The Role of Insulin in the Regulation of PEPCK and Gluconeogenesis *In Vivo*

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Abstract

The regulation of gluconeogenesis by insulin is complex and can involve insulin-mediated events in the liver, as well as in several non-hepatic tissues. Given the complexity of this regulation, it is no surprise that there is considerable debate regarding insulin's ability to regulate the rate of gluconeogenic formation of glucose-6-phosphate (GNG flux to G6P) *in vivo*. Conventional 'textbook' teaching (based on *in vitro* studies of rat liver) depicts that insulin can inhibit this pathway by suppressing the transcription of the enzyme phosphoenolpyruvate carboxykinase (PEPCK). PEPCK is widely considered to be a 'rate-limiting' enzyme with high control strength. Additionally, recent data in rodents have led to the conclusion that hyperinsulinemia in the brain can inhibit GNG flux to G6P, likely through transcriptional regulation of PEPCK. Recent data from the authors' lab have confirmed that the molecular regulation of PEPCK messenger RNA (mRNA) and protein by insulin is conserved in large animals. Acute physiological hyperinsulinemia does not alter gluconeogenic formation of G6P, however, despite substantial reductions in PEPCK protein. This indicates that PEPCK has poor regulatory control over the pathway *in vivo*. A physiological rise in insulin suppresses hepatic glucose production by inhibiting glycogenolysis and promoting glycogen synthesis, stimulating glycolytic flux, and redirecting gluconeogenically derived carbon to glycogen. This review documents the relevant ways in which insulin can regulate GNG flux to G6P *in vivo*.

Keywords

Hepatic glucose production (HGP), insulin, hyperinsulinemia, gluconeogenesis, glycogenolysis, glucose-6-phosphate (G6P), GNG flux to G6P, glycolysis, phosphoenolpyruvate carboxykinase (PEPCK), lipolysis, free fatty acids (FFAs)

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The regulation of hepatic glucose production (HGP) by insulin is critical for the maintenance of desirable blood glucose concentrations.[1] HGP reflects the sum of gluconeogenesis (the synthesis and release of glucose from non-carbohydrate precursors) and glycogenolysis (glucose released from the breakdown of hepatic glycogen). An acute rise in portal vein insulin (such as occurs in response to feeding) causes the rapid suppression of HGP derived from both gluconeogenic and glycogenolytic sources. Standard textbook teaching, based on data culled largely from experiments on isolated hepatocytes, liver slices, and perfused livers from rats, posits that the gluconeogenic pathway is inhibited by insulin via rapid and profound transcriptional regulation of the 'rate-limiting' gluconeogenic enzymes.[2–6] Recent data in rodents are in line with this dogma and in addition suggest that hyperinsulinemia in the brain is sufficient to markedly inhibit flux through the gluconeogenic pathway by downregulating gluconeogenic gene expression.[7–9] By contrast, the exquisite sensitivity of HGP to physiological hyperinsulinemia in humans and dogs *in vivo* is clearly explained by a profound inhibition of glycogenolytic flux, but little (if any) modification of flux through the gluconeogenic pathway.[1,10–14] The purpose of this review is to examine the ways by which insulin brings about its effects on the gluconeogenic pathway *in vivo* and to address discrepancies in the literature concerning sensitivity of the pathway to the hormone in the whole animal.

Potential Regulatory Loci for Insulin's Effects on GNG Flux to G6P

There are three sources that feed into the glucose-6-phosphate (G6P) pool within the hepatocyte:

- G6P formed via glucokinase-mediated phosphorylation of glucose taken up from circulation;
- G6P derived from the breakdown of glycogen; and
- G6P synthesized from three-carbon precursors (lactate, glycerol and certain amino acids) via the gluconeogenic pathway (GNG flux to G6P).

Intracellular G6P has three major cellular fates:

- dephosphorylation by the enzyme G6Pase and export as glucose into the systemic circulation;
- entry into the glycolytic pathway that generates lactate, pyruvate and CO_2 (essentially the reverse of GNG flux to G6P); and
- deposition in liver glycogen (see *Figure 1*).

A rise in insulin can therefore inhibit glucose output derived from gluconeogenesis in several ways. Hyperinsulinemia can:

- suppress the activity of gluconeogenic enzymes, thereby reducing GNG flux to G6P;
- promote the activity of glycolytic enzymes, thereby increasing glycolytic flux (and decreasing the net GNG flux to G6P); and/or
- enhance the deposition of gluconeogenically derived G6P in glycogen.

In the latter two scenarios, hyperinsulinemia can reduce gluconeogenesis without modifying the rate of GNG flux to G6P. The distinction between gluconeogenesis and GNG flux to G6P is therefore crucial in the interpretation of insulin's effects on the pathway. Insulin can also regulate hepatic gluconeogenesis indirectly by mediating events in non-hepatic tissues (see *Figure 2*) such as fat,[15,16] muscle,[17] the pancreatic alpha cell,[18] and the brain.[7–9]

Cellular Mechanisms of Insulin Action in the Liver

Regulation of Cyclic Adenosine Monophosphate Concentration

Insulin, upon binding to its hepatic receptor, activates an intricate signaling cascade that can regulate the enzymes related to glucose uptake and release, gluconeogenesis, glycolysis, and glycogen metabolism (see *Figure 3*). Cyclic adenosine monophosphate (cAMP) is the second messenger responsible for glucagon's stimulatory effects on glucose production *in vitro*[19–21] and *in vivo*.[22,23] Insulin opposes glucagon's action, in part by decreasing the concentration of hepatic cAMP, presumably by activating a phosphodiesterase.[24]

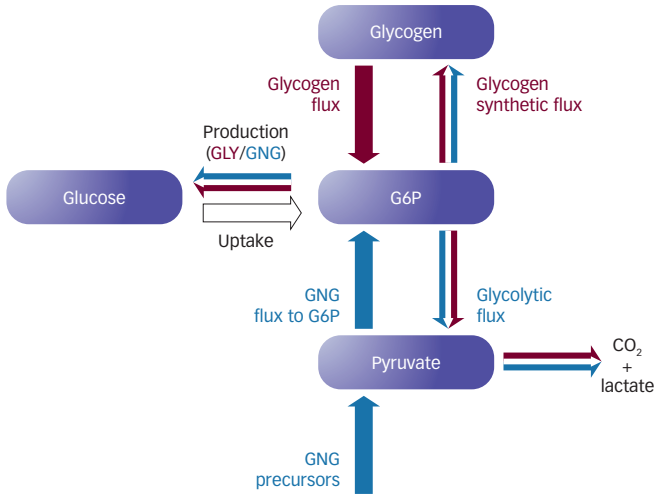
Glucokinase and G6Pase Expression

Insulin can stimulate the transcription of glucokinase[25] and inhibit the expression of G6Pase,[26] leading to long-term changes in the levels of glucokinase and G6Pase protein that favor glucose uptake. The genetic regulation of glucokinase and G6Pase can be modified rapidly in response to insulin in the rat[5,27] and dog,[28] but it has been observed that it takes several hours for the corresponding protein levels to change in the dog model.[28] It is therefore clear that this genetic regulation is not involved in the acute suppression of HGP by hyperinsulinemia, which occurs within minutes. On the other hand, the translocation of glucokinase from the nucleus to the cytoplasm (where it can function in glucose phosphorylaton) is stimulated within minutes by insulin *in vivo*,[29] and may be involved in the rapid transition of the liver from an organ of glucose production to one of glucose uptake.

Transcriptional Regulation of PEPCK

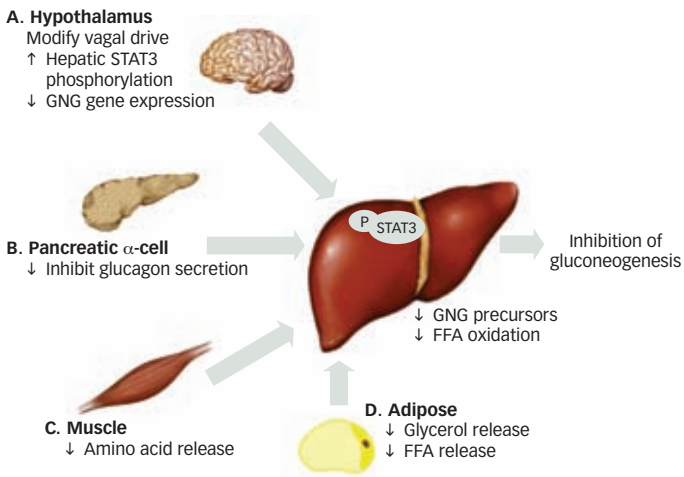
Insulin can also potently and rapidly (within minutes) inhibit the transcription of phosphoenolpyruvate carboxykinase (PEPCK), an enzyme that has long been thought of as the rate-determining enzyme controlling GNG flux to G6P.[3,5,6,26] The genetic control of PEPCK by insulin is complex and involves many signaling intermediates that are also involved in the regulation of G6Pase expression. Studies frequently use

Figure 1: The Sources and Fates of G6P in the Hepatocyte



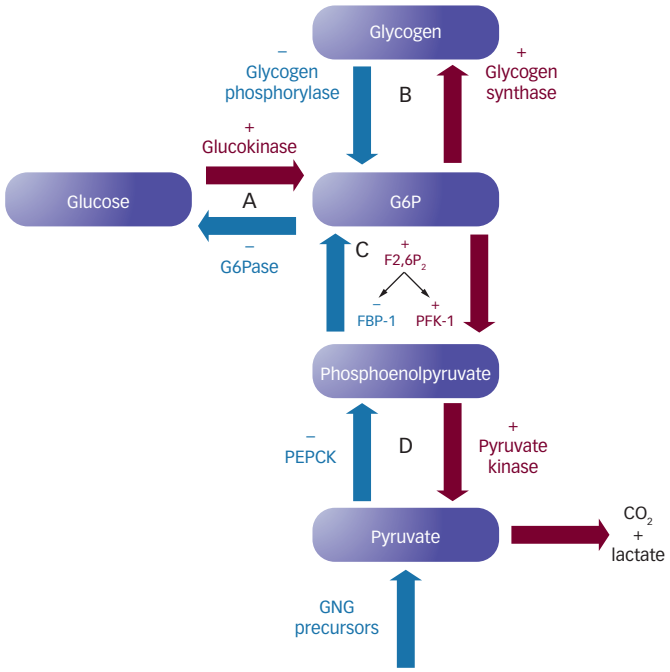
There are three sources that feed into the glucose-6 phosphate (G6P) pool: glucose is taken up from circulation (white); G6P is derived from glycogenolytic flux (red); and G6P is derived from the gluconeogenic pathway (gluconeogenesis [GNG] flux to G6P, blue). There are a number of fates for G6P molecules from this pool, each of which can include G6P derived from all three sources (depicted by a mixture of white, red, and blue): deposition into glycogen, entrance into glycolysis, and exit into circulation as glucose. Note that net hepatic glycogenolytic flux represents the difference between glycogenolytic flux and glycogen synthetic flux. Net hepatic gluconeogenic flux represents the difference between GNG flux to G6P and glycolytic flux. Net hepatic glucose production (consisting of both gluconeogenesis and glycogenolysis) is the difference between glucose uptake and release.

Figure 2: A Physiological Rise in Insulin Can Suppress GNG Flux to G6P by Mediating Effects in Non-hepatic Tissues



Note that the rise in insulin at the liver is usually several-fold that of other organs. Hyperinsulinemia results in: hypothalamic signaling that alters vagal input to the liver, resulting in STAT3 phosphorylation and reduction of gluconeogenesis (GNG) gene expression (A); reduced glucagon secretion from the pancreatic α cell (B); β-protein synthesis and β proteolysis in muscle and reduction of amino acid (GNG substrate) release to liver (C); and inhibition of lipolysis and reduction of release of both glycerol (GNG substrate) and free fatty acids (FFAs) (which stimulate gluconeogenesis and inhibit glycolysis) to the liver (D).

Figure 3: Hepatic Loci of Regulation in Which Insulin Can Inhibit Hepatic Gluconeogenesis (Glucose Release Derived from GNG Flux to G6P)



Arrows marked in red and blue depict pathways that are stimulated and inhibited, respectively, by hyperinsulinemia. Insulin can: mediate transcriptional regulation of both G6Pase ↓and glucokinase ↑ to inhibit the dephosphorylation of G6P and stimulate GK translocation (A); reciprocally regulate glycogen phosphorylase ↓and glycogen synthase ↑ to divert gluconeogenically derived G6P into glycogen (B); stimulate the formation of F2,6P2, a metabolite that regulates key enzymes in the gluconeogenic ↓and glycolytic pathway ↑, respectively (C); and regulate the balance between pyruvate and phosphoenolpyruvate by suppressing the transcription of PEPCK (considered to be the rate-limiting enzyme in the gluconeogenic formation of G6P) and stimulating activity of the glycolytic enzyme pyruvate kinase (D).

PEPCK and G6Pase messenger RNA (mRNA) as indices of insulin's hepatic action. In the fasting state, the transcription factor FOXO1 interacts with the protein PGC1α at the promoter of the gluconeogenic genes PEPCK and G6Pase, driving their mRNA expression.[30–32] PGC1α expression, in turn, is regulated by the protein TORC2.[33,34] A rise in hepatic insulin causes the phosphorylation of FOXO1 and TORC2, leading to their nuclear exclusion and resulting in suppression of GNG mRNA expression.[30–34] In addition, recent studies in rodents have indicated that hyperinsulinemia in the brain can play a role in the suppression of gluconeogenic mRNA expression.[7–9]

Stimulation of Glycolysis

Unlike the long-term (hours to days) genetic control that insulin exerts on the enzymes of the gluconeogenic pathway, hyperinsulinemia can modify the activity of enzymes in the glycolytic pathway in rapid (minute-to-minute) fashion.[4,35,36] There are metabolically specialized hepatocytes within the liver,[37] and GNG flux to G6P (in periportal hepatocytes) and glycolytic flux (in perivenous hepatocytes) can occur simultaneously. Glycolytic flux is essentially the reverse of GNG flux to G6P, so hyperinsulinemia may inhibit G6P formation in the net sense by stimulating the reverse, glycolytic reaction.

Pyruvate kinase is believed to be one of the rate-controlling enzymes in glycolysis and insulin facilitates its desphosphorylation and activation.[36]

Insulin also stimulates the dephosphorylation of the bifunctional enzyme 6-phosphofructokinase-2 (PFK-2)/fructose-2,6-bisphosphatase (FBPase-2), increasing its PFK-2 activity while inhibiting its FBPase-2 activity. PFK-2 and FBPase-2 regulate the synthesis and degradation, respectively, of $F2,6P_2$, a metabolite that potently stimulates glycolytic and inhibits gluconeogenic enzymes *in vitro*.[4,21,35] Thus, it is conceivable that insulin can inhibit GNG flux to G6P *in vivo* by increasing hepatic $F2,6P_2$ concentration.

Glycogen Metabolism

Glycogen phosphorylase (GP) and glycogen synthase (GS) regulate the balance between hepatic glycogen breakdown and deposition and are reciprocally regulated by insulin. In the fasted state, GP and GS are phosphorylated, permitting GP activity and inhibiting GS activity, the net of which favors glycogenolysis.[38,39] Hyperinsulinemia mediates the rapid dephosphorylation of both GP and GS, facilitating the transition from net glycogenolysis to net glycogen synthesis *in vitro*[38] and *in vivo*.[40]

Non-hepatic Mechanisms of Insulin-mediated Regulation of Gluconeogenesis

The Alpha Cell

In addition to reducing the concentration of hepatic cAMP, insulin can also counter glucagon's ability to stimulate HGP by inhibiting glucagon secretion from alpha-cells.[18]

Gluconeogenic Precursor Flux

Insulin can regulate the availability of gluconeogenic precursors to the liver through its effects on peripheral tissues.[20] Insulin has anabolic effects in muscle and fat, inhibiting proteolysis[41] as well as lipolysis,[42] thereby decreasing the availability of gluconeogenic amino acids (GNGAA) and glycerol to the liver. Glucose formation from glycerol accounts for about 3% of hepatic glucose output in 12–14-hour fasted humans.[43] Thus, the reduction of glycerol flux to the liver can be considered to have only a minor influence in the overall gluconeogenic rate. Insulin stimulates hepatic amino acid transport into the liver,[44] thereby increasing the net hepatic fractional extraction of GNGAAs so that the effect on reduction in the flow of GNGAAs from muscle to liver is somewhat offset. Lactate is quantitatively the most important gluconeogenic precursor during fasting. However, lactate production by peripheral tissues (muscle, fat, skin, red blood cells, and the central nervous system), and therefore the availability of lactate to the liver, does not appear to substantially change during physiological hyperinsulinemia.[17,45] Hepatic lactate metabolism, on the other hand, can be significantly altered by insulin in a manner that is secondary to the hormone's inhibition of lipolysis.

Inhibition of Lipolysis

During fasting, lipolysis supplies free fatty acids (FFAs) and glycerol to the liver. While FFAs are not gluconeogenic substrates, they are potent stimulators of gluconeogenesis *in vitro*[46] and *in vivo*.[47] The oxidation of fatty acids by the liver generates energy while preserving circulating glucose for other tissues.[48] Fatty acid oxidation in the liver increases the concentration of citrate, adenosine triphosphate (ATP), nicotinamide adenine dinucleotide (reduced form) (NADH), and acetyl-CoA.[49] Citrate is a potent inhibitor of glycolysis,[50] while ATP and NADH are energy equivalents that can be used to fuel gluconeogenesis.[49] Acetyl-CoA

allosterically stimulates activity of the gluconeogenic enzyme pyruvate carboxylase.[51] The liver in humans fasted for 12–14 hours or longer[52] and in dogs fasted for 22 hours or longer[53] exhibits net lactate uptake, with lactate used primarily for gluconeogenesis. A rise in insulin markedly and rapidly (within 30 minutes) suppresses lipolysis,[54] resulting in reduction of FFA supply to and FFA oxidation by the liver and the removal of cues (citrate, ATP, NADH, and acetyl-CoA) that, at least *in vitro*, stimulate the gluconeogenic pathway and inhibit glycolytic flux. Thus, glycolysis is promoted (as is carbon efflux from the liver in the form of lactate), resulting in a consequent decrease of net hepatic lactate uptake.

Insulin–Brain–Liver Axis

In recent years it has been suggested that hyperinsulinemia in the brain is sufficient to suppress HGP[7–9] strictly by reducing the gluconeogenic rate, with no alteration in glycogenolysis or glucose utilization.[8,9] These observations led the authors to suggest that insulin action in the hypothalamus alters vagal input to the liver, resulting in a hepatic event, suggested to be STAT3 phosphorylation, that causes the reduction of gluconeogenic mRNA expression and suppression of gluconeogenesis.[7–9] As one would predict, this genetic regulation takes several hours to bring about decreased HGP.

Integrating Cellular Effects with Regulation of GNG Flux to G6P *In Vivo*

While there is an abundance of *in vitro* data suggesting that insulin can regulate the gluconeogenic pathway via suppression of PEPCK gene transcription, data in whole animals are conflicting. Certain studies suggest that GNG flux to G6P can be inhibited by insulin in rodents,[7–9] while other rodent studies suggest that flux through the pathway continues unaltered during hyperinsulinemia.[55–57] In humans and dogs, physiological hyperinsulinemia suppresses glucose output (derived from glycogenolytic and gluconeogenic sources) by inhibiting glycogenolytic flux, without altering GNG flux to G6P. This suggests that gluconeogenically derived carbon is redirected into glycogen.[1,10–14] The GNG formation of G6P has been shown to be important in post-prandial glycogen deposition in humans, dogs, and rodents.[56–59] This is in agreement with the concept that GNG flux to G6P is not sensitive to insulin.

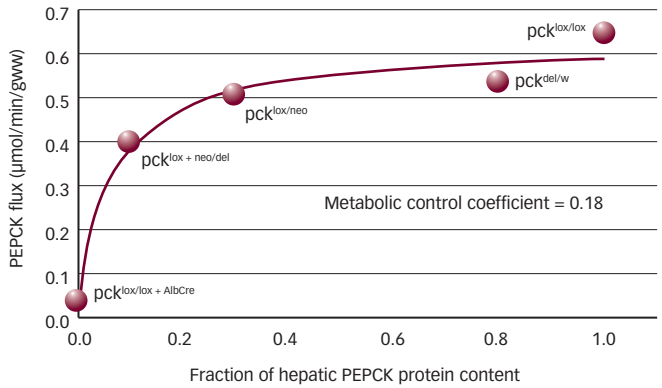
In the dog, two-,[13] four-,[54] and eight-fold[28] increases in insulin have substantial effects on glycogen metabolism without altering GNG flux to G6P. A recent study demonstrated that a supraphysiological dose of insulin (16-fold rise) can reduce GNG flux to G6P rapidly (within 30 minutes) in the canine. This inhibition, however, was still modest relative to the effect on glycogen metabolism.[54] Furthermore, while PEPCK mRNA levels were substantially reduced after five hours, the reduction in PEPCK protein did not occur fast enough to explain the rapid inhibition of GNG flux to G6P.[54]

The rapid suppression of HGP (within 30 minutes) by physiological hyperinsulinemia *in vivo* is associated with reciprocal changes in GP and GS activity that modulate glycogen metabolism and cause a transient increase in glycolytic flux.[28] Glycolysis is essentially the reverse of GNG flux to G6P. Insulin can thus reduce net hepatic GNG flux to G6P (NHGNG flux; see *Figure 1*) by either increasing hepatic F2,6P_2 or decreasing lipolysis, both of which stimulate glycolysis. Sindelar et al.[15,16] brought about a small (~two-fold) selective rise in either systemic (with no alteration in insulin at the liver and, presumably, no alteration in F2,6P_2) or hepatic (with no alteration in peripheral insulin and, presumably, an increase in F2,6P_2) insulin in the dog. Hyperinsulinemia at the liver rapidly inhibited glycogenolysis, but did not alter NHGNG flux (the net of glycolytic flux and GNG flux to G6P). Peripheral hyperinsulinemia, on the other hand, brought about a decrease in HGP that was attributed to reduced NHGNG flux. Interestingly, the time-course of this inhibition correlated with the switch of the liver from an organ of net lactate uptake to an organ of lactate output, an increase in glycolysis, and the inhibition of lipolysis. This observation led to the hypothesis that the fall in FFA was responsible for the decrease in NHGNG flux. In support of this concept, peripheral hyperinsulinemia did not bring about a decrease in NHGNG flux or an alteration in net lactate balance when the fall in circulating FFA was prevented with intravenous intralipid infusion.[16] Thus, insulin-mediated suppression of NHGNG flux was due to the indirect effect of insulin on lipolysis and was not likely related to changes in hepatic F2,6P_2, although F2,6P_2 was not assayed in these studies.

It must be noted that these experiments[15,16] were performed in a setting in which the normal relationship between arterial and portal vein insulin was disrupted. It has recently been confirmed that an eight-fold increase in portally administered insulin (which establishes a physiological gradient of hyperinsulinemia between the periphery and at the liver) can cause a rapid increase in F2,6P_2 in concert with a profound inhibition of fat oxidation by the liver.[28] Although these changes increased glycolysis, GNG flux to G6P was unaltered. During physiological hyperinsulinemia associated with refeeding in the rat, the F2,6P_2 concentration was rapidly increased, yet GNG flux to G6P was not altered.[55] Conversely, insulin-mediated increases in F2,6P_2 stimulated glycolysis in mice[60] and rats.[61,62] Thus, in agreement with findings, it appears that glycolysis is far more sensitive to insulin (and F2,6P_2) *in vivo* than is GNG flux to G6P. While increased F2,6P_2 and decreased fat oxidation were observed in response to four-, eight-, and 16-fold hyperinsulinemia in the dog, only the 16-fold rise in insulin (which caused a ~three-fold higher elevation of F2,6P_2 than observed in response to four- or eight-fold insulin) caused a decrease in GNG flux to G6P.[28,54] Since fat oxidation was similarly suppressed in response to four-, eight-, and 16-fold hyperinsulinemia, it is possible that 16-fold hyperinsulinemia may have elevated F2,6P_2 to a level sufficient to inhibit the gluconeogenic enzyme FBP-1 (see *Figure 3*), whereas four- and eight-fold hyperinsulinemia did not.

Recent data from the authors' laboratory have shown that the molecular inhibition of gluconeogenic gene expression is conserved and intact in large animals, but these signaling events do not correlate to the acute regulation of GNG flux to G6P *in vivo*.[28,54] Furthermore, the time-course of insulin-mediated events suggests that the bulk of inhibition of PEPCK and G6Pase mRNA expression is a result of insulin's direct effects at the liver (mediated through FOXO1). It also suggests that the inhibiton occurs hours prior to regulation through the insulin–brain–liver axis (through STAT3).[28] In response to eight-fold hyperinsulinemia, GNG flux to G6P is not altered after four hours despite a ~50% reduction in PEPCK protein.[28] In response to 16-fold hyperinsulinemia, GNG flux to G6P was suppressed by 30 minutes, but this reduction was too rapid to be explained by a change in PEPCK protein.[54] Furthermore, GNG flux to G6P

Figure 4: Nuclear Magnetic Resonance Analysis of Flux Through the Gluconeogenic Pathway in Perfused Livers Isolated from Transgenic Mice with Varying Amounts of PEPCK Protein Content



Only in livers with 100% PEPCK deletion is flux through the pathway markedly inhibited. Data originally presented by Burgess et al., 2007.[71]

was maintained at this lower rate despite an eventual 60% reduction in PEPCK protein after five hours.[54] Thus, PEPCK appears to have poor control strength over the gluconeogenic pathway during the acute response to insulin.

In several recent rodent studies, the inhibition of gluconeogenesis and suppression of gluconeogenic gene expression (via a pathway that required the vagus nerves and hepatic STAT3) was observed in response to hyperinsulinemia in the brain.[7–9] Data in dogs, however, have indicated that hepatic denervation does not blunt insulin's ability to inhibit HGP,[63] which is similar to what has been observed in humans with liver transplants.[64] Furthermore, insulin introduced into the third ventricle of the brain in dogs during a basal pancreatic clamp, in the same manner and at the same rate as was used in rodents, could not bring about any effect on HGP.[12] Thus, the question arises as to whether the insulin–brain–liver axis that acutely regulates GNG flux to G6P in rodents is conserved in large animals. It is possible that the sensitivity and mechanism of insulin-mediated inhibition of HGP may be species-dependent. Rodents have five to 10 times the basal HGP rates of large animals and they exhaust liver glycogen stores after a relatively short fast. Canines and humans, on the other hand, maintain a significant amount of liver glycogen even after several days of fasting.[65,66] It is therefore conceivable that the drive to maintain GNG flux to G6P during hyperinsulinemia differs between species.

Alternatively, the insulin–brain regulation of GNG flux to G6P in the cited rodent studies may have been due to the experimental design employed.[7–9] Hyperinsulinemia was brought about via insulin infusion in a peripheral vein, resulting in elevated insulin at the brain (and periphery) but basal insulin levels at the liver. At same time, glucagon was not replaced during the clamp protocol, thus depriving the liver of one of its primary physiological signals. Whether hypothalamic hyperinsulinemia can regulate HGP in a physiological circumstance (in the presence of hepatic hyperinsulinemia and physiological levels of glucagon) has not been demonstrated. Even if the insulin–brain–liver signaling axis does exist in large animals and humans, the relevance of this pathway in regulating HGP is debatable. In a study by Edgerton et al.,[14] dogs were subjected to a pancreatic clamp with portal replacement of basal levels of insulin and glucagon and maintenance of euglycemia. Insulin infusion was then switched to a peripheral vessel (resulting in systemic hyperinsulinemia, but also hepatic hypoinsulinemia). This resulted in the elevation of HGP (via increased glycogenolysis) and marked hyperglycemia. Thus, hyperinsulinemia at the brain (and all tissues supplied with arterial blood) could not appropriately inhibit HGP when insulin levels at the liver were deficient. This clearly illustrates that the liver's direct effect is dominant in reducing HGP.

Re-evaluating the Dogma—Does PEPCK Control GNG Flux to G6P?

The notion that PEPCK is the chief rate-limiting enzyme in the gluconeogenic pathway is based largely on several early *in vitro* and *in vivo* studies in rats. The compound 3-mercapopicolinate (3-MP) inhibited glucose formation from lactate, pyruvate, and alanine in perfused liver slices.[67] Oral or intravenous administration of 3-MP caused hypoglycemia *in vivo* in several model systems, including rats, mice, and guinea pigs.[67–69] Cross-over plot analysis of liver metabolite concentrations suggested PEPCK was the target of inhibition, which was supported by the observation that hepatic oxaloacetate levels increased during 3-MP treatment.[68,69] These studies were typically performed after a long fasting period (a condition in which liver glycogen levels were depleted and glycogenolysis was assumed to be zero). This led to the conclusion that 3-MP inhibited gluconeogenesis by pharmacological blockade of PEPCK.

Other evidence casts doubt on the degree of control that PEPCK exerts on GNG flux to G6P. Metabolic control analysis of the pathway using rat hepatocytes determined that PEPCK has poor control strength on the gluconeogenic process under various conditions *in vitro*.[70] A recent study evaluated the gluconeogenic pathway in perfused livers (isolated from transgenic mice) with different amounts of PEPCK protein content (see *Figure 4*). Only in the almost complete absence of PEPCK protein was the gluconeogenic process markedly inhibited.[71] This is in line with the results observed with 3-MP, which presumably resulted in a virtually complete blockade of PEPCK at the enzymatic level. These observations[71] are also in agreement with the physiological regulation of PEPCK protein by insulin. Here even marked, sustained hyperinsulinemia (16-fold for five hours) was only able to reduce the amount of protein by ~60% and thus was unable to alter GNG flux to G6P.[54] Thus, acute physiological hyperinsulinemia does not suppress PEPCK protein expression to a large enough extent to alter GNG flux to G6P *in vivo*. This supports the view that the pathway is active and important in glycogen formation during the post-prandial (hyperinsulinemic) state.[56–59] The only evidence that a physiological elevation in insulin can suppress the GNG flux to G6P comes from rodent studies[7–9] in which the effect was observed in decidedly non-physiological circumstances.

Elevated HGP in the diabetic state is associated with increased gluconeogenesis and also typically increased levels of PEPCK mRNA expression in animal models. This supports the belief that this enzyme has a rate-limiting influence on GNG flux to G6P in a chronic, insulin-resistant state.[72] A recent study, however, demonstrated that in rat

models of diabetes, elevated HGP (associated with increased gluconeogenesis) was observed without elevation in PEPCK or G6Pase mRNA expression.[73] Furthermore, analysis of liver biopsies from human patients with type 2 diabetes had the same level of gluconeogenic mRNA expression as non-diabetic controls.[73] This counters the notion that gluconeogenic enzyme mRNA levels are appropriate markers for the gluconeogenic pathway, even in a chronic disease state.

Conclusions

The regulation of gluconeogenesis by insulin in whole animals is complex, so it is no surprise that there has been controversy regarding the sensitivity and mechanism of insulin's effects on the gluconeogenic pathway *in vivo*. Recent evidence in rodents suggesting that the insulin–brain–liver axis can modify GNG flux to G6P (via transcriptional regulation of supposed rate-limiting enzymes) can be questioned based on the experimental design used. In any case, insulin action in the brain is likely to be of limited importance to the physiological regulation of HGP, given that the direct effect of insulin is clearly dominant and occurs within a few minutes.[14]

Even though intricate insulin-mediated mechanisms are involved in the suppression of PEPCK mRNA expression, eventual decreases in PEPCK protein do not modify GNG flux to G6P in response to acute physiological hyperinsulinemia. This indicates that this enzyme has poor control strength over the pathway *in vivo*. Physiological increases in insulin modify HGP *in vivo* by inhibiting glycogenolysis and promoting glycogen deposition, transiently increasing glycolysis by mediating changes in lipolysis and possibly $F2,6P_2$, and redirecting gluconeogenically derived carbon to glycogen. The rate of GNG flux to G6P does not appear sensitive to a physiological hyperinsulinemia. ■

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