., 2001). High doses of leptin decrease the elevation in *SREBP1c* expression in *IRS2*$^{-/-}$ mice and leptin-deficient *ob/ob* mice exhibit decreased *IRS2* and increased *SREBP1c* (Kerouz et al., 1997; Shimomura et al., 1999a; Tobe et al., 2001).

Liver-specific *IRS2*$^{-/-}$ mice have normal serum free fatty acid (FFA) and triglyceride levels, in addition to normal lipogenic gene expression and hepatic triglyceride content (Simmgen et al., 2006). However, acute liver-specific knockdown using RNA interference (RNAi) against *IRS2* in adult mice results in increased *SREBP1c* expression and mild fatty liver changes but no increase in hepatic triglyceride content (Taniguchi et al., 2005). Alternatively, acute liver-specific knockdown of *IRS1* by RNAi does not alter lipogenic gene expression or serum and hepatic triglyceride levels, but does cause an increase in gluconeogenic gene expression and fasting glucose levels, suggesting that IRS1 is more important to insulin's control of glucose metabolism (Taniguchi et al., 2005). However, there is likely overlap between the IRS

proteins as coordinate hepatic knockdown by RNAi of *IRS1* and *IRS2* results in a more substantial increase in lipogenic gene expression, serum and hepatic triglycerides, glucose levels and gluconeogenic gene expression (Taniguchi et al., 2005).

While Taniguchi et al., 2005 suggests that IRS1 controls glucose metabolism and IRS2 is more important to lipid metabolism based on acute hepatic knockdown, Kadowaki and colleagues have argued that the levels of each isoform and therefore their importance in signaling are temporally regulated. IRS1-associated PI3K activity is highest after refeeding, coinciding with the activation of *SREBP1c* and lipogenic gene expression, while IRS2-associated PI3K activity is highest during fasting and immediately after refeeding (Horton et al., 1998a, Kubota et al., 2008). Unlike short-term hepatic knockdown, *IRS1/IRS2* liver-specific null mice have normal fasted but decreased refed *SREBP1c* expression, highlighting the potential differences resulting from genetic ablation versus adult knockdown (Dong et al., 2008). These mice lack the normal rise in *GCK*, *FAS*, and *SREBP1c* levels that occurs during refeeding in addition to having decreased serum triglycerides. The effects on serum triglycerides are reversed with the presence of one allele of either *IRS1* or *IRS2*, while the effect on lipogenic gene expression is partially reversed with one allele of *IRS1* (Guo et al., 2009). Additionally, though both *IRS1/IRS2* liver-specific null mice have impairments in the induction of lipogenic genes during the fasted to fed transition, these impairments appear to be greater for the most part in the absence of *IRS1* (Guo et al., 2009). When fed a high-fat diet, *IRS1/IRS2* liver-specific null mice have a decrease in hepatic triglycerides, an effect that is also observed in the *IRS1* liver-specific null, but not in the *IRS2* liver-specific null (Guo et al., 2009). These data are consistent with the idea that the importance of each isoform to gluconeogenesis vs. lipogenesis is determined by when it is expressed, but provide no real insight into the mechanism of selective insulin resistance (Guo et al., 2009).

Phosphatidylinositol-3-kinase

There is evidence that PI3K may mediate insulin's effects on both VLDL secretion and lipogenesis. Acute inhibition of PI3K by wortmannin reverses insulin's suppression of hepatic VLDL production, resulting in increased serum VLDL apoB protein and triglyceride in mice (Chirieac et al., 2006). Adenoviral expression of a dominant-inhibitory PI3K p85 regulatory subunit results in a marked decrease in the phosphorylation of Akt, decreased *SREBP1c* and *GCK* expression, as well as lower serum triglycerides and fatty acids, though the reduction in serum lipids is likely due to increased hyperinsulinemia driving uptake into adipose tissue (Miyake et al., 2002). A similar result is found in mice with genetic ablation of all PI3K activity in the liver, which results in decreased serum triglycerides and fatty acids and decreased fasted *SREBP1c* expression as well as elevated glucose and insulin levels and increased gluconeogenic gene expression (Taniguchi et al., 2006b). Insulin-dependent induction of *SREBP1c* expression is blocked in rat hepatocytes upon inhibition of PI3K with wortmannin (Li et al., 2010). Additionally, SREBP1c processing is influenced through a PI3K-dependent mechanism as wortmannin treatment of rat hepatocytes results in decreased nSREBP1c levels (Hegarty et al., 2005).

Atypical protein kinase C

Genetic ablation of all PI3K activity in the liver results in decreased serum triglycerides and fasted *SREBP1c* expression and decreased insulin-stimulated activity of its downstream target aPKCs, including PKCλ (Taniguchi et al., 2006b). Adenoviral overexpression of constitutively-active PKCλ dramatically increases fasted *SREBP1c* expression in both liver-specific *PI3K* null and control mice, though the metabolic ramifications of this manipulation were not reported (Taniguchi et al., 2006b). Additionally, overexpression of PKCλ in primary rat hepatocytes results in increased *SREBP1c* and *FAS* expression, while overexpression of kinase-inactive PKCλ results in decreased insulin-stimulated expression (Matsumoto et al., 2003). These findings are corroborated by the liver-specific *PKCλ* null mice, which exhibit decreased hepatic *SREBP1c* expression and triglyceride content and have blunted induction of *SREBP1c*, *FAS*, and *SCD1* expression during refeeding (Figure 2) (Matsumoto et al., 2003; Sajan et al., 2009). In addition, hepatic adenoviral expression of a kinase-inactive aPKC results in decreased *SREBP1c* expression and nSREBP1c levels and decreased serum and hepatic triglycerides (Sajan et al., 2009). However, in the latter two models, decreased aPKC activity resulted in a simultaneous decrease in insulin levels, and thus the effects on triglyceride levels and lipogenic gene expression could be cell non-autonomous, i.e. secondary to decreased insulin levels systemically (Matsumoto et al., 2003; Sajan et al., 2009).

Akt

Akt exists as three highly related isoforms, Akt1–3, each encoded by a distinct gene. Based on studies of mice with interruptions in each locus, Akt2 is the major isoform mediating insulin's effects on glucose metabolism, whereas Akt1 and Akt3 are more important to growth (Chen et al., 2001; Cho et al., 2001a; Cho et al., 2001b; Garofalo et al., 2003; Easton et al., 2005). Each isoform contains a highly similar pleckstrin homology domain responsible for Akt's binding to PIP3, a kinase domain, and carboxyl-terminal regulatory domain (reviewed in Hanada et al., 2004). Akt2 is the most highly expressed isoform of Akt in liver and though its role in glucose metabolism has been well established, its mediation of insulin's action in hepatic lipid metabolism has emerged more recently (Cho et al., 2001a; Garofalo et al., 2003). Akt activates the transcription of *SREBP1c* and its lipogenic targets in isolated hepatocytes and a human epithelial cell line (Fleischmann and Iynedjian, 2000; Ribaux and Iynedjian, 2003; Porstmann et al., 2005). Hepatic overexpression of constitutively-active Akt (myr-Akt) results in

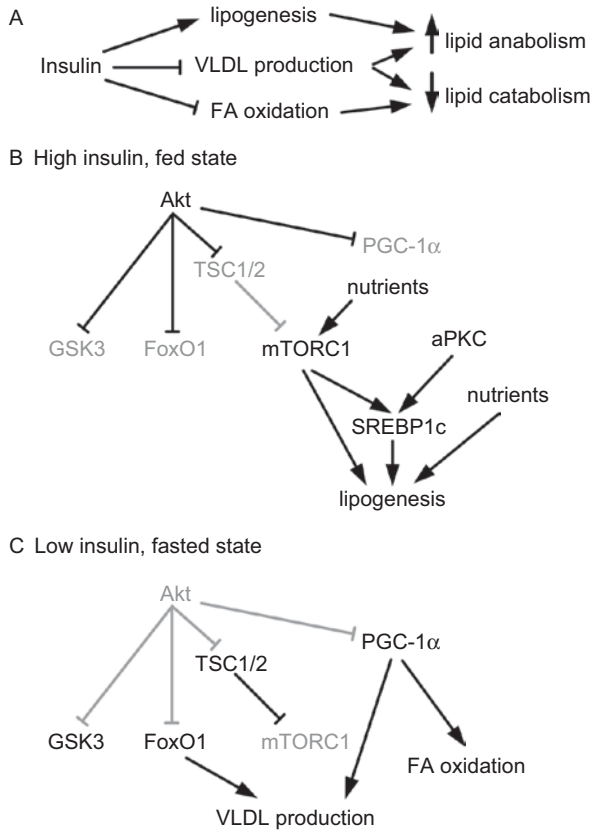

Figure 2. The effects of distal insulin signaling molecules on hepatic lipid metabolism. (A) Insulin promotes *de novo* lipogenesis while inhibiting fatty acid oxidation and very low density lipoprotein (VLDL) export, therefore promoting lipid anabolism and inhibiting lipid catabolism. (B) When insulin is elevated in states of high nutrient influx, *de novo* lipogenesis is stimulated by insulin signaling through Akt and atypical protein kinase C (aPKC). The current data support that this process is largely regulated by mTOR complex-1 (mTORC1) and sterol regulatory element-binding protein-1c (SREBP1c), though the direct action of nutrients themselves on lipogenesis is also important in its regulation. Glycogen synthase kinase-3 (GSK3), FoxO1, and peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) are inhibited (indicated in gray) by insulin signaling through active Akt in fed states. (C) In postabsorptive fasted states when insulin signaling is low, inhibition of GSK3, FoxO1, and PGC-1α is released and these molecules are active. PGC-1α promotes fatty acid oxidation and perhaps VLDL secretion; FoxO1 may also promote VLDL secretion.

hepatic steatosis and hypertriglyceridemia, with induction of *SREBP1c, SCD1*, and *GCK*, a smaller increase in *FAS* and no change in *ACC* or fatty acid oxidation genes (Ono et al., 2003). However, not all of the effects of myr-Akt proceeds through SREBP1c, as the induction of hepatic triglyceride accumulation, *GCK* and *FAS* expression by myr-Akt in *SREBP1* null mice is similar to that in normal mice, though the degree of hypertriglyceridemia and *SCD1* expression is dramatically reduced (Ono et al., 2003). Hepatic deficiency of PTEN, a negative regulator of Akt, also leads to increased lipogenesis, lipogenic gene expression and VLDL secretion, resulting in hepatic steatosis (Horie et al., 2004; Stiles et al., 2004). Though it has been argued that the resulting hepatic steatosis could be an off-target effect of overexpression of Akt, the increased triglyceride accumulation in livers lacking PTEN is suppressed by concomitant removal of Akt2, showing that this is major downstream target of PIP_3 in the evolution of steatosis (Taniguchi et al., 2006b; He et al., 2010).

Consistent with these data, our group recently used an *Akt2* null model to show that this isoform is required for the development of lipid accumulation in the livers of obese, insulin-resistant mice. *ob/ob* mice lacking germline or hepatic Akt2 exhibit decreased hepatic steatosis under both fed and fasted conditions (Leavens et al., 2009). Normalization of triglyceride accumulation correlates with a substantial decrease in *de novo* lipogenesis; lipogenic gene expression is elevated in *ob/ob* mice, an effect that is partially or completely prevented with loss of hepatic Akt2. *ob/ob Akt2*$^{+/-}$ mice also exhibit an intermediate decrease in hepatic lipid accumulation and lipogenesis, arguing that not only is Akt2 required for this process, it is rate-determining. *ob/ob Akt2*$^{-/-}$ mice have worsened diabetes, with dramatically increased fasted glucose and insulin levels as well as increased hypertriglyceridemia. However, *ob/ob* liver-specific *Akt2* null mice exhibit only a slight increase in fasting glucose levels and normal or decreased serum triglycerides, arguing that the hypertriglyceridemia of the *ob/ob Akt2*$^{-/-}$ mouse is secondary to the severity of the diabetes or loss of Akt2 in an extrahepatic tissue. Additionally, *ob/ob* liver-specific *Akt2* null mice do not exhibit any changes in energy expenditure, triglyceride secretion or β-oxidative gene expression. Thus, it appears that Akt2 is required for the increase in *de novo* lipogenesis and hepatic steatosis that occurs in the *ob/ob* mouse model (Leavens et al., 2009).

However, just as liver-specific *Akt2* null mice exhibit decreased steatosis, hepatic triglyceride accumulation resulting from DIO is also reduced in livers lacking Akt2. However, since these livers do not display decreased *de novo* lipogenesis, Akt2 must also control additional lipid metabolic processes beyond fatty acid synthesis related to triglyceride accumulation (Leavens et al., 2009). It is likely that this role of Akt2 is cell-autonomous, as circulating FFA levels are unchanged in the liver-specific *Akt2* knockout. When DIO is produced by feeding mice high-fat high-sucrose Surwit diet, lipogenesis is increased but this does not correlate with lipogenic gene expression in livers regardless of the presence of Akt2. Taken in aggregate, these data suggest that Akt2 is important to the development of steatosis through its role in inducing genes of lipogenesis, but also through post-transcriptional regulation of lipogenesis and other metabolic pathways. How Akt2 is required for the development of hepatic steatosis in this model remains unknown as these mice do not exhibit changes in energy expenditure, triglyceride secretion or β-oxidative gene expression (Leavens et al., 2009).

Peroxisome proliferator-activated receptor-γ coactivator-1α

PGC-1α is a transcriptional co-activator that integrates multiple inputs including insulin signaling to coordinately

regulate many aspects of metabolism (reviewed in Lin et al., 2005). Expression of PGC-1α is highest in the liver during fasting and positively regulates β-oxidation and perhaps VLDL secretion while potentially inhibiting *SREBP1c* expression (Figure 2) (Zhang et al., 2004a). Akt directly phosphorylates PGC-1α, at serine-570, impairing its ability to activate genes involved in fatty acid oxidation, in particular, medium-chain acetyl-CoA dehydrogenase (Li et al., 2007). In primary hepatocytes, expression of PGC-1α stimulates palmitate oxidation and co-expression of myr-Akt significantly blunts this stimulation in cells expressing PGC-1α but not in those expressing a S570A mutant of PGC-1α. Akt's inhibition of PGC-1α serves as a dual regulatory step for the hepatic switch from fasting to feeding, simultaneously turning off fatty acid oxidation and gluconeogenesis, the latter in concert with Akt's phosphorylation and inhibition of FoxO1 (Li et al., 2007).

Mice lacking PGC-1α either in the whole body or specifically in the liver develop hepatic steatosis during fasting due to decreased β-oxidation and increased lipogenic gene expression (Leone et al., 2005; Estall et al., 2009). Additionally, liver-specific *PGC-1α*$^{-/-}$ mice display hypertriglyceridemia, which correlates with increased apoB levels, as well as increased hepatic insulin resistance (Estall et al., 2009). Mice lacking the highly homologous protein PGC-1β also exhibit increased hepatic steatosis when fed a high-fat diet due to decreased β-oxidation (Lelliott et al., 2006; Sonoda et al., 2007).

Mammalian target of rapamycin

mTOR is a serine-threonine kinase that mediates much of insulin/PI3K/Akt's positive effects on protein synthesis through activation of S6 kinase (S6K) and inhibition of the translation repressor eIF4E-binding protein (4E-BP). The activities of both mTOR and S6K are increased in obese rats, and S6K1 activity is increased in *ob/ob* mice (Um et al., 2004; Khamzina et al., 2005). Inhibition of mTOR by rapamycin in isolated rat hepatocytes results in decreased *de novo* lipogenesis and esterification of fatty acids and increased fatty acid oxidation (Brown et al., 2007). Furthermore, rapamycin blocks the stimulation of *SREBP1c* expression by insulin in isolated rat hepatocytes and by fasting/refeeding in rats *in vivo* (Figure 2) (Li et al., 2010). Expression of myr-Akt *in vitro* activates SREBP1 processing, *SREBP1, FAS* and *ACL* expression and leads to increased *de novo* lipogenesis, all of which are prevented by mTOR inhibition with rapamycin (Porstmann et al., 2008). This is a highly-conserved pathway for nutrient storage as *Drosophila melanogaster* deficient in dTOR have decreased lipid levels while those deficient in 4E-BP have normal triglyceride stores but deplete them more rapidly and thus have reduced survival during starvation (Teleman et al., 2005; Luong et al., 2006). In non-hepatocyte cell lines, mTORC1 appears to mediate much of Akt's effect on SREBP1c processing and gene expression (Düvel et al., 2010). In fibroblasts, this was responsible for the increased *de novo* lipogenesis associated with growth, while mTORC1-dependent increases in hypoxia-inducible factor (HIF) mediated the accelerated glucose metabolism. However, while mTORC1 is regulated by insulin signaling, it is also an important nutrient sensor, and likely coordinates the influences of both insulin and nutrients on lipid metabolism (reviewed in Sengupta et al., 2010).

Mice deficient for S6K1 are hyperglycemic due to reduced insulin secretion, but are small and have reduced fatty acid levels associated with increased β-oxidation in adipose tissue (Pende et al., 2000; Um et al., 2004). However, it is unknown whether S6K plays a similar role in hepatic β-oxidation (Um et al., 2004). Mice lacking 4E-BP1/4E-BP2 exhibit hepatic steatosis, increased S6K activity in liver, muscle, and adipose, and increased adiposity due to a combination of decreased energy expenditure and lipolysis and an increase in re-esterfication in adipose tissue on high-fat diet (Le Bacquer et al., 2007).

Forkhead box O1

FoxO1 is a transcription factor that is negatively regulated by insulin signaling through phosphorylation by Akt; much of insulin's ability to suppress hepatic glucose output has been attributed to this pathway (Brunet et al., 1999; Gross et al., 2008). Overexpression of normal or constitutively-active FoxO1 in liver increases serum triglycerides during fasting or following an oral lipid load (Altomonte et al., 2004; Kamagate et al., 2008). This has been attributed to increased VLDL production and decreased clearance of triglycerides from the bloodstream (Figure 2). FoxO1 directly activates the expression of microsomal triglyceride transfer protein (MTP), a protein that transfers lipids to growing VLDL particles in the liver; inhibition of MTP *in vivo* results in decreased serum triglyceride but increased hepatic triglyceride levels (Liao et al., 2003; Kamagate et al., 2008). Mice expressing a constitutively-active *FoxO1* transgene have increased MTP expression, VLDL production and serum triglyceride levels (Kamagate et al., 2008). Adenoviral expression of *FoxO1* in the liver also results in increased hepatic expression of apoC-III, the apolipoprotein that inhibits LPL, which is responsible for the hydrolysis of circulating triglycerides, and thus inhibits clearance of VLDL triglycerides (Altomonte et al., 2004). Conversely, RNAi knockdown of *FoxO1* in the liver increases hepatic triglyceride levels but decreases VLDL production, suggesting that the absence of FoxO1 can secondarily cause hepatic steatosis by impairing triglyceride secretion from the liver (Kamagate et al., 2008).

FoxO1 also has an influence on lipogenic and β-oxidative gene expression, though it is unclear whether this is a primary action of FoxO1. RNAi knockdown of *FoxO1* in the liver decreases *FAS* and *ACC* expression, while hepatic expression of constitutively-active *FoxO1* by adenovirus results in increased lipogenic gene and decreased β-oxidative gene expression (Matsumoto et al., 2006; Kamagate et al., 2008). However, hepatic steatosis occurs in both cases, attributed to decreased VLDL secretion in the absence of *FoxO1* and to increased

lipid synthesis and decreased oxidation in the presence of constitutively-active FoxO1 (Matsumoto et al., 2006; Kamagate et al., 2008). Interestingly, the latter mice also exhibit a compensatory increase in IRS2 protein levels and phosphorylation of Akt, so the hepatic steatosis resulting from constitutively-active *FoxO1* expression may be secondary to the upregulation of another Akt-dependent pathway. This same group later reported that FoxO1 deletion fails to reverse the blunted refeeding-stimulated expression of *SREBP1c* or decrease in serum triglyceride levels in double *IRS1*/*IRS2* null livers, though it is able to partially restore VLDL triglyceride secretion, suggesting that FoxO1 is a component of IRS-dependent VLDL secretion but not of IRS-dependent lipogenesis (Dong et al., 2008). However, Zhang et al. (2006) later reported that transgenic mice expressing constitutively-active *FoxO1* in liver have decreased hepatic expression of *SREBP1c* and its lipogenic targets and reduced lipogenesis following feeding and decreased serum triglyceride levels during fasting and feeding. Though these mice do not exhibit differential activation of other insulin signaling pathways, they are markedly glucose-intolerant and insulin-resistant; thus it is unclear whether a directly action of FoxO1 is to suppress the expression of lipogenic genes (Zhang et al., 2006). Taken as a whole, it is unlikely that FoxO1 is an important transcription factor in the regulation of hepatic lipid metabolism.

Glycogen synthase kinase-3

As its name indicates, GSK3 phosphorylates and inhibits glycogen synthase in the absence of insulin signaling; upon nutrient influx, GSK3 is inhibited via phosphorylation by Akt, potentially mediating insulin's stimulation of glycogen production (Cross et al., 1995) (reviewed in Roach, 2002). A series of reports by Ericsson and colleagues have shown that GSK3 can directly phosphorylate SREBP1, which promotes the ubiquitination and degradation of SREBP1 protein (Sundqvist et al., 2005; Bengoechea-Alonso and Ericsson, 2009, Punga et al., 2006). Additionally, expression of constitutively-active GSK3 blocks the accumulation of nSREBP protein that occurs in cells expressing myr-Akt (Porstmann et al., 2008). This implicates a role for GSK3 in the downregulation of lipogenesis during fasting, a process that would then be turned off by insulin during feeding; however, the relevance of this pathway has yet to be shown either in liver cells or *in vivo*.

Forkhead box A2

Forkhead box A2 (Foxa2) is a transcription factor and may serve as a potential pathway through which insulin regulates lipid metabolism during the fasting to feeding transition. Stoffel and colleagues have published that adenoviral overexpression of constitutively-active Foxa2 in either lean or obese mice leads to increased VLDL secretion and β-oxidation, as assessed by gene expression and calorimetry, resulting in decreased hepatic triglyceride accumulation (Wolfrum et al., 2004; Wolfrum and Stoffel, 2006). Mice lacking one allele of *Foxa2* exhibit decreased hepatic ketogenesis and increased serum triglyceride and fatty acid levels, an effect that is enhanced by high-fat diet-feeding (Wolfrum et al., 2004). Activity of Foxa2 is highest in fasted low-insulin states, but whether this and its effects on lipid metabolism are due to lack of insulin regulation or coactivation with PGC-1β remains unclear (Wolfrum et al., 2004; Zhang et al., 2005; Wolfrum and Stoffel, 2006). Additionally, whether Foxa2 is negatively regulated by insulin through phosphorylation by Akt and nuclear exclusion during refeeding remains contested, as does its role in the regulation of hepatic lipid metabolism (Wolfrum et al., 2004; Zhang et al., 2005).

Concluding remarks

While the underlying pathogenesis of hepatic steatosis remains elusive, its development is clearly associated with insulin resistance (Fabbrini et al., 2009). The characterization of the insulin signaling transduction pathways that control hepatic lipid metabolism is essential to revealing novel therapeutic targets for the treatment of dyslipidemia and hepatic steatosis associated with T2DM. These signaling pathways appear to be distinct from those controlling hepatic glucose metabolism and are preserved in insulin-resistant states, leading to selective insulin resistance in the liver (Figure 3). Specifically, insulin's stimulation of *de novo* lipogenesis is preserved in T2DM, and as such, individuals with hepatic steatosis have inappropriately elevated rates of lipogenesis.

Most evidence points toward the PI3K/Akt/mTOR pathway as the dominant mediator of insulin's stimulation of *de novo* lipogenesis. Much of this regulation is transmitted through the activation of the transcription factor SREBP1c, which drives the coordinated transcription of genes encoding lipogenic enzymes. However, not all regulation proceeds through this pathway; almost certainly, insulin also influences *de novo* lipogenesis through SREBP1c-independent pathways and probably has even more profound effects on lipid accumulation post-transcriptionally. *In vivo*, insulin exerts substantial effects on liver metabolism non-cell autonomously through controlling available substrate. Substrate itself, especially glucose, regulates transcription of lipogenic genes but the precise contributions of insulin- and glucose-dependent gene expression postprandially and during pathological states has yet to be clarified. Lastly, an important challenge is understanding the biochemical mechanisms that underlie selective insulin resistance, in which insulin can no longer suppress hepatic glucose output but continues to promote lipid synthesis and accumulation. The predominant notion today is that insulin's Akt-dependent antagonism of FoxO1 activity is impaired, accounting for the elevated glucose output, while continued mTORC1 activity drives lipogenesis and steatosis (Figure 3). Yet problems with this model have to be resolved. For example, if nutrient excess is responsible for mTORC1 activity in obese, insulin-resistant

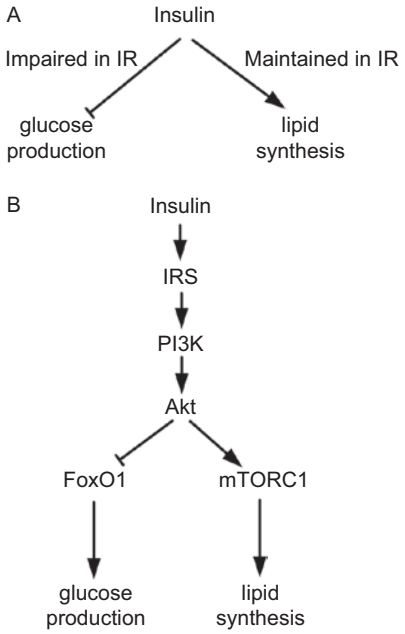

Figure 3. Proposed model of selective insulin resistance occurring downstream of Akt signaling. (A) Insulin signaling inhibits glucose production while promoting lipid synthesis; in insulin-resistant states (IRS), regulation of this first action is lost while that to the second is maintained. This proposed model has been termed selective insulin resistance. (B) Current evidence supports that the bifurcation of insulin signaling to glucose production and lipid synthesis, and therefore selective insulin resistance, occurs at or below the level of Akt, with FoxO1 largely responsible for signaling to glucose production and mTOR complex-1 (mTORC1) controlling signaling to lipid synthesis. However, these processes are likely not controlled through a single linear pathway and may have inputs from other insulin signaling mediators as well as from other signaling pathways.

states, why does this pathway depend on the continued presence of Akt2 in the mouse? The resolution of these and other questions in the upcoming years will provide important insights into the causes and potential treatments for the cluster of diseases associated with obesity and insulin resistance.

Declarations of interest

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