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Insulin regulation of gluconeogenesis

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Abstract

The coordinated regulation between cellular glucose uptake and endogenous glucose production is indispensable for the maintenance of constant blood glucose concentrations. The liver contributes significantly to this process by altering the levels of hepatic glucose release, through controlling the processes of *de novo* glucose production (gluconeogenesis) and glycogen breakdown (glycogenolysis). Various nutritional and hormonal stimuli signal to alter hepatic gluconeogenic flux, and suppression of this metabolic pathway during the postprandial state can, to a significant extent, be attributed to insulin. Here, we review some of the molecular mechanisms through which insulin modulates hepatic gluconeogenesis, thus controlling glucose production by the liver to ultimately maintain normoglycemia. Various signaling pathways governed by insulin converge at the level of transcriptional regulation of the key hepatic gluconeogenic genes *PCK1* and *G6PC*, highlighting this as one of the focal mechanisms through which gluconeogenesis is modulated. In individuals with compromised insulin signaling, such as insulin resistance in type 2 diabetes, insulin fails to suppress hepatic gluconeogenesis, even in the fed state; hence, an insight into these insulin-moderated pathways is critical for therapeutic purposes.

Keywords

glycogenolysis; glucose; regulation; gluconeogenesis; insulin

Introduction

Glucose is a major metabolic fuel that serves the energetic demands of mammalian tissues. During periods of starvation, glucose can be generated through the gluconeogenesis pathway, which is highly evolutionarily conserved from microorganisms to vertebrates. In the human body, the liver is the main site of gluconeogenesis. Increased gluconeogenesis in the liver of patients with type 2 diabetes is considered a major contributor to hyperglycemia and subsequent diabetic organ damage. Insulin is a key hormone that inhibits gluconeogenesis, and insulin resistance is a hallmark of type 2 diabetes. Understanding the regulation of gluconeogenesis and the role of insulin signaling in this pathway is important

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Competing Interests

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to developing new therapies for type 2 diabetes. Here, we aim to depict the role of insulin in the framework of hepatic gluconeogenesis.

Gluconeogenesis contributes to hepatic glucose production

Hepatic glucose production is a sum of gluconeogenesis, which is the formation of glucose from pyruvate or other 3- or 4-carbon compounds, and glycogenolysis, which is the breakdown of glycogen to glucose. The main substrates of gluconeogenesis in humans are lactate, glycerol, alanine, and glutamine. Together, these account for 90% of gluconeogenic substrates; however, other amino acids and citric cycle intermediates can also serve as substrates for gluconeogenesis.[1,2] Starting from lactate or an α-keto acid derived from amino acid breakdown, pyruvate can be generated for gluconeogenesis. Pyruvate is converted via carboxylation to oxaloacetate in the mitochondria. This reaction is stimulated by high levels of acetyl-CoA, which is produced via β-oxidation of fatty acids in the liver, and inhibited by high levels of ADP and glucose. After an intermediate step that allows oxaloacetate to leave the mitochondria via malate, oxaloacetate is decarboxylated and then phosphorylated to form phosphoenolpyruvate by phosphoenolpyruvate carboxykinase (PEPCK). This enzyme is a regulator of the rate of gluconeogenesis, and its transcription is targeted by multiple factors, including glucagon and insulin.[3] After several steps of reverse glycolysis, fructose 1,6-bisphosphatase converts fructose 1,6-bisphosphate to fructose 6-phosphate. Fructose 2,6-bisphosphate and AMP inhibit this reaction, while citrate activates the fructose 1,6-bisphosphatase enzyme. Glucose-6-phosphate (G-6-P) is formed from fructose 6-phosphate by phosphoglucoisomerase. G-6-P can be used in other metabolic pathways or dephosphorylated by glucose-6-phosphatase (G-6-Pase) to form free glucose. Whereas free glucose can easily diffuse into and out of the cell, the phosphorylated form (G-6-P) cannot, providing a mechanism by which intracellular glucose levels are controlled. The final reaction of gluconeogenesis—the formation of glucose—occurs in the lumen of the endoplasmic reticulum, where G-6-P is hydrolyzed by G-6-Pase to produce glucose and release an inorganic phosphate. Like the two previous step, this step is a reversal of glycolysis, in which hexokinase catalyzes the conversion of glucose and ATP into G-6-P and ADP. Glucose is then shuttled into the cytoplasm by glucose transporters located in the endoplasmic reticulum membrane.

Enhanced hepatic glucose production leads to increased glucose release to the blood, which can cause hyperglycemia. Type 2 diabetes is characterized by persistent hyperglycemia. In patients with type 2 diabetes, gluconeogenesis has been identified as the primary source of glucose production, while glycogenolysis was found not to contribute.[4] These findings underscore the importance of maintaining normal gluconeogenic rates to avoid disease pathophysiology. Insulin is a major hormone regulator of gluconeogenesis, so understanding its role in determining gluconeogenesis rates is essential to understanding the cause of and potential treatments for type 2 diabetes.

Insulin action on gluconeogenesis is both direct and indirect

A role for insulin in the regulation of hepatic glucose output is widely accepted. In healthy individuals, physiological hyperinsulinemia suppresses gluconeogenesis by 20%, while

glycogenolysis is completely suppressed.[5] Hyperglycemia alone suppresses hepatic glycogenolysis with only minimal effects on glycogen storage. Only the combination of hyperglycemia and hyperinsulinemia has a significant effect on hepatic glycogen synthesis.[6] Thus, insulin plays a crucial role in hepatic glucose metabolism.

The dominant mechanism of insulin-mediated regulation of hepatic gluconeogenesis is not clear. Insulin exerts direct control of gluconeogenesis by acting on the liver, but also indirectly affects gluconeogenesis by acting on other tissues. The direct effect of insulin was demonstrated in fasted dogs, where portal plasma insulin suppressed hepatic glucose production. even without changes in glucagon or gluconeogenic precursors.[7] However, in mouse models, insulin was found to have more potent effects on hepatic glucose production *in vivo* rather than *in vitro.*[8–10] Moreover, indirect effects of insulin on extrahepatic tissues have been shown to be sufficient to maintain normal glucose metabolism, suggesting an important role for indirect insulin regulation of gluconeogenesis.[11]

Suppression of gluconeogenesis through indirect effects of insulin is known to involve multiple tissues and cell types, with pancreatic α cells, adipose tissue, skeletal muscle, and the brain exerting known effects on hepatic gluconeogenesis. In pancreatic α cells, insulin inhibits the secretion of glucagon, which can indirectly lead to suppression of hepatic glucose production by reducing hepatic glucagon signaling.[12] Glucagon has effects on the transcriptional regulation of gluconeogenesis, primarily through the transcription factor CREB, but also through metabolite flux by affecting the activity of phosphofructokinase 1 (PFK1) in a PKA-dependent manner.[13,14] Insulin decreased plasma glucagon levels *in vivo* and inhibited glucagon secretion from pancreatic αcells *in vitro.*[15] However, in mice lacking the insulin receptor in the liver, insulin did not suppress glucagon secretion or hepatic glucose production, highlighting the importance of intrahepatic insulin signaling.[16]

Other indirect mechanisms by which insulin suppresses hepatic gluconeogenesis are through reducing gluconeogenic substrate release from adipose tissue and skeletal muscle or by acting on the brain. Insulin has inhibitory effects on lipolysis and proteolysis and thus decreases plasma levels of non-esterified fatty acids (NEFAs) and glycerol derived from adipose tissue, as well as amino acids from skeletal muscle.[10,14] A reduction of free fatty acid delivery to the liver has been shown to decrease hepatic glucose output.[17] However, NEFA failed to reduce hepatic glucose production in liver-specific insulin receptor gene knockout mice, suggesting that NEFAs are substrates that depend on intrahepatic insulin signaling to regulate hepatic gluconeogenesis.[16] The role of the central nervous system in gluconeogenesis is complex and has been recently reviewed,[18] but insulin has been found to inhibit gluconeogenesis by acting on the brain in an insulin receptor–dependent manner.[19]

The idea that extrahepatic insulin signaling can control hepatic glucose production (HGP) is supported by the fact that insulin can suppress HGP in mice where the canonical hepatic insulin signaling components Akt and FOXO1 are depleted.[20] Moreover, acute depletion of the insulin receptor and FOXO1 in liver does not prevent insulin from suppressing HGP.[21] It is important to mention, however, that in these experiments insulin can still signal through the IGF receptor, which might be sufficient to suppress HGP. A recent study further supports the idea that insulin's main effect on HGP is through suppression of lipolysis in white

adipose tissue.[22] Here, intrahepatic acetyl-CoA levels were shown to be elevated in HFD-fed rodents, resulting in an increase in pyruvate carboxylase activity and gluconeogenesis. The increase in hepatic acetyl-CoA is a result of increased lipolysis due to insulin resistance in fat. In support of the importance of the fat lipolysis–hepatic acetyl-CoA axis in controlling HGP, reducing lipolysis by inhibition of adipose triglyceride lipase or by neutralizing interleukin (IL)-6, a cytokine known to promote lipolysis in fat, normalizes hepatic acetyl-CoA levels and pyruvate carboxylase activity as well as HGP.[22]

Insulin regulation of hepatic gluconeogenesis through transcriptional modulation

Insulin can regulate hepatic gluconeogenesis via transcription of genes involved in gluconeogenic control, including *PCK1* and *G6PC.*[23] Changes in transcription may not contribute to acute regulation of hepatic glucose output, but they determine the gluconeogenic capacity of the liver and may have long-term effects, especially in pathological states.

Whether transcriptional regulation of gluconeogenic enzymes can be observed in human livers is controversial. A recent study showed no induction of *PCK1* and *G6PC* in liver biopsies of patients with type 2 diabetes.[24] However, another study in patients found a clear correlation between insulin resistance and *PCK1*, *G6PC,* and *FOXO1* mRNA levels.[25] An explanation for these findings may be that lesions in nonalcoholic steatohepatitis are unequally distributed over the liver, and significant sampling errors might occur.[26] Also, the degree of liver damage and other parameters, such as total body weight and tissue cross talk, may affect whether changes in gene expression are observed. Nonetheless, since gluconeogenesis is a primary driver of hepatic glucose production in type 2 diabetic patients[4,27] and insulin affects gluconeogenesis through transcription, determining the insulin signaling pathways that alter gluconeogenic gene transcription and ultimately gluconeogenesis can contribute to our understanding of type 2 diabetes pathophysiology.

Insulin activates several signaling pathways that regulate gluconeogenesis

Insulin binds to and initiates signaling through the insulin receptor, which is a tyrosine kinase that is activated upon ligand binding. This activation leads to phosphorylation of a variety of intracellular substrates. Two major downstream pathways of insulin action are the PI3K and MAPK pathways.[28] However, the activities of several other pathways are modulated by insulin action, with implications for transcriptional control of gluconeogenesis (Fig. 1).

IRS1 and IRS2

Among the numerous downstream signaling components of the insulin receptor, the insulin receptor substrates (IRS) play a key role in the regulation of hepatic glucose production. Mice lacking the IRS2 protein exhibited diabetes-like symptoms and increased hepatic glucose production.[29] Mice with knockout of *Irs1* had decreased insulin sensitivity, but not diabetes per se.[30] Double knockout of *Irs1* and *Irs2* in the liver leads to severe

hyperglycemia, hyperinsulinemia, and induction of gluconeogenic genes, such as *Pck1* and *G6pc*.[31] However, the double knockout also had severe growth defects, so interpretation of data from this animal model must be cautious. Taken together, these data demonstrate that IRS proteins are important for insulin signal transduction, and that IRS1 and IRS2 can compensate for each other.

PI3K

The activation of IRS proteins results in the recruitment of the lipid kinase PI3K to the plasma membrane, where it phosphorylates PI-(4,5)-bisphosphate (PtdIns(4,5)P2/PIP2) to generate PtdIns(3,4,5)P3 (PIP3), an important second messenger of several growth factor receptors and mediators of PEPCK and G-6-Pase expression levels. Adenoviral overexpression of the dominant-negative mutant of the PI3K regulatory subunit p85α, which lacks the binding site for the PI3K catalytic subunit, increased *Pck1* and *G6pc* gene expression, as well as hepatic glucose production *in vivo*.[32] Pharmacological inhibition of PI3K also inhibited the insulin-mediated suppression of *Pck1* and *G6pc* expression.[33] Therefore, there is evidence that PI3K is a necessary downstream component of insulin-suppressed gluconeogenesis.

PDK1

Downstream of PI3K, the generation of PIP3 is known to increase the activity of 3-phosphatidylinositol-dependent kinase-1 (PDK1).[28] Mice deficient for PDK1 in the liver have decreased glucose tolerance, decreased pyruvate tolerance, and fail to normalize blood glucose levels 2 h after insulin administration,[34] probably because livers from these mice display a massive defect in glycogen storage. Furthermore, hepatic glucose output and gluconeogenic gene expression were not suppressed by feeding in these mice. Acute insulin-triggered Akt signaling was also impaired in mice lacking PDK1 in the liver. These findings suggest that PDK1 is important for insulin signaling but also plays a major role in glucose metabolism under physiological conditions.

Akt

PIP3 generated by PI3K allows Akt/PKB to bind and be phosphorylated by PDK1.[35] Insulin-stimulated PI3K-mediated phosphorylation of Akt at Ser473 activates the kinase.[36] Akt kinases control diverse functions, including cell growth, survival, proliferation, and metabolism. However, the mechanisms through which Akt activity is specified to particular cellular functions in response to extracellular stimuli are not fully understood. Studies of Akt isoform–specific gene knockout mice suggested that Akt signaling diversity might in part be due to the different functions of the three Akt family members AKT1, AKT2, and AKT3.[37] Among the three, AKT2 appears to play the major role in glucose metabolism; however, redundancy in Akt functions also appear to occur. *Akt2* knockout mice developed a type 2 diabetes–like phenotype, and cells derived from these mice had impaired glucose utilization, suggesting a central role for AKT2 in the maintenance of glucose homeostasis.[38] Simultaneous deletion of *Akt1* and *Akt2* caused lethality shortly after birth,[39] and *Akt1* and *Akt3* double-knockout mice were embryonic lethal.[40] However, mice with knockout of *Akt2* and *Akt3* with a single functional allele of *Akt1* (*Akt1*$^{+/-}$*Akt2*$^{-/-}$*Akt3*$^{-/-}$) were viable despite reduced body weight and insulin and glucose intolerance.[37] While knockout of *Akt2*

in mice caused insulin resistance and diabetes-like symptoms, including increased hepatic glucose production, AKT1 was dispensable for glucose homeostasis.[38,39] Of note, a role for increased *Pck1* and *G6pc* transcription was not demonstrated in these mouse models, suggesting regulation of acute glucose output *in vivo* rather than of chronic transcriptional changes. However, overexpression of Akt in hepatoma cells and primary hepatocyte cultures decreased *Pck1* and *G6pc* gene transcription, demonstrating that Akt can regulate transcription of gluconeogenic enzymes.[41]

Several proteins have been identified to mediate suppression of *Pck1* and *G6pc* by Akt, such as FOXO1, a master regulator of gluconeogenesis.[42] Akt seems to be the key regulator of FOXO1 activity.[43] Ablation of *Akt1* and *Akt2* in the livers of mice leads to insulin resistance and diabetes and increased expression of several FoOXO1 target genes.[20] *Pck1* and *G6pc* expression levels in the knockout livers were not different from that in the control livers during fasting, but the normal suppression of these genes after feeding was lost. Deletion of *Foxo1* in these mice normalized the hyperglycemia, glucose intolerance, hyperinsulinemia, and the response to feeding despite defective insulin signaling.[20] This study elegantly showcased the regulatory effect of the Akt–FOXO1 network on hepatic gluconeogenesis.

Activated Akt can phosphorylate and inhibit glycogen synthase kinase-3 (GSK-3), which inhibits glycogen synthase and therefore enhances the release of G-6-P from glycogen. However, whether this is a direct insulin-mediated effect remains unclear. Pharmacological inhibition of GSK-3 was shown to suppress *Pck1* and *G6pc* transcription, but to a lesser extent than insulin.[40] Furthermore, overexpression of GSK-3 had no effect on insulin-mediated suppression of *Pck1* and *G6pc* transcription, suggesting different mechanisms of action for GSK-3 and insulin on *Pck1* and *G6pc* expression.[40]

MAPK

Insulin is a potent simulator of the Raf/MEK/ERK1/2 pathway, and this pathway might be involved in the regulation of gluconeogenesis. Thus far, neither pharmacological inhibition nor overexpression of components of the Raf/MEK/ERK1/2 pathway have been shown to be efficient in suppressing *Pck1* and *G6pc* transcription or hepatic glucose production.[33,44,45] However, the downstream effector p38 has gained attention in the regulation of gluconeogenesis. Inhibition of p38 by siRNA or a pharmacological inhibitor reduced glycemia in mice and suppressed gluconeogenesis in liver, along with suppression of *Pck1* and *G6pc* transcription.[46] Activation of p38 by free fatty acids showed similar results on *Pck1* and *G6pc* transcription.[47,48] Mice lacking MAPK phosphatase 1 (MKP-1), a negative regulator of p38 and JNK activity, exhibited increased gluconeogenesis and hepatic insulin resistance.[49] P38 inhibition also decreased transcription of peroxisome proliferator–activated receptor γ coactivator 1α (*Ppargc1a*) and *Creb*, suggesting transcriptional control of these major regulators of gluconeogenic gene expression.[46] P38 can also activate PGC-1α by phosphorylation.[50] Along with PGC-1α and CREB, p38 also phosphorylates and activates FOXO1, implicating a broader role for p38 in the transcriptional regulation of glucose homeostasis and gluconeogenesis.[46,47,51] Additionally, insulin promotes glycogen synthesis through the activation of protein phosphatase-1.[52] This is mediated through the Ras/MAPK pathway, and protein phosphatase-1 in turn activates glycogen synthase while

simultaneously inactivating phosphorylase a and phosphorylase kinase to control glycogen metabolism.[52]

CDC-like kinase

CDC-like kinase 2 (CLK2) belongs to a large and highly conserved family of kinases. Among the CLKs, CLK1–4 play roles in mRNA splicing and nuclear recruitment of proteins.[53] However, CLK2 is the only kinase known to be regulated by insulin and to have an effect on hepatic gluconeogenesis. CLK2 is directly phosphorylated and thereby stabilized via insulin-activated Akt.[54] Overexpression of CLK2 decreased hepatic glucose output in mice and corrected hyperglycemia in *db/db* mice.[55] On the contrary, in liver-specific *Clk2* knockout mice, no diabetic phenotype was observed, suggesting a compensatory adaptive mechanism during chronic CLK2 deficiency.[56] CLK2 potentially mediates its effects through modulation of the PP2A–phosphatase complex and phosphorylation of PGC-1α, leading to decreased transcriptional activity of PGC-1α.[54,56]

Cyclin-dependent kinases

Cyclin-dependent kinases (CDKs) are a family of protein kinases first characterized for their roles in the cell cycle.[57] They are also involved in regulating transcription, mRNA processing, and differentiation.[58] They are present in all known eukaryotes, and their regulatory function in the cell cycle has been evolutionarily conserved.[59] The mechanism by which insulin affects the activity of CDKs is not completely understood. Reports suggested a role for insulin in the activity of CDK regulatory subunit 1 (CKS1), CDK4, and CDK5. CKS1 is upregulated by insulin via the insulin receptor to promote cell proliferation in insects, but no effect on gluconeogenesis was reported.[60] Two independent reports recently showed the activation of CDK4 through refeeding and insulin in the liver. Both groups also observed profound effects on hepatic gluconeogenesis. This very likely occurs via the inhibition of PGC-1α transcriptional activity.[61,62] The role of CDK5 in insulin signaling is unclear. CDK5 activity is increased by insulin, and this effect can be abolished by a PI3K inhibitor in primary adipocytes.[63] In adipose tissue, activated CDK5 promotes adipogenesis through phosphorylation and activation of PPAR-γ.[64] A role for CDK5 in regulating hepatic glucose production remains elusive.

Transcriptional regulation of gluconeogenesis by insulin

FOXO1

In mammals, the Forkhead protein family comprises four proteins, FOXO1, FOXO3 (FOXO3a), FOXO4, and FOXO6. Among these, FOXO6 shows a high specificity to neuronal localization, while the other three factors are widely distributed and are present in most tissues. There seems to be a considerable overlap in the transcriptional targets of the first three, and there is also evidence that each of these can compensate for loss of the others to some degree.[65,66] While *Foxo1* knockout is embryonically lethal owing a the failure of angiogenesis, *Foxo3* knockout produces premature ovarian failure, and *Foxo4* knockout has no obvious phenotype.[28] Triple conditional knockouts resulted in lymphomas, hemangiomas, and angiosarcomas, which did not occur with double-knockout combinations.[65] FOXO1 specifically plays a major role in the regulation of insulin sensitivity.[66] FOXO1

transcriptional activity is regulated by a complex array of posttranslational modifications (PTMs). With regards to the gluconeogenic function of FOXO1, the primary regulatory event is Akt-mediated phosphorylation of three conserved residues, one threonine and two serines (Thr24, Ser253, and Ser316 in *Mus musculus*), that results in binding to 14-3-3 proteins and nuclear export of FOXO1.[66]

Mice with specific deletion of *Foxo1* in the liver show reduced fasting glucose and gluconeogenic gene expression, and glucose clamp studies demonstrate that *Foxo1* ablation impairs fasting- and cAMP-induced glycogenolysis and gluconeogenesis. Furthermore, *Foxo1* deletion prevents neonatal diabetes and steatohepatitis in insulin receptor gene knockout mice.[67] When FOXO1 is constitutively expressed in the murine liver, fasting blood glucose increases.[68] There is strong evidence that FOXO1 directly regulates *Pck1* and *G6pc* transcription. FOXO1 has been reported to bind to the insulin-responsive sequences of the *Pck1* and *G6pc* promoters *in vitro.*[69,70] The importance of Akt phosphorylation on FOXO1 regulation is striking. In the fed state, insulin signaling activates PI3K and subsequently Akt. Akt then phosphorylates FOXO1, leading to its inactivation though its export from the nucleus, with subsequent suppression of gluconeogenesis.[20] Hyperglycemia following Akt gene deletion can be corrected by concomitant hepatic deletion of *Foxo1.*[20,38] These data indicate that the regulation of hepatic glucose metabolism and the maintenance of glucose homeostasis are strongly mediated though the Akt–FOXO1 axis. In this context, the main function of insulin-mediated signaling is to counteract FOXO1 and thus reduce glucose production during the fed state. However, FOXO1 does not inhibit the insulin-mediated upregulation of anabolic processes, such as glycogen and lipid synthesis.[20]

As mentioned briefly earlier, the activity of FOXO1 is also modulated by processes other than Akt-mediated phosphorylation. Its acetylation and deacetylation provide a second tier of regulation, and deacetylation is generally considered to enhance FOXO1 activity to promote gluconeogenesis. FOXO1 can be deacetylated by SIRT1 under conditions of cellular stress, in the process overriding the nuclear exclusion effect of Akt and causing nuclear retention and expression of FOXO1 target genes, including *Pck1* and *G6pc.*[71] Class IIa HDACs can also contribute to deacetylation of FOXO1 and have been shown to be positive regulators of hepatic FOXO1 in response to glucagon signaling during fasting.[72] Once these HDACs are activated through AMPK-dependent phosphorylation, they translocate to the nucleus, where they deacetylate and activate FOXO1, inducing transcription of gluconeogenic genes.[66,71]

Several other mechanisms of modulating FOXO1 activity, such as regulation of its glycosylation, ubiquitination, and proteosomal degradation, have recently been described. The transcription factor XBP-1, involved in the unfolded protein response, has been shown to increase insulin sensitivity. This activity is independent of its transcriptional effects, and may be mediated through its indirect interaction with FOXO1, acting as a chaperone to direct it toward proteosomal degradation.[73] The herpesvirus-associated ubiquitin-specific protease 7 (USP7; also known as herpesvirus-associated ubiquitin-specific protease (HAUSP)) is capable of mono-deubiquinating FOXO1, resulting in suppression of FOXO1 transcriptional activity through decreased FOXO1 occupancy on the promoters of gluconeogenic genes.[74] Another mechanism that can be utilized to modulate FOXO1

activity is through the glucose-derived *O*-linked β-*N*-acetylglucosamine (*O*-GlcNAc) modification. In diabetes, this specific modification is increased on hepatic FOXO1. It regulates FOXO1 activation in response to glucose, resulting in increased expression of gluconeogenic genes, while concomitantly inducing expression of genes involved in the ROS detoxification pathways.[75,76] Paradoxically, it is induced by hyperglycemia and appears to result from PGC-1α binding to *O*-GlcNAc transferase and targeting it to nuclear FOXO1.[77] Other signaling pathways, such as thyroid hormone signaling, retinoid signaling, and p53-regulated pathways, have been linked to modulation of the FOXO1 transcriptional activity on gluconeogenic genes.[55,78,79] The physiological relevance of these findings has yet to be determined.

CREB

CREB was first identified as a transcription factor that binds to cyclic AMP–binding element (CRE) on the promoter of the somatostatin gene.[80] Mice that express a dominant-negative inhibitor of CREB in the liver (*alb-ACREB* transgenic mice) showed decreased blood glucose levels and decreased expression of gluconeogenic genes, emphasizing the role of CREB in hepatic gluconeogenesis as a progluconeogenic factor.[81] Since then, the role of CREB as a crucial transcription factor in liver metabolism has been studied extensively. The key event in CREB activation is its phosphorylation at Ser133 following an increase in intracellular cAMP levels and subsequent activation of PKA. Activated PKA translocates to the nucleus and phosphorylates CREB, which is critical for the interaction of CREB with CBP/p300 to promote its transcriptional activity.[13] Apart from PKA, other kinases have been reported to phosphorylate CREB, including calmodulin-dependent kinases and MAPK kinases, such as p38.[13] Reversal of CREB phosphorylation can be mediated through PP1 and PP2 phosphatases, with potential tissue- and cell-specific regulatory patterns.[13] However, the interaction of CREB with its coactivators, rather than the phosphorylation of Ser133, appears to be the critical event that promotes its transcriptional activity.[13] CBP and p300 are closely related histone acetyl transferase orthologs that catalyze the transfer of the acetyl group from acetyl CoA to lysine residues of histone or non-histone proteins. The formation of a transcriptional complex of CREB and CBP/p300 seems to be critical for the selective transcriptional induction of cAMP-responsive genes.[13,82] Insulin has also been found to inhibit gluconeogenesis by selectively disrupting the CREB–CBP interaction. Refeeding triggers the phosphorylation of CBP at Ser436 by the atypical PKC-ι/γ (aPKCι/γ). This modification on CBP appears to block binding of CREB that is phosphorylated at Ser133. However, the regulatory Ser436 site in CBP is not conserved in p300, suggesting that CBP and p300 perform distinct roles in the liver.[13,83] CBP/p300 can additionally be phosphorylated and thereby inactivated by salt-inducible kinase (SIK).[84] It has also been reported that active CBP/p300 can stabilize CRTC2 through acetylation of a lysine residue, thereby increasing its activity.[85] The CRTC family consists of three members (CRTC1, CRTC2, and CRTC3) that have similar modular structures: they all contain an N-terminal CREB-binding domain (CBD), a central regulatory domain (REG), a splicing domain (SD), and a C-terminal transactivation domain (TAD).[13] In the basal state, CRTCs are sequestered in the cytoplasm through phosphorylation-dependent interactions with 14-3-3 proteins, such as SIK.[13] Exposure to cAMP and calcium, but not other signals, triggers the calcineurin-mediated dephosphorylation and nuclear translocation of CRTCs, which then bind to CREB

over relevant gene promoters. Among the different family members, CRTC2 is highly expressed in the liver. In fasting conditions, PKA-dependent inactivation of SIK leads to dephosphorylation of CRTC2 and its nuclear translocation.[86] Mice with a *Crtc2* knockout have decreased circulating glucose concentrations during fasting, lowered circulating blood glucose concentrations, and improved insulin sensitivity in the context of diet-induced obesity.[87] Hyperglycemia affects the activity of CRTC2 by enhancing *O*-glycosylation, resulting in reduced phosphorylation at Ser171, nuclear retention, and subsequent increased activity.[13,88]

CREBH

CREBH is an endoplasmic reticulum (ER)-bound transcription factor that is controlled by regulated intramembrane proteolysis (RIP) of the ER, which is known to maintain sterol homeostasis and to mediate the unfolded protein response (UPR). It is mainly expressed in the liver and is activated upon acute ER stress, when it promotes the transcription of acute phase response genes, such as *Crp* (C-reactive protein) or *Sap* (serum amyloid P-component). CREBH has been reported to induce the transcription of gluconeogenic genes, and acute knockdown of CREBH reduces fasting blood glucose levels in mice.[89] In mice with hepatic knockout of CREBH, a decrease in hepatic lipid content was observed in those on normal chow diet, while those on a high-fat diet displayed increased steatosis, suggesting that CREBH is involved in lipogenesis and lipolysis; however, no abnormalities in blood glucose were observed.[90] The same study reported that insulin treatment promotes cleavage and thereby activation of CREBH.[90]

PGC-1α

PGC-1α was initially identified in brown adipocytes as a coactivator of the transcription factor PPAR-γ, where it is critical for the control of thermogenesis and mitochondrial biogenesis.[91,92] The family of PGC-1 transcriptional coactivators includes various isoforms of PGC-1α as well as PGC-1β and PGC-1–related co-activator (PRC). They share similar structural features, comprising an N-terminal activation domain, a central regulatory domain, and a C-terminal RNA-binding domain.[93] Following PPAR-γ, PGC-1α has been shown to interact with various transcription factors, such as PPAR-α, PPAR-δ, FOXO1, and SREBP, and nuclear receptors, such as estrogen related receptors (ERRs), hepatocyte nuclear factor 4α (HNF4α), and glucocorticoid receptor (GR).[93] In addition, PGC-1α interacts with several other proteins, including HAT domain–containing proteins, such as CBP and p300, via the N-terminal domain, and the mediator complex via the C-terminal region.[94,95] Knockdown or knockout of *Ppargc1a* in primary hepatocytes results in decreased glucose production and reduced expression of gluconeogenic genes, while *Ppargc1a* null mice show increased gluconeogenic gene expression insensitive to normal feeding controls.96 Overexpression of PGC-1α in the liver of mice promotes the expression of gluconeogenic genes by enhancing the activity of various transcription factors, such as CREB, HNF4α, and FOXO1.[81,97,98] Insulin-stimulated Akt-mediated phosphorylation of FOXO1 obstructs its interaction with PGC-1α and interferes with the activation of hepatic gluconeogenic gene expression in mice.[97] Insulin can also regulate PGC-1α activity via the alteration of its acetylation and phosphorylation status. Acetylation of hepatic PGC-1α is controlled by counteracting effects of the GCN5 acetyltransferase and the SIRT1 deacetylase, and this

PTM impairs the ability of PGC-1α to promote gluconeogenic gene expression.[99,100] Insulin activates the cyclin D1/cyclin-dependent kinase 4 complex, which increases GCN5 acetyltransferase activity through phosphorylation, to promote the acetylation of PGC-1α that results in the suppression of hepatic glucose production.[62] The transcriptional co-regulator CITED2 (CBP- and p300-interacting transactivator with glutamic acid– and aspartic acid–rich COOH-terminal domain 2) activates PGC-1α by decreasing its acetylation through blocking its interaction with GCN5, resulting in increased gluconeogenic gene expression; however, this event is negatively regulated by insulin in an Akt-dependent manner.[101] Additionally, activation of Akt through insulin signaling enables it to phosphorylate PGC-1α directly at Ser570, thereby inhibiting its ability to be recruited to gluconeogenic gene promoters.[102] The insulin-regulated CLK2 phosphorylates PGC-1α in its serine- and arginine-rich (SR) domain, impairing its ability to function as a transcriptional coactivator, specifically toward FOXO1.[55] The insulin-inducible corepressor small heterodimer partner–interacting leucine zipper protein (SMILE) directly competes with PGC-1α for HNF4α to suppress its hepatic transcriptional activity and gluconeogenesis.[103]

Glucocorticoid receptor

GR has been linked to hepatic gluconeogenesis for a long time. It is activated by endogenous cortisol as well as exogenous corticosteroids. Upon fasting, increased secretion of glucocorticoids activates GR in the liver, which promotes transcription of gluconeogenic genes.[104] Even though insulin does not directly interact with GR, the transcriptional activity of GR is important for modulating insulin-mediated signaling. GR increases the transcription of *Mapk14* (p38 MAPK) and thereby counteracts the insulin-mediated effects on gluconeogenesis. Glucocorticoids also diminish IR and IRS1 phosphorylation in response to insulin in liver.[105]

Sterol response element–binding protein

Members of the sterol response element–binding protein (SREBP) family were initially characterized as transcription factors that are activated by low cholesterol levels in the cell.[106] The SREBP-1c isoform in the liver has been demonstrated to be regulated by nutritional stimuli *in vivo*, such as a carbohydrate-rich diet, and insulin has been found to rapidly stimulate the transcription of *Srebp1* in hepatocytes and adipose and muscle tissue. The effect of insulin on the expression level of SREBP-1c protein in hepatocytes appears to be mediated through activation of PI3K. In addition, by employing a version of Akt that can be conditionally regulated, studies in hepatoma cells provide evidence that activation of Akt is sufficient to mimic this biological response to insulin.[107] While mice with siRNA-mediated knockdown of *Srebp1* show decreased gluconeogenic gene expression, no effect was observed on fasting glucose levels.[108] A suggested mechanism of SREBP-1 action on gluconeogenesis is its interference with the HNF4α-mediated recruitment of PGC-1α to gluconeogenic gene promoters.[109] Coimmunoprecipitation experiments demonstrate that these two transcription factors (SREBP-1 and HNF4α) directly interact through the transactivation domain of SREBP and the ligand binding/AF2 domains of HNF4α.[109] In the same study, SREBP-1 does not bind the *Pck1* promoter, supporting an indirect effect of SREBP-1 on gluconeogenic gene expression, possibly through altering the localization of

HNF4α and PGC-1α.[109] Overall, the role of SREBP-1 in gluconeogenesis appears to be moderate, and the main functional role lies in the regulation of lipid metabolism.

STAT3

STAT3 is a member of the signal transducer and activator of transcription (STAT) protein family. STAT3 is phosphorylated by receptor-associated Janus kinases (JAKs) in response to cytokines and growth factors. Activated STAT3 proteins form homo- or heterodimers, and translocate to the cell nucleus, where they act as transcription activators.[110] In the liver, STAT activation is mainly linked to inflammation and cancer. Interestingly, STAT3 has the ability to directly bind to the promoters of *Pck1* and *G6pc* to inhibit the promoter activity of these gluconeogenic genes.[111] In mice, liver-specific deletion of *Stat3* increases the expression of *Pck1*, *G6pc,* and *Ppargc1a*. Conversely, liver-specific overexpression of a constitutively active form of STAT3 decreases hepatic glucose production and blood glucose levels in diabetic mice.[112] A significant mechanism through which insulin is able to modulate STAT3 activity is its hypothalamic action. Insulin action in the hypothalamus stimulates IL-6 production in the liver, and IL-6 in turn suppresses gluconeogenesis by activating STAT3.[113] Another point of cross talk between insulin and STAT3 is through GSK-3β. STAT3 suppresses the expression of GSK-3β, a negative regulator of the insulin signaling pathway. Mice lacking STAT3 in the liver do not exhibit a physiological decrease of *Gsk3b* mRNA or GSK-3β protein levels in response to feeding, which suggests that these mice display an impaired response to insulin.[114]

DAX1

Dosage-sensitive sex reversal, adrenal hypoplasia congenita critical region on the X-chromosome, gene 1 (DAX1) is an atypical nuclear receptor that lacks a classical DNA-binding domain. It was initially described in hypogonadotropic hypogonadism and is mainly expressed in adrenal glands, the pituitary gland, and the testes.[115] DAX1 has been shown to be expressed in the liver, and its expression is increased by insulin and SIK1, whereas it is decreased in diabetic and high-fat diet–fed mice.[116] It has been shown that DAX1 blocks the association of HNF4α and PGC-1α with the gluconeogenic gene promoters, suggesting a novel mechanism for feeding-induced repression of gluconeogenesis.[116] However, the mechanism through which insulin or insulin-related signaling increases DAX1 is not clear. Current data suggest an increase in *Dax1* transcription. In testicular Leydig cells, *Dax1* transcription is increased by insulin in an Akt-dependent manner. However, high-fat diet suppresses hepatic DAX1 levels in mice, suggesting that DAX1 regulation is distinct in different tissues.[116,117]

Insulin signaling and its impact on hepatic gluconeogenesis in diabetes and obesity

In obesity and diabetes, tissue homeostasis is disturbed in many compartments, such as skeletal muscle, adipose tissue, liver, kidney, and connective tissues. Eventually, these changes lead to tissue damage and remodeling, as seen in liver fibrosis, kidney fibrosis, or atherosclerosis. Additionally, these changes are major drivers of insulin resistance and thereby fuel a vicious cycle leading to organ damage, morbidity, and mortality.

Inflammation is a widely recognized occurrence in obesity and diabetes and has been linked to the pathogenesis of diabetes, mainly through impaired insulin signaling.[118] Chronic as well as acute inflammation have been clearly linked to insulin resistance and elevated levels of cytokines, such as TNF-α, and have been described and extensively studied in this context.[119,120] TNF-α signaling can activate various intracellular pathways, such as those involving JNK and IKK, and this inhibits insulin receptor signaling through serine phosphorylation of IRS1.[121] In contrast to the activating tyrosine phosphorylation, this modification leads to inactivity of IRS1 and eventual degradation of the protein.[122] Serine phosphorylation of IRS1 can come about as a result of several events, including through mTOR, S6 kinase, PKC-θ, and JNK signaling.[123]

Disturbed hepatic lipid metabolism is also known to affect insulin signaling and hepatic glucose production. Several studies support a role for hepatic diacylglycerol (DAG) accumulation and PKC-ε activation as factors for impaired hepatic insulin action. DAG has been shown to promote PKC activation with a subsequent decrease in insulin receptor tyrosine kinase activity. Mice lacking PKC are protected from diet-induced insulin resistance, even though hepatic lipid content is increased.[124] In humans, FOXO1 expression and transcriptional activity are increased in nonalcoholic steatohepatitis (NASH) liver, emphasizing a role for inflammation and disturbed lipid metabolism in the dysregulation of hepatic gluconeogenesis.[25]

Non-alcoholic fatty liver disease (NAFLD) is a growing subclass of liver disease, as it is closely related to obesity, metabolic syndrome, and diabetes. Patients diagnosed with hepatic steatosis have an increased risk for cardiovascular disease, as well as for diabetes and related conditions.[125] An important trigger may be hepatic insulin resistance, as it may be sufficient to promote dyslipidemia and atherosclerosis.[126] In addition, excess lipid accumulation in the liver has negative effects on the progression of liver disease. Interestingly, hepatic insulin signaling is impaired, leading to not only an increase in HGP but also an ongoing hepatic lipid production that results in hepatic steatosis, steatohepatitis, and cirrhosis. The phenomenon where impaired insulin signaling blocks one of its actions (decrease of HGP) while promoting another action (lipogenesis) is termed *selective insulin resistance*. A potential mechanism leading to this paradox in insulin action in the brain and the adipose tissue, which results in increased lipolysis and excess triglyceride synthesis in the liver, has been recently reviewed elsewhere.[127]

Additionally, insulin action in the central nervous system can indirectly regulate metabolism in the liver. Insulin signaling in the brain through hypothalamic PI3K and ATP-sensitive potassium channels has been suggested to suppress *Pck1* and *G6pc* expression in the liver, which results in the blunting of gluconeogenesis; activation of the hepatic IL-6/STAT3 pathway has been demonstrated to be mechanistically involved in this process.[112,128–130] Insulin-mediated FOXO1 nuclear exclusion through the PI3K–Akt axis in pro-opiomelanocortin (POMC) neurons has also been suggested to suppress basal HGP.[131] However, a study by Ramnanan *et al.* indicated that, in dogs, regulation of hepatic gluconeogenesis mediated by the action of insulin on the brain is debatable.[132] Scherer *et al.* additionally showed that insulin signaling in the rat brain is able to modulate triglyceride secretion and lipid content in the liver.[133] Thus, we highlighted the promise of potentially

targeting insulin resistance in the central nervous system for the treatment of type 2 diabetes.[134]

Concluding remarks

An intact insulin signaling system is paramount to maintaining blood glucose levels within a narrow normal glycemic range during periods of fasting or excess nutrient availability, and this is achieved in particular via regulation of metabolic flux through the hepatic gluconeogenic pathway. This review summarizes some of the mechanisms through which insulin can modulate hepatic gluconeogenesis. A significant node of control involves the transcriptional regulation of expression of the key hepatic gluconeogenic genes *Pck1* and *G6pc*, which occurs primarily through the transcription factor FOXO1 and nuclear receptor HNF4α and their transcriptional coactivator PGC-1α. Understanding these regulatory pathways is of extreme importance in terms of identifying potential targets and devising treatments for disorders involving insulin resistance and dysregulated hepatic gluconeogenesis, such as in type 2 diabetes.

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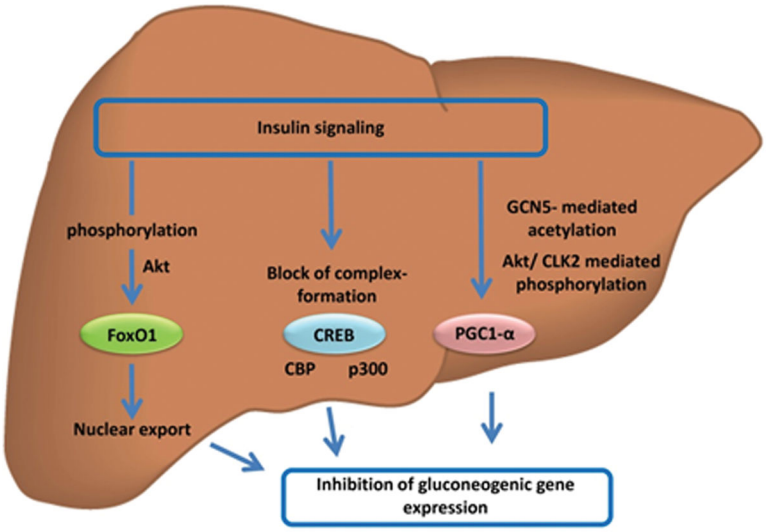

Figure 1.

Regulation of gluconeogenic gene expression by hepatic insulin signaling. Insulin action regulates the activity of trancription factors controlling gluconeogenic gene expression. AKT-mediated phosphorylation leads to nuclear export of FOXO1. Inhibitory phosphorylation of CBP/p300 blocks trancription-complex formation of CREB. Modification of PGC-1α by GCN5-mediated acetylation or AKT/CLK2-mediated phosphorylation decreases PGC-1α transcriptional activity.

Table 1

Extrahepatic effects of insulin that regulate hepatic gluconeogenesis.

Organ/tissue	Action	Effect on liver	Reference
Pancreatic alpha cells	Secretion of glucagon ↓	Transcriptional regulation of gluconeogenic genes	12–14, 16
White adipose tissue	Lipolysis ↓	Reduction of free fatty acid delivery to the liver	10,14,17
Skeletal muscle	Proteolysis ↓	Reduction of amino acid flux to the liver	10,14
Central nervous system	Pleiotropic manner	Multiple effects	18

Table 2

Animal models of targets of insulin signaling

Insulin target	Phenotype of (liver-specific) KO mice	Potential mechanism of action	Reference
IRS1, IRS2	Hyperglycemia, hyperinsulinemia	Induction of gluconeogenic genes	29–31
PI3K (p110-α and p110-β subunits)	Diet-induced liver steatosis ↓ Glucose intolerance (p110-α) ↑	Insulin-induced Akt phosphorylation at Ser473 ↓	135
PDK1	Glucose tolerance ↓ Pyruvate tolerance ↓ Insulin sensitivity ↑	Disruption of acute AKT-dependent insulin signaling	34
Akt2	Insulin resistance ↑ Hepatic glucose production ↑	Increased expression of FOXO1 target genes	20, 38, 39
p38 MAPK	Glycemia ↓ Gluconeogenesis ↓	Decreased transcription of *Pparg*, *Ppargc1a*	46
FoxO1	Fasting glucose ↓ Gluconeogenic gene expression ↓	Direct regulation of *G6pc* and *Pck1* transcription	67–70
PGC-1α	Gluconeogenic gene expression ↑ Loss of feeding-induced regulation	Regulation of *G6pc* and *Pck1* transcription	96