Resolving the Paradox of Hepatic Insulin Resistance

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SUMMARY

This review describes the signaling pathways involved in the regulation of liver metabolism by insulin. In addition, it explores the molecular mechanisms underlying hepatic insulin resistance, highlighting the contribution of intrahepatic and extrahepatic pathways.

Insulin resistance is associated with numerous metabolic disorders, such as obesity and type II diabetes, that currently plague our society. Although insulin normally promotes anabolic metabolism in the liver by increasing glucose consumption and lipid synthesis, insulin-resistant individuals fail to inhibit hepatic glucose production and paradoxically have increased liver lipid synthesis, leading to hyperglycemia and hypertriglyceridemia. Here, we detail the intrahepatic and extrahepatic pathways mediating insulin’s control of glucose and lipid metabolism. We propose that the interplay between both of these pathways controls insulin signaling and that mis-regulation between the 2 results in the paradoxic effects seen in the insulin-resistant liver instead of the commonly proposed deficiencies in particular branches of only the direct hepatic pathway. (Cell Mol Gastroenterol Hepatol 2019;7:447–456; https://doi.org/10.1016/j.jcmgh.2018.10.016)

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Metabolic disorders such as obesity and type II diabetes mellitus (T2DM) have reached epidemic proportions and continue to be a leading cause of death worldwide.1 The liver plays a central role in the systemic regulation of glucose and lipid metabolism and aberrant hepatic insulin action is thought to be a primary driver of insulin resistance, in which higher circulating insulin levels are necessary to adequately control blood glucose levels. During a normal physiologic fasting period, a high glucagon-to-insulin ratio decreases the rate of glucose consumption and shifts the liver to glucose production, first by consuming its stores of glycogen (glycogenolysis) and then from glucogenic precursors in a synthetic pathway (gluconeogenesis).2 In the postprandial state, decreasing glucagon and increasing insulin levels signal the liver to increase glucose consumption, stop glucose production, and store excess nutrients in the form of glycogen and lipids.3 In pathologic states, such as obesity and T2DM, insulin fails to appropriately regulate hepatic metabolism, leading to excess production of glucose despite accelerated rates of lipid synthesis, a condition now commonly referred to as selective hepatic insulin resistance.4 As a consequence, insulin-resistant disorders such as obesity and T2DM are closely linked to nonalcoholic fatty liver disease (NAFLD), a disorder that can lead to liver dysfunction and progress to deadly nonalcoholic steatohepatitis.5

Increased rates of glucose production and lipogenesis are well documented in insulin-resistant human beings. Patients with NAFLD almost universally show hyperinsulinemia.6 In addition, both obese and diabetic human beings show a higher prevalence of NAFLD than lean ones.7 Isotope labeling experiments in subjects with NAFLD showed that subjects with increased hepatic steatosis had 2-fold higher rates of de novo lipogenesis and increased plasma levels of free fatty acids (FFAs) and insulin.8 In addition to increased lipid synthesis, insulin-resistant individuals have increased rates of hepatic glucose production (HGP).9 Indeed, there is a significant correlation between rates of gluconeogenesis and the extent of liver fat in NAFLD patients.10 Therefore, during the progression of insulin resistance, insulin fails to suppress HGP yet continues to drive excess lipid synthesis, leading to the sequelae of NAFLD, hyperglycemia, and hypertriglyceridemia.

Experiments in both mice and human beings have shown the essential role for hepatic insulin action in the regulation of glucose production and lipogenesis. Liver insulin resistant knockout mice (LIRKO) mice fail to inhibit glucose production and cannot induce de novo lipogenesis.11-14 In addition, LIRKO mice fail to accumulate lipids and do not develop fatty liver, even when fed a high-fat diet, despite increased blood glucose and insulin levels.12 These

Abbreviations used in this paper: ChREBP, carbohydrate response element binding protein; FFA, free fatty acid; Gck, glucokinase; GSK3, glycogen synthase kinase 3; GYS2, glycogen synthase; HGP, hepatic glucose production; IRS, insulin-receptor substrate; LIRKO, liver insulin resistant knockout mice; mTORC, mechanistic target of rapamycin complex; NAFLD, nonalcoholic fatty liver disease; PIP3, phosphatidylinositol (3,4,5)-trisphosphate; PIP3K, phosphoinositide-3-kinase; PTEN, phosphatase and tensin homolog; SCAP, SREBP cleavage-activating protein; SREBP1c, sterol regulatory element binding protein 1c; TAG, triacylglycerol; T2DM, type II diabetes mellitus; TSC, tuberous sclerosis complex; VLDL, very-low-density lipoprotein.

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liver-specific knockout mouse models resemble human beings that lack a functioning insulin receptor and show extremely high blood glucose levels, however, hepatic steatosis fails to arise. The clinical findings corroborate the concept that the liver is the key driver of insulin’s whole-body action on glucose and lipid homeostasis. Further supporting this statement, fat-specific deletion of the insulin receptor results in lipodystrophy along with insulin resistance and hyperglycemia. However, these mice are not protected from NAFLD, and eventually develop nonalcoholic steatohepatitis, unlike the LIRKO mice and human beings with insulin-receptor mutations. Experiments using congenital mouse models can pose some issues because off-target effects of genetic manipulation can develop over time and obscure results. For example, LIRKO mice are typically smaller than wild-type mice, possibly because of defects in the insulin-like growth factor axis, and eventually the observed effects, such as hyperglycemia, disappear as a result of liver failure. In these instances, inducible genetic knockouts hold some benefit because one can observe the direct effects of the knockout before the off-target effects begin to manifest. In this case, inducible knockout of the insulin receptor reciprocates the glucose intolerance and hyperinsulinemia of the LIRKO mice without the off-target metabolic effects. These mice also fail to promote hepatic lipogenesis in response to a high carbohydrate meal. Resolving what specific factors mediate insulin action on the liver to generate these paradoxical effects has become a major focus in obesity and T2DM studies and has provided many insights into the molecular mechanisms of insulin action and hepatic metabolism. Here, we discuss these pathways in depth and suggest an integrated model to deconvolute the paradox of hepatic insulin action that integrates the direct effects of insulin action on the liver with many extrahepatic pathways from peripheral metabolic organs.

**Hepatic Insulin Signaling and Lipid Metabolism**

Strong evidence has indicated that the phosphoinositide-3-phosphate kinase (PI3K)/Akt pathway is the key signaling pathway that mediates the effects of insulin on anabolic metabolism in all organisms. When insulin binds to the insulin receptor (IR), it recruits and activates PI3K through insulin-receptor substrates (IRS), generating phosphatidylinositol (3,4,5)-trisphosphate (PIP3). IRS proteins link the PI3K pathway to the insulin receptor by binding to phosphotyrosine residues on the insulin receptor. Knockout of multiple insulin-receptor substrates prevents activation of the pathway in response to insulin, leading to insulin resistance and hyperglycemia, but not hepatic steatosis. Knockout of multiple insulin-receptor substrates prevents activation of the pathway in response to insulin, leading to insulin resistance and hyperglycemia, but not hepatic steatosis. Knockout of mutant mice lacking hepatic Akt2, the most abundant hepatic isoform, have decreased lipid accumulation, and decreased de novo lipogenesis in the liver of ob/ob mice or mice subjected to a high-fat diet. However, despite its abundance, liver-specific deletion of Akt2 only results in mild insulin resistance owing to residual Akt1 activity. Knockout of both Akt1 and 2 is necessary to fully suppress Akt activity in the liver and leads to severe insulin resistance, glucose intolerance, and a reduction in hepatic lipid synthesis.

Because several studies support an obligate role of hepatic insulin action to regulate lipogenic metabolism, defining the mechanisms downstream of Akt are essential for understanding the pathogenesis of NAFLD during insulin resistance. One major downstream target of Akt is the mechanistic target of rapamycin complex 1 (mTORC1). Akt activates mTORC1 through inhibition of the tuberous sclerosis complex (TSC), a protein that inhibits mTORC1 localization to and activation at the lysosome through inhibition of Rheb. Activation of mTORC1 shifts the cell from a catabolic to an anabolic and proliferative state in which protein, lipid, and nucleic acid synthesis become greatly enhanced. Because one of the hallmarks of T2DM and insulin resistance is enhanced de novo lipogenesis, research has focused on determining the role of mTORC1 in de novo lipogenesis and hepatic lipid metabolism. Studies have shown that activation of mTORC1 is required for de novo lipogenesis, however, activation of mTORC1 alone is not sufficient to induce lipogenesis in the absence of hepatic insulin signaling. This is consistent with the phenotype of mice lacking Tsc specifically in hepatocytes because liver-specific TSC knockout fails to induce lipogenesis and lipogenic gene expression despite constitutive mTORC1 signaling, suggesting Akt regulates hepatic lipid metabolism via mTORC1-dependent and independent pathways.

Sterol regulatory element binding protein 1c (SREBP1c) is a member of the SREBP class of transcription factors that are key players in controlling cellular expression of genes required for lipid and cholesterol metabolism. Insulin regulates SREBP1c by both enhancing its gene expression and post-translational processing. Akt mediates these processes through multiple downstream pathways. mTORC1, in particular, is a key activator of SREBP1c because inhibiting
mTORC1 blocks insulin-dependent cleavage and activation of SREBP1c (Figure 1). For example, SREBP1c processing in transgenic rats requires S6K1, a target of mTORC1. Consistent with increased lipogenesis in insulin-resistant models, several models for diabetes in mice, such as ob/ob, involve heightened levels of SREBP1c activity. SREBP cleavage-activating protein (SCAP) is a major regulator of SREBP activity because it chaperones SREBP proteins from the endoplasmic reticulum to the Golgi where it is cleaved, releasing the active part of SREBP to the nucleus where it regulates transcription. SCAP is required for activation of all isoforms of SREBP and its deletion significantly reduces cholesterol and fatty acid synthesis in the liver. In addition, eliminating SCAP specifically in hepatocytes reduces lipid accumulation in the liver and is sufficient to prevent hepatic steatosis in ob/ob mice and sucrose-fed hamsters. Therefore, SREBP1c is a necessary factor in lipogenic gene expression and in the development of fatty liver.

In addition to SREBP1c, carbohydrate response element binding protein (ChREBP) is a well-studied, glucose-responsive transcription factor that may play a role in controlling hepatic lipid metabolism. Glucose-6-phosphate is the key activator of ChREBP, facilitating its migration to the nucleus (Figure 1). Because insulin signaling enhances glucose uptake in the liver, ChREBP becomes activated. As a transcription factor, ChREBP activates similar lipogenic genes to SREBP1c, although its roles in insulin sensitivity remain controversial. Normal mice with ChREBP deleted globally show decreased lipogenesis as well as mild insulin
resistance. However, ChREBP deficiency in obese mice also results in decreased lipid accumulation and improved insulin sensitivity. Moreover, increased ChREBP is sufficient to increase fatty liver progression because overexpression of hepatic ChREBP in mice results in steatosis. Consistent with these mouse studies, obese human beings typically have higher ChREBP expression in the liver, which correlates with fatty liver. Recently, studies deleting ChREBP specifically in mouse hepatocytes showed mild insulin resistance and protection from hepatic steatosis when challenged with a high-carbohydrate diet, but had no effect on lipogenesis and lipogenic gene expression under normal chow. Hepatic deletion of ChREBP in mice following a high-carbohydrate diet caused a reduction in glycolytic and lipogenic gene expression, including a partial loss of SREBP1c expression. Restoration of nuclear SREBP1c signaling in liver-specific ChREBP knockout mice increased the expression of the lipogenic genes ACLY, ACC2, SCD1, and GPAT, but failed to restore them to control levels, suggesting that both SREBP1c and ChREBP are needed to fully regulate lipogenesis in the liver. In addition, SREBP1c overexpression had no effect on restoring glycolytic gene expression. Moreover, overexpressing ChREBP was not sufficient to regain any significant lipogenic gene induction in mice lacking SREBP after SCAP deletion, showing that SREBP is required for the induction of lipogenic expression. The interplay between ChREBP and SREBP1c in regulating lipogenic gene expression helps ensure that the liver does not initiate lipid synthesis unless both glucose and insulin are present, and future studies will continue to unravel their coordinated regulation of lipid synthesis.

Alongside de novo lipogenesis, insulin action also regulates lipid homeostasis by regulating triacylglycerol (TAG) secretion from the liver via very-low-density lipoprotein (VLDL)-TAG export. Enhanced secretion of VLDL-TAG is another hallmark of people with insulin-resistant conditions, such as obesity or NAFLD. In particular, a failure of insulin to facilitate degradation of apolipoprotein B, a major protein in VLDL synthesis, as well as increased levels of FFAs and increased lipogenesis in insulin-resistant disorders, are believed to stimulate VLDL secretion.

The last point potentially carries the most weight because it may not be insulin resistance per se that stimulates VLDL secretion, but instead the hyperinsulinemia that results from it. Studies in rats have shown that hyperinsulinemia stimulates TAG turnover and VLDL secretion. In addition, disrupting insulin signaling in mouse livers by deleting Akt or the insulin receptor reduces VLDL secretion. Downstream of Akt, inhibiting or activating mTORC1 in the liver leads to decreased or increased VLDL secretion, respectively, through the regulation of phosphatidylinositol synthesis, a crucial part of VLDL synthesis and secretion. As such, insulin regulation of VLDL-TAG secretion is complex and the coordinated control of apolipoproteins, phospholipids, and TAG synthesis are essential for proper control of VLDL-TAG secretion.

In addition to mTORC1, strong evidence exists for FoxO1's ability to regulate liver lipid synthesis downstream of hepatic Akt signaling. When activated by insulin, Akt phosphorylates FoxO1 and inactivates it via phosphorylation, leading to nuclear exclusion (Figure 1). Transgenic mice with livers expressing a constitutively active form of FoxO1 that cannot be phosphorylated by Akt due to its three active serine residues being mutated to alanines, FoxoAAA, fail to initiate transcription of lipogenic genes after feeding, leading to a reduction in lipogenesis and triglyceride secretion. Conversely, deletion of all FoxO isoforms from the liver activates lipogenic gene expression and induces de novo lipogenesis correlating with hepatic steatosis. Because FoxO1 is thought to be a transcriptional activator, the specific mechanisms governing its inhibition of lipogenesis is unclear. However, recent studies have argued that FoxO1 directly represses the transcription of SREBP1c. In addition, FoxO1 has been implicated in regulating the expression of glucokinasine (Gck) through a repression mechanism mediated by Sin3a and Sin3b59,62,64,65 (Figure 1). Importantly, Gck expression depends on insulin signaling via Akt, and deletion of FoxO1 partially increases Gck expression. In addition to FoxO1, full activation of Gck also requires activation of mTORC1 (Figure 1). It is attractive to speculate that FoxO1 inhibition of Gck could affect expression of lipogenic factors such as ChREBP, which are dependent on intracellular glucose concentrations for activation. Mechanistically, both activation of mTORC1 and inhibition of FoxO1 are required and sufficient to regulate hepatic lipogenesis in the absence of insulin signaling in vivo. In summary, both human and mouse data support an obligate role for hepatic insulin signaling via Akt in the regulation of hepatic lipid synthesis and fatty liver. For the remainder of this review, we focus on the molecular mechanisms mediating insulin's control of HGP.

### Direct Regulation of HGP by Insulin

Together with enhanced lipogenesis, insulin-resistant livers fail to suppress glycogenolysis and gluconeogenesis despite hyperinsulinemia resulting in increased HGP. Activation of Akt by insulin inhibits both glycogenolysis and gluconeogenesis through multiple downstream pathways including glycogen synthase kinase 3 (GSK3) and FoxO1. The canonical model of insulin suppression of glycogen synthesis is Akt-mediated phosphorylation and inhibition of GSK3 (Figure 1). However, recent studies in mice with a mutant form of GSK3 that cannot be phosphorylated and inhibited by Akt, still induce glycogen synthesis in response to insulin, indicating that Akt can suppress glycogenolysis through pathways separate from Akt-dependent GSK3 phosphorylation. One such independent pathway involves direct activation of glycogen synthase (GYS2). GYS2 is considered a downstream target of GSK3, however, studies have indicated that glucose-6-phosphate also directly can activate GYS2 (Figure 1). Because insulin signaling increases Gck expression and glucose uptake and restoration of Gck expression in the absence of Akt is sufficient to restore glycogen content, insulin signaling via Akt to Gck may represent a GSK3 phosphorylation-independent mechanism for glycogen synthesis.

Classic studies in vivo and in the perfused liver have shown that insulin's direct action on glucose regulation
suppresses HGP in a fashion dependent on Akt. Along with its roles in inhibiting lipogenesis, FoxO1 also regulates HGP downstream of Akt. FoxO1 promotes gluconeogenesis by regulating expression of glucose-6-phosphatase and phosphoenolpyruvate carboxykinase (Figure 1), and its inhibition improves glycemia in insulin-resistant and diabetic mice. Hepatic deletion of FoxO1 in mice results in significant decreases in glyco gensynthesis and gluconeogenesis. Surprisingly, deletion of FoxO1 in IR, IRS, and Akt knockout mice is sufficient to restore insulin’s suppressive effects on HGP in vivo despite a lack of autonomous insulin action. These data provide genetic evidence that supports classic physiological studies by Cherrington and Bergman that extrahepatic mechanisms contribute to the regulation of HGP in vivo. Together with the obligate role of hepatic insulin action for lipid metabolism, these data challenge the classic model of selective insulin resistance in the liver and instead implicate the role of extrahepatic mechanisms in the control of HGP by insulin.

### Insulin’s Indirect Regulation of HGP

The central nervous system plays an integral role in glucose and lipid homeostasis. Nutrients, metabolites, and hormones signal in various regions of the hypothalamus to control metabolism. Insulin can act on neurons in the hypothalamus, particularly agouti-related peptide– and proopiomelanocortin-expressing neurons. Knockout of the insulin receptor in agouti-related peptide–expressing neurons results in a failure of insulin to inhibit HGP but had no impact on insulin’s effects on body weight. In response to insulin, activation of potassium adenosine triphosphate channels in the hypothalamus signals through the vagus nerve to the liver, which inhibit hepatic gluconeogenesis. Studies from several labs, including the Rosetti Lab, have identified a brain–liver axis involving signal transducer and activator of transcription 3 signaling in hepatocytes (Figure 2). However, denervation of the hepatic branch of the vagus nerve fails to prevent insulin’s ability to suppress HGP in mice during a peripheral infusion of insulin under euglycemic clamp conditions. In addition, mice lacking hepatic Akt and FoxO1 suppress glucose production during hyperinsulinemic–euglycemic clamp conditions after a hepatic vagotomy, questioning the role of the brain–liver axis in the regulation of HGP. Moreover, recent studies in dogs have shown that blocking brain insulin signaling does not have any effect on insulin’s inhibition of HGP during clamp conditions. Glucagon is the principal counter-regulatory hormone that stimulates glyco gensynthesis and gluconeogenesis during fasting and opposes the hepatic actions of insulin. Glucagon increases HGP by acutely stimulating gluconeogenesis and chronically promoting gluconeogenesis (Figure 2). Under euglycemic clamp conditions, increased insulin concentrations led to a reduction in glucagon secretion. Moreover, human studies have indicated a close correlation of insulin action and decreased glucagon concentrations, implying some effect of insulin on glucagon secretion. Genetic evidence also supports this correlation, indicating that deletion of the insulin receptor from α cells in mouse pancreas leads to enhanced glucagon secretion, leading to mild glucose intolerance, hyperglycemia, and hyperglucagonemia. Because of hyperglucagonemia’s long association with diabetes, many commercial antidiabetic drugs target some part of the glucagon signaling mechanism with some success. Despite this well-established effect on glycemia, increasing glucagon levels acutely or blocking hepatic glucagon action fails to negate insulin’s ability to suppress glucose production, indicating that insulin’s actions on suppressing glucagon are not required to acutely inhibit HGP. Insulin acts on adipose tissue to increase glucose uptake, suppress lipolysis, and drive lipid synthesis. As a result,
insulin suppresses circulating levels of FFAs and glycerol, which correlates with changes in HGP\(^92\) (Figure 2). Work in the canine model has shown that insulin inhibition of lipolysis contributes to the acute inhibition of hepatic glucose production.\(^93\) Increased gluconeogenic flux largely contributes to this effect on HGP.\(^94\) Recent genetic studies from several groups have supported these classic physiology studies and assert that FFA action on the liver drives HGP in insulin-resistant livers or livers completely devoid of hepatic insulin signaling.\(^32,72,95\) Perry et al\(^95\) proposed that insulin’s indirect action on the liver negates the requirement for direct hepatic insulin signaling in the control of HGP. However, other work has shown that insulin’s direct action on the liver dominates.\(^32,72\)

Differences in experimental clamp conditions could underlie these contrasting results on the role of FFAs in the control of HGP. The experiments of Perry et al\(^95\) involved overnight fasting mice, which left their glycogen stores depleted and made them dependent on gluconeogenesis,\(^96\) skewing the impact of insulin’s direct action on glycogenolysis.\(^96\) In addition, Perry et al\(^95\) used acetate to mimic the effects of FFAs on HGP, which blocked insulin’s ability to suppress HGP. Other groups, including the Shulman laboratory, have used the physiological substrate FFAs to directly test the contribution of adipocyte lipolysis to HGP and found insulin can suppress HGP despite increased FFAs, confirming a dominant role for hepatic insulin action in the control of HGP.\(^32,72,97\) Moreover, studies comparing the effects of peripheral vs portal insulin infusion show significant differences in hepatic insulin levels.\(^98\) Peripheral insulin infusion is commonly performed during hyperinsulinemic–euglycemic clamp conditions in mice, but fails to recapitulate the proper portal insulin concentrations and may lead to an underinsulinated liver, minimizing the direct effect of insulin on HGP.\(^32,98\) At the same time, increased insulin levels at the periphery exaggerates insulin’s indirect effects. Accounting for these factors in the clamp conditions shows that the direct effects of insulin on the liver prevail.\(^73\) Despite these experimental differences, an agreement has emerged that FFAs from the adipose tissue play essential roles in modulating HGP during the progression of insulin resistance and metabolic disease.

Extensive studies have outlined the major processes of direct insulin action on the liver via the PI3K/Akt pathway and its various methods of regulating glucose and lipid homeostasis. With this knowledge, investigators have put forth a massive effort to elucidate the mechanism of hepatic insulin resistance associated with conditions such as obesity and T2DM. An attractive hypothesis in the field suggests that hepatic insulin action is selective, suggesting a bifurcation occurs distal to Akt to control lipogenesis and HGP via distinct and independent pathways. However, directly testing this model using mouse models fails to explain the pathophysiology of the insulin-resistant liver. It is becoming increasingly clear that insulin’s direct action on the liver is the driving force of hepatic de novo lipogenesis and that both direct and indirect mechanisms exist to control insulin’s regulation of hepatic glucose production. Going forward, unraveling the mechanisms of how these extrahepatic factors communicate to and regulate the liver and its ability to promote HGP in the face of increased hyperinsulinemia and subsequent lipogenesis will be paramount to fully disentangling the paradox of hepatic insulin resistance during metabolic disease.

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