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PATHOGENESIS OF TYPE 2 DIABETES AND INSULIN RESISTANCE (M-E PATTI, SECTION EDITOR)

Regulation of Glucose Production in the Pathogenesis of Type 2 Diabetes

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Abstract

Purpose of Review Increased glucose production associated with hepatic insulin resistance contributes to the development of hyperglycemia in T2D. The molecular mechanisms accounting for increased glucose production remain controversial. Our aims were to review recent literature concerning molecular mechanisms regulating glucose production and to discuss these mechanisms in the context of physiological experiments and observations in humans and large animal models.

Recent Findings Genetic intervention studies in rodents demonstrate that insulin can control hepatic glucose production through both direct effects on the liver, and through indirect effects to inhibit adipose tissue lipolysis and limit gluconeogenic substrate delivery. However, recent experiments in canine models indicate that the direct effects of insulin on the liver are dominant over the indirect effects to regulate glucose production. Recent molecular studies have also identified insulin-independent mechanisms by which hepatocytes sense intrahepatic carbohydrate levels to regulate carbohydrate disposal.

Summary Dysregulation of hepatic carbohydrate sensing systems may participate in increased glucose production in the development of diabetes.

Keywords Glucose production · Diabetes · Gluconeogenesis · Glycogenolysis · Glucose 6 phosphate · ChREBP

Introduction

Glucose is the major circulating sugar in mammals. It plays an essential anabolic role and is a primary energetic substrate. In many physiological circumstances, certain tissues such as the brain, red blood cells, and kidney medulla rely predominately on glucose to meet their energetic needs [1]. However, excess circulating glucose is toxic and can damage cells and organ systems. Hyperglycemia, which defines T2D, causes microvascular disease including retinopathy, nephropathy, and ophthalmopathy [2]. This hyperglycemia is also associated with macrovascular disease including markedly increased risk of coronary artery disease and peripheral vascular disease [3]. On the other hand, hypoglycemia also poses a severe health risk. Hypoglycemia acutely impairs neuronal function and can rapidly lead to coma and death. Mammals have evolved complex, dynamic regulatory control systems to maintain circulating glucose within a narrow range.

The metabolic regulatory systems that control blood glucose within a tight range and prevent toxicity of hyper- or hypoglycemia also function to efficiently store excess glucose following consumption of digestible carbohydrate. This storage can be in the form of glycogen, a multi-branched polysaccharide of glucose that can be rapidly catabolized to make glucose when needed. Alternatively, excess carbohydrate can be converted to fatty acids through de novo lipogenesis (DNL); these fatty acids are esterified and stored as triglyceride in adipose tissue. However, conversion of glucose to fat is unidirectional as acetyl-CoA derived from fatty acid oxidation cannot be converted back to glucose. Following absorption and disposal of a meal's carbohydrates, the organism rapidly and smoothly transitions from net glucose disposal to net glucose production to maintain euglycemia. In this review, we will survey major physiological and molecular mechanisms

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that participate in the regulation of glucose production and how these may go awry in the development of diabetes. We will highlight gaps in our knowledge and ongoing controversies concerning aberrant glucose production in association with insulin resistance and the progression to diabetes.

Glucose production is achieved via glycogenolysis, breaking down glycogen to its constituent glucose monomers, or by gluconeogenesis, the synthesis of new glucose molecules from other carbohydrate precursor molecules. The relative contribution of glycogenolysis versus gluconeogenesis to glucose production depends in part on the duration of fasting and abundance of glycogen stores. The liver plays a central role in these processes and accounts for about 80 to 90% of endogenous glucose production in the post-absorptive state [4]. The liver is the only organ that stores abundant amounts of glycogen that can also directly release hydrolyzed glycogen as glucose into circulation. Skeletal muscle can also store significant amounts of glycogen, which can be mobilized via glycolytic metabolism to lactate. Lactate released from muscle can circulate to the liver where it can be converted to glucose through gluconeogenesis.

In humans, the liver extracts as much as 30% of an oral glucose load and stores 19% of ingested carbohydrate as hepatic glycogen after a mixed meal [5, 6]. In the early postprandial period, glycogen breakdown accounts for approximately 50% of hepatic glucose output, and an overnight fast decreases liver glycogen by about 20 to 40% in healthy individuals [6, 7]. In humans, during a prolonged fast, the contribution of glycogen breakdown to hepatic glucose output steadily declines from 50% after 14 h fasting to about 5% at the 42-h mark [8, 9]. Whereas glycogenolysis is the major contributor to glucose production in healthy individuals in the early postprandial period, the contribution of gluconeogenesis to glucose production increases to 95% with prolonged fasting. Although the contribution of gluconeogenesis to glucose production increases over the duration of a fast, the net hepatic glucose output decreases, i.e., gluconeogenesis does not fully substitute for the decreased glycogenolysis [10]. Thus, with prolonged fasting, when gluconeogenesis predominates, net glucose production may be limited by substrate availability [11].

Fasting also induces hydrolysis of triglyceride in adipose tissue, which supplies glycerol and non-esterified fatty acids (NEFAs). Glycerol released from hydrolyzed triglyceride in adipose tissue or elsewhere is readily used in the liver as gluconeogenic substrate [12]. NEFAs can be oxidized directly in many tissues for energetic purposes. In the liver, energy and reducing equivalents generated by NEFA oxidation can support gluconeogenesis [13]. Hepatic NEFAs can also be converted into ketones which circulate as a preferred fuel for neurons and other specific tissues during prolonged fasts [1]. To summarize, in normal physiology, with consumption of an oral carbohydrate load, glucose production is suppressed and lipogenesis is stimulated. Conversely, in the fasted condition, glucose production increases whereas lipogenesis declines in favor of lipolysis and fatty acid oxidation.

Since Banting and Best's discovery that deficiency of insulin causes diabetes mellitus, the pathogenesis of diabetes is commonly framed in terms of this hormone's actions to regulate glycemia and fuel homeostasis [14]. Insulin, by binding and activating the ubiquitously expressed insulin receptor, has pleiotropic effects on glycemia, systemic fuel metabolism, and other anabolic cellular processes [15]. Insulin potently stimulates glucose uptake in skeletal muscle and adipose tissue while inhibiting adipose tissue lipolysis and stimulating adipose lipogenesis and fat deposition [16]. In the liver, insulin suppresses glucose production and stimulates lipogenesis. Genetic experiments in mice prove that hepatic insulin action is essential for the regulation of glucose production and lipogenesis [17]. Selective knockout of the insulin receptor in the liver produces hyperglycemia and aberrantly increases glucose production and reduces hepatic lipogenesis [17]. Absent the hepatic insulin receptor, hepatic lipogenesis cannot be fully reactivated with carbohydrate feeding [18]. In the obese, hyperinsulinemic condition that commonly precedes development of overt hyperglycemia and T2D, lipogenesis is increased consistent with increased insulin signaling in the liver [19, 20]. However, glucose production is increased or inappropriately "normal" for the degree of hyperinsulinemia [21]. With the progression to T2D, lipogenesis persists with overt increases in glucose production contributing to hyperglycemia [22, 23]. The failure to appropriately suppress glucose production while lipogenesis is activated is termed "the pathogenic paradox of hepatic insulin resistance" [24]. Defining the mechanisms that give rise to this paradox and contribute to increased glucose production in the setting of hyperinsulinemia will be essential to understand the pathogenesis of T2D.

Methodological Concerns in Studying Glucose Production

Physiological and molecular mechanisms underlying the effects of insulin to regulate glucose production have been studied extensively across a range of experimental paradigms in animals and humans. These studies often implement the euglycemic-hyperinsulinemic clamp or related procedures with the use of labeled glucose infusions to distinguish the effects of insulin or other hormones on glucose disposal versus glucose production. This procedure has been the de facto gold standard for measurement of glucose production in vivo for 40 years [25]. Yet, the key molecular mechanisms that lead to variation in glucose production among healthy individuals across different physiological conditions remain uncertain. Likewise, the specific molecular derangements that lead to

aberrant glucose production in the setting of overnutrition, obesity, or T2D remain unclear.

Many factors contribute to these persistent uncertainties. One major factor is that the accessibility of tissue, particularly liver tissue, is restricted in both healthy and metabolically compromised humans. Additionally, the conditions in which human liver biopsy samples can be obtained are limited. For safety reasons, percutaneous and surgical liver biopsies are typically performed only after an overnight fast. Thus, the molecular correlates of aberrant glucose production in humans have essentially only ever been examined in fasted tissue samples. As impaired suppression of glucose production during the prandial phase may be a major contributor to prandial hyperglycemia and prandial glycemia may be a better predictor of progression to T2D and adverse outcomes compared to fasting glucose, studying exclusively fasted liver is a significant limitation [26, 27]. Moreover, recent evidence (discussed below) suggests that key regulatory events that may determine glucose production or hepatic insulin sensitivity as measured in the fasted state may be established in the fed state. Thus, studying fasted human liver tissue exclusively may present more of a limitation to understanding the molecular pathophysiology of glucose production than is widely recognized. The importance of studying molecular events in both fed and fasted conditions necessitates animal experiments.

However, even in animal studies where experimental conditions can be better controlled and tissues are more accessible, livers are most frequently harvested at the end of tracer infusions and/or euglycemic-clamp procedures which are performed after fasting. Such fasts are essential to ensure that the intestines clear digestible carbohydrates which would confound measurements of glucose turnover. Aside from the issue that studies largely focused on fasted conditions may miss the dynamic regulatory events that are established during feeding to regulate glucose production, fasts of similar duration are likely to have profoundly different metabolic effects across the different species commonly used to study this physiology. Animals ranging from dogs to rats to mice may share common metabolic regulatory themes, but have evolved specialized metabolic adaptations related to their distinct ecological niches [28]. There is wide variation in metabolic and basal glucose turnover rates in comparison to glycogen stores across these species [9, 29]. The basal metabolic rate and glucose turnover rate normalized to body weight is an order of magnitude greater in mice then in humans. Thus, glycogen depletion and the transition to a fatty acid-dependent fuel economy occur much more quickly in mice than humans. These differences limit extrapolation of conclusions drawn from experiments performed in small animals to larger animals and humans, particularly after fasting. Despite these concerns, rodents remain favored models to define molecular mechanisms related to glucose homeostasis due to their tractability for genetic intervention studies.

The molecular and physiological state after sustained, hours-long peripheral insulin and glucose infusions accompanying euglycemic-hyperinsulinemic clamp procedures may reverse or prevent progression into fasting-like physiology in part by preventing lipolysis. However, this clamped state is far from a normal physiological state. It is also quite different from a state induced by normal dynamics of transient hyperinsulinemia and hyperglycemia that occurs after eating a discrete meal, which is usually of mixed macronutrient content. Moreover, high-dose peripheral insulin and glucose infusions commonly used in these procedures do not mimic the portal hyperinsulinemia and hyperglycemia that occurs with feeding where glucose enters the circulation from the intestines via the portal vein and insulin is secreted directly into the hepatic portal circulation. Thus, with feeding, liver insulin and glucose concentrations are significantly higher than arterial levels. This is not commonly replicated in most clamp procedures. Some sophisticated laboratories can perform portal insulin infusions in live, conscious animals, often dogs, and these studies may be essential for clarifying key physiological and mechanistic aspects of the regulation of glucose production [30•]. With these caveats in mind, we will discuss hormonal and non-hormonal mechanisms regulating glucose production in physiological and pathophysiological conditions with an emphasis on lessons from genetic mouse models. We will consider the implications of these lessons and additional questions that relate to mechanisms that may mediate aberrantly increased glucose production in human insulin resistance and T2D.

Insulin-Mediated Regulation of Hepatic Glucose Production

Insulin regulates endogenous glucose production through multiple molecular mechanisms that operate through divergent signaling pathways on distinct time scales. Upon binding to its receptor, insulin elicits phosphorylation and activation of insulin receptor substrates that lead to activation of PI3-kinase and accumulation of phosphatidylinositol-3,4,5-triphosphate [31]. 3-Phosphoinositide-dependent protein kinase-1 (PDK1) phosphorylates AKT, a critical mediator of insulin signaling and regulator of glucose production [32].

A key downstream substrate of insulin action is FOXO1, an insulin-responsive transcription factor that localizes to the nucleus during fasting where it drives the expression of gluconeogenic enzymes including G6PC and PCK1 (also known as PEPCK) [33, 34]. Hepatic deletion of FOXO1 alone or in combination with FOXO3 and FOXO4 leads to hypoglycemia [35, 36]. Activated AKT phosphorylates FOXOs resulting in their translocation to the cytosol and degradation [37, 38]. Consistent with this, deletion of AKT2 with or without hepatic AKT1 results in hyperglycemia attributed to

constitutive FOXO1 activity [39]. Unrestrained activity of FOXO1 is also observed in liver-specific insulin receptor knockout mice [17], and glycemia can be normalized by liver-specific deletion of FOXO1 [40]. These genetic experiments conclusively demonstrate the ability of insulin through regulation of hepatic AKT and FOXOs to regulate gluconeogenic enzyme expression and glucose production. However, the importance of these observations to dysregulated glucose production in association with obesity and T2D is less certain.

One source of uncertainty arises from observations that in liver-specific insulin receptor and FOXO1 double knockout animals, insulin is still able to suppress glucose production [40•]. Similarly, in mice lacking AKT and FOXO1, glucose production can be suppressed by feeding and insulin [41]. Restoration of hepatic insulin signaling in whole-body insulin receptor knockout mice fails to fully suppress glucose production [42]. Altogether, these studies indicate that extrahepatic insulin signaling can be important for regulation of endogenous glucose production in mice in some contexts.

Regulation of delivery of gluconeogenic substrate to the liver is one mechanism by which extrahepatic insulin action might regulate glucose production. Insulin-mediated suppression of adipose tissue lipolysis may limit delivery of NEFAs and glycerol to the liver. In individuals with diabetes and healthy controls, circulating NEFAs correlate with increased gluconeogenesis [13, 43]. Preventing the fall in circulating glycerol and NEFAs after insulin administration substantially blunts insulin-induced downregulation of HGP in a canine model [44, 45]. Perry et al. have suggested that increased hepatic FA oxidation associated with adipose insulin resistance enhances hepatic acetyl-CoA accumulation, which allosterically activates pyruvate carboxylase to enhance gluconeogenic flux [46•]. Acetyl-CoA can also activate pyruvate dehydrogenase kinase, which inhibits pyruvate dehydrogenase activity [20]. This would spare pyruvate from oxidation and preserve its use as gluconeogenic substrate. However, insulin is still capable of suppressing glucose production when circulating NEFAs are present in excess [30•]. Thus, the direct effects of insulin to suppress glucose production at the liver dominate over substrate delivery from the periphery. Other indirect effects of insulin to regulate glucose production may include insulin's actions in the hypothalamus as demonstrated in a variety of rodent models [47–52]. However, the inability of insulin infused into carotid arteries to regulate glucose production in a canine model indicates this is not a major mode of insulin action on glucose production in larger animals [53, 54].

Most experiments assessing glucose production in genetic mouse models have been performed in fasted conditions where glycogen is largely depleted [29]. In the glycogen-depleted state, glucose production may be dependent on gluconeogenesis and the availability of gluconeogenic substrate. This may enhance the apparent effect of extrahepatic insulin to regulate glucose production by limiting delivery of substrate from adipose tissue or other stores. It is less clear how important these extrahepatic effects of insulin are in larger animals or humans, particularly in overnourished subjects where there will be abundant glycogen and potential gluconeogenic substrate after an overnight fast [9, 55]. Edgerton et al. have recently shown that in dogs, the direct effects on insulin on the liver are sufficient to fully mediate suppression of glucose production in response to physiological portal insulin levels independently of indirect effects of insulin [30•].

Increased gluconeogenesis, in part through increased FOXO1 activity, might contribute to the pathogenesis of increased basal glucose production in individuals with T2D and increased fractional gluconeogenesis in both pre-diabetes and diabetes [56, 57]. However, the acute and potent effect of insulin to suppress glucose production is primarily mediated through inhibition of glycogenolysis with little effect on gluconeogenesis which is inconsistent with FOXOs mode of action [58]. Thus, understanding the mechanisms which prevent insulin's ability to suppress glycogenolysis may be of primary importance in understanding the pathogenesis of hepatic insulin resistance as detected in euglycemic-hyperinsulinemic clamp procedures.

These considerations have led to studies aimed at identifying defects in insulin action or insulin signaling that might increase glycogenolysis leading to hepatic insulin resistance. Glycogenolysis reflects the net flux of potential glucose through glycogen phosphorylase (PYGL, also known as PG) which catabolizes glycogen to glucose monomers, and is opposed by flux through glycogen synthase (GYS2, also known as GS) which uses UDP glucose to add glucose moieties to the growing glycogen polymer [59, 60]. In addition to the action of AKT to inhibit FOXO1, activated AKT phosphorylates and inactivates glycogen synthase kinase 3 (GSK3), which inhibits the activation of GS. However, mice carrying mutations in GSK3 that prevent its phosphorylation still respond to insulin with increased glycogen synthesis indicating that the AKT-GSK3 signaling pathway is dispensable for insulin-mediated regulation of glycogenolysis [61]. Insulin also triggers the deactivation of GS and activation of GP by recruiting protein phosphorylase 1 (PPP1CA also known as PP1) to the glycogen regulatory complex [62]. PP1 dephosphorylates GS and GP, activating the former and inactivating the later [63]. PP1 is directed to glycogen by interactions with PP1 regulatory subunits PPP1R3A and PPP1R3B [64, 65]. PPP1R3A and PPP1R3B mRNA and protein levels decrease during fasting, suggesting longer-term regulation of glycogenolysis at the level of transcription [66]. This coordinated signaling through GSK3, PP1, and other regulators of GS and GP shifts the liver towards glycogen synthesis and away from glycogenolysis and glucose production.

Because of the strong association between hepatic steatosis and hepatic insulin resistance, numerous investigators have

sought the identity of lipid mediators which may contribute to the development of insulin resistance. As an example, intrahepatic accumulation of diacylglycerols (DAGs) activates protein kinase Cε (PKCε) which can bind and inhibit the insulin receptor [67]. Knockdown of protein kinase Cε protected mice from high-fat feeding-induced insulin resistance [67, 68]. However, selective deletion of PKCε in mouse liver did not enhance hepatic insulin signaling [69]. Whether steatosis is a causal contributor to hepatic insulin resistance or a biomarker associated with hepatic insulin resistance remains a controversial topic [70].

Non-hormonal Mediated Regulation of Hepatic Glucose Production

Whether direct or indirect, pancreatic hormones play a central role in the regulation of glucose production and systemic fuel homeostasis. However, there are mechanisms that modulate glucose production independently of hormones. One important mode of non-hormonal regulation is termed hepatic "autoregulation." This phenomenon initially referred to the regulation of gluconeogenesis in isolated, perfused livers dependent on the concentration of glucose in the perfusate and independent of hormone levels [71]. With pancreatic hormones clamped at basal levels, infusion of gluconeogenic substrates like lactate, alanine, or glycerol in dogs and humans can markedly increase gluconeogenesis from these substrates without affecting total glucose production, indicating a corresponding reduction in glycogenolysis [72–76]. Additionally, glucose infusion alone is capable of significantly decreasing hepatic glucose production independently of hormonal regulation [77, 78]. These observations suggest rapid, substrate-mediated regulation of glucose production that occurs independently of hormonal and in some experiments neural input. However, the mechanisms that mediate autoregulation can be compromised. For instance, the ability of glucose to suppress glucose production in normal rats is lost in insulin-deficient, diabetic rats [79]. Similarly, the ability of hypoglycemia per se to stimulate glucose production is attenuated in insulin-deficient, type 1 diabetic (T1D) patients [80]. Results evaluating whether autoregulation may or may not be impaired in diabetic patients have been inconsistent [43, 81].

Ultimately, the rate of glucose production is dependent on interactions between the concentration of glucose-6-phosphate (G6P), the immediate substrate for glucose production, its availability to G6PC, the enzyme that catalyzes its transformation to glucose, and the activity of this enzyme. Intracellular G6P levels are determined by multiple competing and collaborating fluxes producing or utilizing cellular G6P. G6P is produced by glucokinase-mediated phosphorylation of glucose, hydrolysis of glycogen, or synthesis via gluconeogenesis. G6P can be consumed via glucose production or glycogen synthesis. It can be catabolized via glycolysis and used for either energetic or anabolic purposes via anaplerotic or oxidative mitochondrial pathways.

Emerging evidence indicates that hepatic intracellular G6P levels are homeostatically controlled [82•, 83–85]. As an example, dosing mice with large amounts of fructose or glycerol, both of which are rapidly phosphorylated by hepatocytes and transformed into G6P, does not appreciably increase hepatic G6P levels [82•]. Like most complex biological systems, homeostatic control of G6P is likely regulated at multiple levels (Fig. 1). One major and well-described mode of regulation is that G6P allosterically and potently stimulates GS and inhibits GP [86•, 87]. Indeed, the allosteric effects of G6P on GS and GP are dominant over the effects of insulin to regulate glycogen synthesis and glycogenolysis [86•]. Thus, increasing cellular G6P will promote its own reduction by increasing net glycogen synthesis.

We and others have begun to elucidate additional molecular mechanisms by which hepatocytes and other "glucose-sensing cells" mediate intracellular G6P homeostasis via regulatory effects of the carbohydrate sensing transcription factor—carbohydrate responsive-element binding protein (ChREBP, also known as Mlxipl) [82•, 84]. The balance of studies indicates that ChREBP transcriptional activity is allosterically activated by G6P, although other carbohydrate metabolites may also participate [82•, 88, 89]. Activated ChREBP stimulates expression of glycolytic and lipogenic enzymes that might contribute to catabolism of increased G6P. While the function of ChREBP in regulating glycolysis and lipogenesis is well-established [84], it also potently upregulates selected enzymes involved in glucose production including G6PC and the endoplasmic reticulum G6P transporter (Slc37a4) [82•]. Hepatic G6PC activity is markedly reduced in ChREBP KO mice [82•]. Thus, activation of ChREBP may enhance utilization of G6P by enhancing both glycolysis and glucose production simultaneously [82•, 90]. G6P levels are elevated in livers from ChREBP KO mice, and this is accompanied by markedly increased glycogen levels—presumably due to the allosteric effects of increased G6P to impair net glycogenolysis. Activation of ChREBP is able to increase G6PC expression even in FOXO1 knockout mice indicating that upregulation of G6PC through activation of ChREBP is dominant over the ability of insulin to suppress it [82•].

Fructose is a preferred hepatic metabolic substrate and acutely stimulates hepatic glycogen synthesis [91–93]. High doses of fructose can be used to rapidly simulate the effects of hepatic carbohydrate overload. Fructose also acutely and potently activates hepatic ChREBP [82•]. Whereas fructose gavage does not produce detectable increases in G6P in normal mice, in ChREBP KO mice, it profoundly increases G6P levels demonstrating that ChREBP plays an essential role in G6P homeostatic feedback control. To assess the role of ChREBP in glucose production, we performed glucose turnover studies in wild-type and ChREBP KO mice of varying

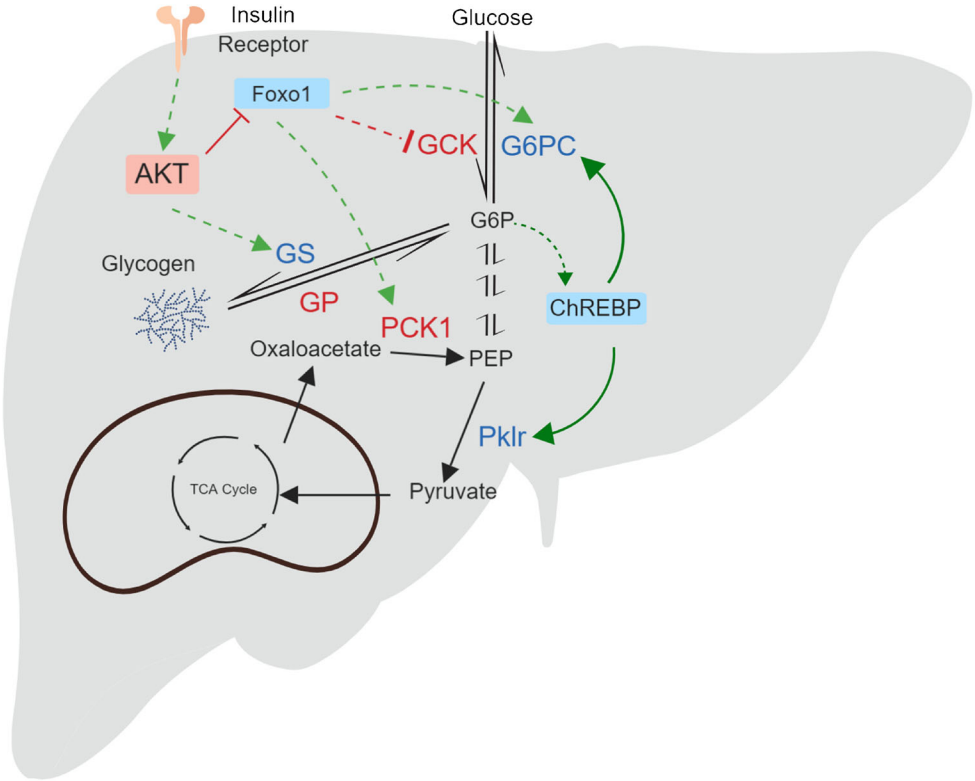

Fig. 1 Insulin binds the insulin receptor and activates AKT, which enhances glycogen synthesis by activating glycogen synthase (GS) and repressing glycogen phosphorylase (GP). Activated AKT also inhibits the transcription factor FOXO1 leading to decreased expression of gluconeogenic enzymes including glucose-6-phosphatase (G6PC) and phosphoenolpyruvate carboxykinase (PCK1) and increased expression of glucokinase (GCK) which enhances hepatic glucose uptake. The transcription factor ChREBP senses cellular glucose-6-phosphate (G6P) levels and mediates cellular G6P homeostasis by enhancing expression of enzymes that dispose of G6P including G6PC and liver pyruvate kinase (Pklr). Enzymes labeled in *red* increase G6P production, whereas enzymes labeled in blue contribute to G6P disposal

ages subjected to chow or high-fructose feeding for 2 weeks. Glucose turnover was measured after 4-h fasting, when ample glycogen stores remain. Fructose feeding increased glucose production in wild-type mice and this strongly correlated with the activity of G6PC measured in liver samples from these mice. Although fructose gavage acutely increases ChREBP activity in control mice implying increased G6P levels, in the mice exposed to 2 weeks of high-fructose diet, hepatic G6P levels decline quickly following food removal [82•]. In fact, they become significantly lower than that in chow fed control mice [82•]. As glucose production is increased concordant with increased G6PC activity, but in the setting of reduced G6P levels, these results demonstrate that increased G6PC activity can dominate over the availability of its immediate substrate, G6P, to drive glucose production. The marked reductions in G6P levels that occur following food removal may facilitate increased glycogenolysis allowing for ongoing substrate delivery to support increased glucose production in the short term. Thus, chronic hepatic carbohydrate overload resulting from fructose feeding or other form of overnutrition may induce modifications that can reset the hepatic autoregulatory mechanisms to support increased rates of glucose production. These results are consistent with short term, fructose feeding studies in humans which increase both glucose production and glycogenolysis [94•].

The effects of ChREBP on hepatic autoregulation and hepatic glucose production may extend beyond the setting of diets high in fructose. Expression of the potent ChREBP-beta isoform, a surrogate marker of tissue ChREBP activity, is increased in patients with obesity and insulin resistance, suggesting that activated hepatic ChREBP may contribute to hepatic insulin resistance in human subjects [95, 96]. Consistent with this, knockdown of ChREBP in livers of genetically obese mice on chow diet downregulates G6PC and normalizes glycemia [97]. These observations are also consistent with human studies where carbohydrate overfeeding in healthy volunteers can increase glucose production via increased glycogenolysis suggesting a modified autoregulatory set point [98, 99].

Our studies demonstrated insulin-independent regulation of G6PC as a mechanism that modifies basal glucose production. We have not yet directly tested whether this mechanism contributes to the inability of insulin to suppress glucose production as detected by clamp procedures in "insulin-resistant" states. Our results do not exclude the possibility that defects in hepatic insulin signaling also contribute to aberrant glucose production in the setting of generalized overnutrition or progression to T2D.

However, our results do provide an alternative perspective on this issue. As noted above, insulin's acute effects to reduce glucose production are predominately mediated through insulin's ability to reduce glycogenolysis. The reduction in glucose production following acute inhibition of glycogenolysis is thought to result from reducing the availability of G6P as substrate for G6PC-mediated glucose production. However, the effects of low G6P levels to stimulate glycogenolysis are dominant over insulin's ability to suppress it [86•]. Thus, if G6PC activity is high, and this drives glucose production in the face of low hepatic G6P levels, hyperinsulinemia may be unable to suppress glycogenolysis and glucose production because G6P is already low. It will be important to measure hepatic G6P levels with and without insulin infusion to determine whether this mechanism may contribute to "hepatic insulin resistance" as routinely detected by the euglycemic-hyperinsulinemic clamp procedure in the setting of obesity and T2D.

Is it possible that increased hepatic G6PC activity whether induced by ChREBP or other factors is a major determinant of glucose production and insulin resistance in obese humans or those with T2D? The mRNA expression levels of hepatic gluconeogenic enzymes have been measured in well-phenotyped humans in only a few studies [100, 101]. In general, G6PC mRNA levels or those of other gluconeogenic enzymes do not correlate with hepatic glucose production or other indices of insulin resistance. However, as noted above, all of these biopsy samples are obtained in fasted subjects. This is important because G6PC mRNA has the highest turnover rate of any mRNA species measured in the liver [102•]. Indeed, we have observed that in fructose-fed mice, after 4-h food removal, when G6PC activity is highly elevated and glucose production is increased, G6PC mRNA levels are as low as or lower than chow fed-controls (unpublished data). The acute reduction in G6PC mRNA, which is discordant with the G6PC activity, is consistent with acute, dynamic reductions in ChREBP activity induced by profound decreases in hepatic G6P levels following food removal. This rapid downregulation of ChREBP and its transcriptional targets including G6PC would ultimately serve to restore G6P levels to close the homeostatic feedback loop. These results suggest that it will be important to assess molecular parameters in the fed state, which may set the metabolic tone for glucose production after food removal. Moreover, it will be critically important to measure key enzymatic activities in conjunction with metabolite levels rather than mRNA in human liver samples to define mechanisms regulating glucose production in the pathogenesis of T2D.

Conclusions

The molecular mechanisms accounting for increased glucose production in the development of diabetes remain poorly understood. This may be, in part, due to technical limitations in obtaining relevant tissue specimens from human subjects, and also due to differences in physiology that limit extrapolation from small animal models to humans. Nevertheless, recent, elegant genetic work in mice demonstrates that components of the hepatic insulin signaling cascade including the insulin receptor, AKT, the FOXO transcription factors play important roles in the direct effects of insulin-mediated regulation of glucose production. These studies also reveal a role for extrahepatic insulin action in limiting delivery of gluconeogenic substrate. Recent evidence also suggests that non-hormonal, G6P sensing mechanisms involving the nutrient sensing transcription factor ChREBP may affect hepatic autoregulation and glucose production. In the setting of overnutrition and particularly hepatic carbohydrate overload, activation of ChREBP may enhance glucose production independently of hepatic insulin signaling in both mice and humans. We anticipate that additional studies to further elucidate mechanisms mediating hepatic autoregulation of glucose production will improve understanding of the pathophysiology of glucose production and may allow for the development of new strategies to treat and prevent T2D.

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Compliance with Ethical Standards

Conflict of Interest Ashot Sargsyan declares that he has no conflict of interest.

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