Review

Unraveling the Regulation of Hepatic Metabolism by Insulin

Paul M. Titchenell,¹,* Mitchell A. Lazar,¹ and Morris J. Birnbaum¹,²,*

During insulin-resistant states such as type 2 diabetes mellitus (T2DM), insulin fails to suppress hepatic glucose production but promotes lipid synthesis leading to hyperglycemia and hypertriglyceridemia. Defining the downstream signaling pathways underlying the control of hepatic metabolism by insulin is necessary for understanding both normal physiology and the pathogenesis of metabolic disease. We summarize recent literature highlighting the importance of both hepatic and extrahepatic mechanisms in insulin regulation of liver glucose and lipid metabolism. We posit that a failure of insulin to appropriately regulate liver metabolism during T2DM is not exclusively from an inherent defect in canonical liver insulin signaling but is instead due to a combination of hyperinsulinemia, altered substrate supply, and the input of several extrahepatic signals.

The Pathological Diabetic Liver
Excess fat accumulation in metabolic organs, altered glucose homeostasis, and the development of insulin resistance are hallmarks of T2DM. The liver plays a fundamental role in coordinating systemic metabolic homeostasis and the adaptation to nutrient availability and deprivation. During the fasting state, the combination of high glucagon and low insulin levels increases hepatic glucose production (HGP) to meet the metabolic demands of peripheral tissues. This increase in HGP initially originates from the breakdown of stored glycogen, termed glycogenolysis. As glycogen stores are reduced, decreased glycolytic flux, increased substrate supply, and the augmented expression and activity of genes involved in the gluconeogenic pathway enable the liver to generate glucose from 3-carbon precursors [1]. This process is opposed in response to a meal, as levels of glucagon decline and insulin concentrations rise, which signal to increase liver glucose uptake, synthesize glycogen, suppress HGP, and induce the synthesis of fatty acids for storage and subsequent utilization [2].

During insulin-resistant disorders such as T2DM, insulin fails to stimulate liver glucose uptake and to adequately suppress HGP in both the fasted and postprandial state [3]. The inability of insulin to inhibit HGP results from an initial failure to inhibit glycogenolysis followed by an inability of insulin to appropriately suppress gluconeogenesis [3]. Excess HGP coupled with the well-documented defect in muscle glucose uptake leads to hyperglycemia [3]. However, despite the inability of insulin to regulate glucose homeostasis, insulin-resistant individuals exhibit increased de novo lipogenesis and re-esterification, inducing fat accumulation in the liver. In addition, insulin-resistant individuals have increased secretion and decreased clearance of triglycerides, leading to elevations in blood lipid levels [4–7]. Therefore, during the progression of insulin resistance, the lipid-promoting effects of insulin are maintained but the control of HGP by insulin is defective, leading to hyperglycemia and hypertriglyceridemia [8]. In this review we focus on recent findings detailing the molecular mechanisms governing lipid synthesis in liver and HGP, and highlight the essential role of both direct and indirect effects of insulin in coordinating

¹Institute for Diabetes, Obesity, and Metabolism, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, USA
²Present address: Internal Medicine, Pfizer Inc., Cambridge, MA, USA

*Correspondence: pttc@mail.med.upenn.edu (P.M. Titchenell) and morris.birnbaum@pfizer.com (M.J. Birnbaum).

Trends
Insulin action in liver coordinates regulation of liver glucose and lipid production by cell-autonomous and non-autonomous mechanisms.

Liver insulin action is required for lipid synthesis and development of fatty liver.

The ability of insulin to suppress hepatic glucose production is determined by both direct and indirect mechanisms, including the contribution of adipose tissue lipolysis and gluconeogenic regulation by glucagon.

Defining the molecular mechanisms responsible for selective insulin control of lipid synthesis and glucose production may allow the development of therapeutic agents that reduce hyperglycemia without inducing hypertriglyceridemia.
hepatic metabolism. We suggest a unified model to reconcile the selective defects in insulin action in liver, a model that explains increased HGP and lipid synthesis independently of defects in canonical insulin signaling as a result of a combination of hyperinsulinemia, altered substrate supply, and the input of several extrahepatic signals including free fatty acids.

Liver Insulin Signaling and Hepatic Lipid Metabolism

In the postprandial state, insulin controls the synthesis and storage of lipids in liver in part by increasing de novo lipogenesis, suppressing fatty acid oxidation, and promoting triglyceride esterification and secretion [9]. Studies in rodent models and humans have defined the significance of hepatic insulin action to the regulation of hepatic lipid metabolism and the development of steatosis during insulin resistance. To address directly the role of hepatic insulin signaling in the regulation of lipid homeostasis, Kahn and colleagues generated mice lacking the insulin receptor in hepatocytes (LIRKO). Despite significant alterations in glucose homeostasis and pronounced insulin resistance, LIRKO mice are protected from the lipid abnormalities commonly associated with T2DM. When challenged with a high-fat diet or genetic forms of obesity, mice lacking the hepatic insulin receptor neither accumulate excess hepatic triglycerides nor develop fatty liver disease [10,11]. Mechanistically, LIRKO mice have reduced serum triglycerides, increased fatty acid oxidation, and fail to induce de novo lipogenesis and the lipogenic gene program in response to a meal [10,11]. These rodent studies are supported by human studies demonstrating that patients with insulin receptor loss-of-function mutations display extreme insulin resistance and hyperglycemia, but are protected from the development of fatty liver [12]. These clinical studies support the genetic loss-of-function mouse experiments highlighting the essential role of direct insulin action in the liver in the initiation and progression of fatty liver disease during insulin resistance and T2DM. To summarize, studies in both humans and animal models indicate that hepatic insulin signaling is required for both de novo lipogenesis and hepatic lipid synthesis. As a result, there has been considerable interest in the metabolism field in unraveling the specific signaling pathways mediating liver insulin action to explain the paradoxical increase in lipid synthesis simultaneously with enhanced HGP.

During the past decade many studies have pointed to one signaling cascade in particular as being integral to the metabolic actions of insulin – the phosphoinositide 3-kinase (PI3K)–Akt pathway. Upon insulin engagement of its cognate receptor, PI3K is recruited to insulin receptor substrates (IRSs) and and phosphorylates phosphatidylinositol (3,4) bisphosphate (PIP2) to generate phosphatidylinositol (3,4,5)-triphosphate (PIP3). PIP3 generation promotes the recruitment of pyruvate dehydrogenase kinase 1 (PDK1) and Akt (also known as protein kinase B), leading to the subsequent phosphorylation of Akt by PDK1 on Thr308 and by mammalian target of rapamycin (mTORC) 2 on Ser473 [13]. Akt then signals to multiple downstream pathways to control cell growth, survival, and glucose and lipid metabolism including mTORC1, glycogen synthase kinase, and the FoxO transcription factors [14,15] (Figure 1). In support of the Akt pathway as a mediator of insulin control of hepatic lipid metabolism, mice with hepatic deficiency of phosphatase and tensin homolog (PTEN, a negative regulator of Akt) or overexpression of a myristoylated, constitutively active form of Akt, display hepatomegaly and a dramatic increase in liver fat [16,17]. Consistent with this, germline Akt2 knockout mice (Akt2KO) as well as liver-specific Akt2KO mice are protected from the development of fatty liver in response to genetic or diet-induced obesity and insulin resistance, despite marked hyperinsulinemia [18]. Moreover, in mice lacking Pten in liver, enhanced Akt2 signaling is required for the induction of the lipogenic gene program and hepatic steatosis. This direct requirement for Akt2 signaling acting downstream of Pten was nicely demonstrated using combined genetic knockout of Akt2 and Pten specifically in hepatocytes [19]. Genetic loss-of-function experiments have uncovered the signaling pathways coordinating the effects of insulin on hepatic lipid metabolism. These data strongly support the notion that Akt signaling
downstream of the insulin receptor is essential to increase hepatic lipid synthesis and accumulation of liver fat during insulin resistance.

With the role of Akt in hepatic lipid metabolism being clearly defined, attention has turned to elucidating the more distal signaling events downstream of Akt in the regulation of lipid metabolism. Recent studies have focused on the transcriptional control of de novo lipogenesis by insulin, highlighting the importance of several lipogenic transcription factors including the sterol regulatory element binding protein 1c (SREBP1c) and the carbohydrate response element binding-protein (ChREBP). SREBP1c is ubiquitous transcription factors that play a crucial role in the synthesis of fatty acids, triglycerides, and cholesterol [20], and hepatic SREBP1c activity is increased in mouse models of insulin resistance such as ob/ob (obese) mice and lipodystrophic diabetes [21]. In hepatocytes, insulin activation of Akt enhances both the synthesis and processing of the predominant SREBP isofom, SREBP1c [22,23]. The requirement for SREBP1c in mediating hepatic steatosis and fatty liver during insulin resistance and carbohydrate-induced hypertriglyceridemia has been demonstrated in vivo by Moon et al. who generated mice with liver-specific knockout of Scap, an escort protein essential for cleavage of all SREBPs [24]. These mice were protected from hepatic steatosis when fed a high-fat diet or crossed with mice deficient for leptin, consistent with an essential role for SREBPs in mediating hepatic lipid accumulation downstream of insulin.
The role of ChREBP downstream of hepatic insulin action is less well defined. ChREBP is a glucose-responsive transcription factor and activates similar lipogenic genes to SREBP1c, including Fasn, Scd1, and Elovl6 [25]. Dietary fructose administration induces hepatic steatosis, an effect that is dependent on ChREBP [26]. Hepatic overexpression of ChREBP is sufficient to induce fatty liver in mice, and increased ChREBP has been correlated with non-alcoholic fatty liver disease (NAFLD) in humans [27]. Germline deletion of ChREBP reduces basal amounts of fatty acid synthesis independently of changes in SREBP [28]. Moreover, liver-specific knockdown of ChREBP reduces de novo lipogenesis and hepatic triglyceride in ob/ob mice [29]. Although it is now well-established that de novo lipogenesis and fat accumulation are increased in the livers of insulin-resistant people [4–7], the activation status of SREBP1c and ChREBP is not known. Future studies will be necessary to better understand the specific effects of these key lipogenic transcription factors and how these molecules interact in the regulation of lipogenic gene expression and de novo lipogenesis in humans.

In addition to SREBPs/ChREBP, the FoxO transcription factors have been implicated as an important downstream transcriptional regulator of hepatic lipid metabolism [14]. Akt controls the activity of the FoxO proteins through a well-characterized phosphorylation mechanism that plays a central role in multiple metabolic processes [30]. Many of the studies of FoxO transcription factors have focused on the predominant FoxO transcription factor in liver, FoxO1. Transgenic mice that express in liver a mutated version of FoxO1 (FoxoAA) that cannot be phosphorylated by Akt, and therefore is constitutively active [31,32], display reduced de novo lipogenesis and triglyceride secretion, and fail to induce the lipogenic gene program in response to a meal [33–35]. Consistent with this, hepatic deletion of all three FoxO transcription factors induces de novo lipogenesis, hepatic steatosis, and increases in lipogenic gene expression [36]. The transcriptional mechanisms responsible for direct control of hepatic lipid metabolism by FoxO1 are not completely defined. Studies by Deng et al. suggest that FoxO1 can bind directly to the SREBP1c promoter and affect the transcriptional activity of Sp1 and SREBP1c [33]. FoxO1 also controls glucokinase gene expression, and this likely contributes to the lipogenic response by increasing glucose-6-phosphate levels and subsequently activating ChREBP [37]. Collectively, the data support the hypothesis that FoxO transcription factors, in particular FoxO1, play an important role in mediating insulin control of hepatic lipid synthesis.

Several studies have reported that mTORC1 is downstream of Akt in the activation of SREBP in cell culture models [23,38]. Experiments in mice confirm that mTORC1 is required although not sufficient to induce de novo lipogenesis and the lipogenic gene program [39,40]. Moreover, in the absence of Akt, genetic activation of mTORC1 is not sufficient to induce de novo lipogenesis nor the SREBP1-dependent lipogenic gene program without concomitant inactivation of FoxO1 [37]. In addition to SREBP1, activation of mTORC1 and inhibition of FoxO1 are both essential to induce glucokinase expression, which contributes to increased glycolytic flux and activation of ChREBP. These changes, together with the activation of SREBP1 and other genes involved in lipogenesis such as Fasn, are necessary to induce de novo lipogenesis downstream of Akt [37,41]. Despite this complexity downstream of Akt, the most crucial point is that the regulation of lipid metabolism and the development of fatty liver require insulin signaling via Akt. We now turn our attention to defining the mechanisms governing the control of HGP by insulin.

**Direct Regulation of HGP by Insulin**

In response to a meal, increased insulin concentrations in the blood signal the liver to regulate glucose metabolism by stimulating liver glucose uptake and glycogen storage, and suppressing HGP (Figure 1). The precise coordination of glucose fluxes by insulin is essential to adequately control postprandial glucose concentrations. In response to oral glucose challenge, the liver is responsible for ~30% of the disposal of glucose. However, this does not take into
account the acute suppression of hepatic production, which further contributes to the important role of the liver in postprandial glycemic control [42,43]. During the progression of insulin resistance, defects in both hepatic glucose uptake and production contribute to postprandial hyperglycemia [42]. Given the well-documented defect in the ability of insulin to suppress HGP in T2DM, in the remaining sections we will discuss recent data defining the mechanisms of regulation of HGP by insulin in vivo.

Similar to its control of hepatic lipid metabolism, insulin acts directly on hepatocytes to suppress HGP via multiple mechanisms, including acute changes in metabolic pathways and long-term alterations in gene expression [2]. Elegant physiological studies have demonstrated that insulin directly suppresses HGP [43] by acutely inhibiting glycolysis [44]. Importantly, direct control of HGP by insulin can occur independently of changes in glucagon and circulating free fatty acids, further suggesting that the direct action of insulin on hepatocytes is essential for proper regulation of HGP and hepatic glucose homeostasis [45,46]. Mechanistically, studies both in vivo and in the perfused livers of mice lacking hepatic Akt2 demonstrate that Akt is required for the direct effects of insulin on glycolysis [47]. Insulin alters liver gluconeogenic gene expression through multiple mechanisms including the allosteric regulation of pyruvate carboxylase and potentially by inhibiting the transcription of gluconeogenesis genes including glucose-6-phosphatase (G6pc) and phosphoenolpyruvate carboxykinase (Pck1) [2,48]. Both genetic and pharmacological approaches have clearly demonstrated the requirement for hepatic Akt signaling in mediating direct insulin control of hepatic gluconeogenic gene expression [23,49]. These data implicate direct action of insulin on hepatocytes in the physiological response to nutrient intake and in the regulation of blood glucose.

This conventional view of insulin regulation of HGP in the liver suggests that Akt phosphorylates and inactivates glycogen synthase kinase 3β (GSK3) and FoxO1 to control glycolysis and gluconeogenic gene expression, respectively [2]. However, recent experiments have questioned this canonical view. For example, insulin still suppresses HGP and glycolysis in a mouse with mutant GSK3 that cannot be phosphorylated by Akt, suggesting that Akt mediates the control of glycolysis by insulin through GSK3-dependent as well as GSK3-independent mechanisms [47]. Moreover, and surprisingly, although regulating gluconeogenic gene expression and HGP are the best-characterized metabolic roles of FoxO1 in liver [50], hepatic deletion of FoxO1 is sufficient to normalize glucose tolerance and peripheral insulin sensitivity in mice lacking the insulin receptor, insulin receptor substrates, or Akt isoforms in liver [49,51–53]. Nevertheless, deletion of FoxO1 in liver alleviates the requirement for direct insulin action to suppress HGP, suggesting that direct liver insulin action is not essential for in vivo control of HGP [37,49,52]. Lastly, transgenic expression of the insulin receptor in the livers of mice lacking insulin receptor in all other tissues failed to correct systemic insulin resistance and glucose intolerance despite restoring insulin signaling in hepatocytes [54]. These data contradict the canonical model of hepatic insulin action and provide genetic evidence that insulin may regulate HGP by additional extrahepatic mechanisms.

Extrahepatic Control of HGP

Central Nervous System (CNS)

Several studies indicate that the CNS plays a crucial role in the regulation of peripheral metabolism and energy balance by insulin [55]. Direct intracerebral infusion of insulin suppresses HGP and gluconeogenic gene expression in rodents without activating hepatic insulin signaling [56]. Moreover, deletion of the insulin receptor in agouti-related peptide (Aggrp)-expressing neurons in the arcuate nucleus of the hypothalamus prevents insulin-mediated suppression of HGP during hyperinsulinemic–euglycemic clamps with peripherally infused insulin [57]. However, HGP was found to be suppressed by insulin in mice devoid of hepatic insulin signaling that were subjected to selective hepatic vagotomy, suggesting that non-
autonomous regulation of HGP can occur independently of neural signaling [37]. These results are consistent with the observation that hepatic vagotomy does not alter HGP during peripheral infusion of insulin [58], although hepatic vagotomy does not completely rule out central regulation by vagus-independent neuronal pathways or indirect mechanisms. Moreover, the effect of insulin action in the CNS to acutely suppress HGP is not observed in canine models, where insulin action in the CNS induced a slow and modest reduction in hepatic gluconeogenic genes without altering HGP or gluconeogenesis [59,60]. These data indicate that acute effects of insulin to suppress HGP occur independently of a direct brain–liver mechanism.

**Glucagon**

Glucagon is the major hormone that opposes the effects of insulin on hepatic glucose metabolism, functioning as a positive regulator of gluconeogenesis and glycogenolysis [61]. Physiological studies in dogs demonstrate an antagonistic role of insulin on glucagon secretion during euglycemic clamp [1,62]. Moreover, modern genetic studies support the importance of glucagon signaling in the control of HGP [63–65]. Deletion of the insulin receptor gene in pancreatic β cells enhances glucagon secretion and leads to mild glucose intolerance, hyperglycemia, and hyperglucagonemia in the fed state, supporting a role for insulin in intraislet communication [66]. Surprisingly, the metabolic abnormalities associated with streptozotocin-induced insulin deficiency are abrogated by loss of the glucagon receptor [67,68]. However, antagonism of glucagon action in liver failed to prevent the ability of insulin to suppress HGP during hyperinsulinemic-euglycemic clamp in mice also lacking hepatic insulin signaling, indicating that peripheral actions of insulin to regulate HGP are independent of acute changes of liver glucagon signaling [37]. This is consistent with older studies that show extrahepatic action of insulin suppresses HGP regardless of the portal concentrations of glucagon [69]. Although insulin controls HGP independently of acute changes in glucagon, several clinical studies have demonstrated reductions in blood glucose levels with glucagon receptor antagonists [70].

**Free Fatty Acids (FFAs)**

Insulin markedly suppresses adipocyte lipolysis, reducing the levels of circulating FFAs and glycerol. It has been known for decades that an increase in circulating FFAs impairs the ability of insulin to suppress endogenous glucose production and may directly enhance HGP in humans [71]. The ability of FFAs to augment HGP is likely mediated by multiple metabolic pathways that stimulate gluconeogenesis, including increased ATP production, mitochondrial acetyl-CoA, and changes in the NAD+/NADH ratio [43]. The timecourse of insulin-mediated suppression of HGP correlates with the reduction of FFAs [72], and prevention of the decline in FFA levels during hyperinsulinemic–euglycemic clamp in the presence of somatostatin partially blocks the reduction in HGP by peripheral insulin infusion in dogs [73]. Moreover, lowering serum FFA during somatostatin infusion is sufficient to reduce HGP independently of exogenous insulin [74], whereas elevating FFAs fails to increase HGP [73]. These data support the notion that an increase in the delivery of FFAs from visceral adipose depots results in increased HGP and may explain the disproportionate association of abdominal compared to subcutaneous adiposity with metabolic disease [75].

Recent reports have reinforced the conclusion that FFAs act as an extrahepatic signal, and reductions in circulating free fatty acids contribute to the ability of insulin to suppress HGP in vivo. Infusion of acetate to mimic an elevation in FFA by increasing intrahepatic acetyl-CoA effectively blocked the ability of insulin to suppress HGP in fasted mice as well as in mice lacking hepatic insulin signaling [48]. Similarly, preventing the reduction in FFAs during a hyperinsulinemic–euglycemic clamp blocks the ability of insulin to suppress HGP in mice lacking hepatic insulin signaling [37].
While these studies agree on the main point that insulin suppression of lipolysis underlies its non-hepatic-autonomous effects on HGP, an important difference in these studies concerns the role of the extrahepatic pathway when insulin signaling is intact in hepatocytes. Perry et al. suggest that the effect of insulin on lipolysis is the predominant mechanism of action by which insulin suppresses HGP, regardless of whether the hepatocyte remains capable of responding directly to insulin [48], whereas Titchenell et al. found that an intact insulin signaling pathway in liver was sufficient to mediate the suppression of HGP even when the decrease in FFA was abrogated [37]. One explanation for the discrepancy between the physiological role of FFAs in controlling HGP may relate to the use of acetate to mimic the reduction in FFAs and the subsequent reduction in hepatic acetyl-CoA levels [48]. In this regard, previous studies using FFAs instead of acetate demonstrated that the direct actions of insulin on the liver are dominant over peripheral effects [76–78]. These mouse experiments are also supported by experiments in dogs, which similarly found that the direct actions of insulin on the liver are dominant in the control of HGP [45]. In addition, the study by Perry et al. used overnight fasted mice, which are glycogen depleted. In doing so, the direct inhibition of glycogenolysis by insulin may be under-represented. In conclusion, this suggests that indirect control of the regulation of HGP becomes the major determinant only under conditions in which the direct pathway is defective. However, in the setting of obesity-associated diabetes, where direct liver insulin regulation of HGP is defective, there is agreement that adipocyte-derived FFAs drive HGP independently of the direct actions of insulin on hepatocytes. Future work will be necessary to address this issue in more depth.

Concluding Remarks and Future Perspectives

Recent understanding of signaling mechanisms downstream of the hepatic insulin receptor have illuminated the crucial role of activation of Akt and its downstream effectors in the direct control of lipid metabolism by insulin. In addition, multiple lines of evidence using distinct models have demonstrated the important contributions of hepatic and extrahepatic pathways in the regulation of glucose metabolism in the liver. Thus, the metabolism of the enigmatic diabetic liver may be explained by a new paradigm wherein hyperinsulinemia directly drives hepatic lipogenesis and lipid accumulation while indirect mechanisms, including excess circulating FFAs, block the ability of insulin to suppress HGP. Several questions remain unanswered, however, particularly with regard to the mechanism by which FFAs are increased during insulin resistance and obesity, and how they act as a dominant regulator of HGP independently of direct action of insulin (see Outstanding Questions). It will be essential to unravel these underlying mechanisms to devise effective strategies to combat hepatic insulin resistance and improve the metabolic dysfunction that is rampant in modern society.

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References

4. Lambert, J.E. et al. (2014) Increased de novo lipogenesis is a distinct characteristic of individuals with nonalcoholic fatty liver disease. Gastroenterology 146, 726–735

Outstanding Questions

What are the relative contributions of SREBP1c and ChREBP to de novo lipogenesis and hepatic steatosis?

How does the insulin-resistant liver become insensitive to the direct regulation of HGP during T2D?

How does adipose tissue become insensitive to insulin action during metabolic disease?

What is the mechanism driving hyperglucagonemia during metabolic disease?
38. Porstmann, T. et al. (2008) SREBP activity is regulated by mTORC1 and contributes to Akt-dependent cell growth. Cell Metab. 8, 224–236.
40. Yecies, J.L. et al. (2011) Akt stimulates hepatic SREBP1c and lipogenesis through parallel mTORC1-dependent and independent pathways. Cell Metab. 14, 21–32.
60. Ramnanan, C.J. et al. (2012) Evidence against a physiologic role for acute changes in CNS insulin action in the rapid regulation of hepatic glucose production. Cell Metab. 15, 656–664
64. Ozcan, L. et al. (2012) Calcium signaling through CaMKII regulates hepatic glucose production in fasting and obesity. Cell Metab. 15, 739–751
68. Lee, Y. et al. (2011) Glucagon receptor knockout prevents insulin-deficient type 1 diabetes in mice. Diabetes 60, 381–397
72. Rebrin, K. et al. (1995) Free fatty acid as a link in the regulation of hepatic glucose output by peripheral insulin. Diabetes 44, 1038–1045