MINIREVIEW

Sweet Changes: Glucose Homeostasis Can Be Altered by Manipulating Genes Controlling Hepatic Glucose Metabolism

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The liver is responsible for glucose synthesis in the fasting state, and glucose uptake, storage, and utilization in the fed state. A phenotypic switch, normally initiated by insulin or glucagon, controls the transition between the two states, which includes transcriptional alterations that regulate metabolic enzyme abundance for multiple metabolic pathways in a coordinated manner. A network of transcription factors, coactivators, and corepressors direct these changes, thus acting as transcriptional sensors of the nutritional status of an organism. The inability of the hepatocyte to undergo this metabolic reprogramming is characteristic of diabetes mellitus. Modulations that control the amount of individual metabolic enzymes or transcription factors can initiate the fasting-to-fed transition of the hepatocyte in an insulin-independent manner. Alternatively, overexpression of key regulators of metabolism can lock hepatocytes in the fasted state. These manipulations alter hepatic glucose flux, leading to either amelioration or induction of diabetes mellitus. These maneuvers reveal the complexity of the coordinated mechanisms used by the liver to alter its phenotype and provide evidence for the control strength of metabolic signaling. (Molecular Endocrinology 18: 1051–1063, 2004)

The liver is strategically positioned to influence glucose homeostasis through a delicate balance between hepatic glucose uptake and utilization (HGU) and hepatic glucose production (HGP). Physiologically, insulin and glucose act jointly to influence the fasted-to-fed transition in the liver by promoting expression of genes normally induced in the fed state, including glucokinase (GK), liver pyruvate kinase (L-PK), 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFK2), acetyl-coenzyme A carboxylase, and fatty acid synthase (FAS) (Refs 1–3 and Fig. 1). Conversely, genes that are activated during the fasted state, such as phosphoenolpyruvate carboxykinase (PEPCK), fructose-1,6-bisphosphatase, and carnitine palmitoyl transferase (CPT) I and II are down-regulated (1–3). This phenotypic switch requires genes to be stimulated or repressed by a coordinated process. Normally, various hormones contribute significantly both to the regulation of these metabolic genes and to modifications in specific proteins that control hepatic metabolic processes. However, by simply overexpressing key metabolic enzymes, or the factors that regulate their abundance, these alterations in gene transcription can be achieved regardless of hormonal status (1, 4–9).

Abbreviations: ChREBP, Carbohydrate response element binding protein; CPT, carnitine palmitoyl transferase; DM PFK2, double-mutant PFK2; FAS, fatty acid synthase; F-2,6-P₂, fructose-2,6-bisphosphate; GFAT, glutamine fructose-6-phosphate amidotransferase; GK, glucokinase; GKRP, GK-regulatory protein; GlcNaC, N-acetyl glucosamine; G6Pase, glucose 6-phosphatase; HGP, hepatic glucose production; HGU, hepatic glucose uptake and utilization; L-PK, liver pyruvate kinase; PEPCK, phosphoenolpyruvate carboxykinase; PGC-1α, PPAR-γ coactivator-1α; PKA, protein kinase A; PKF1 and -2, 6-phosphofructo-1-kinase and 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase; PPAR, peroxisomal proliferator-activated receptor; PP1 and PP2A, protein phosphatases 1 and 2A; SREBP, sterol-regulatory element-binding protein; STZ, streptozotocin; Xu-5-P, xylulose 5-phosphate.

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can either promote or prevent the development of diabetes in a hormone-independent fashion, and these manipulations are considered on an individual basis in this review (Fig. 2). The effects of insulin and glucagon on hepatic glucose metabolism are well documented and have been reviewed elsewhere (20–26). The present discussion will focus on how alterations of glucose metabolism potently affect hepatocyte function. During this process, two main questions will be addressed: 1) How can altering metabolic enzyme abundance influence both the phenotype of the hepatocyte and whole-body glucose homeostasis? 2) What are the coordinating mechanisms required to mediate this process?

HEPATIC ENZYME ELEVATIONS LEADING TO DIABETES

PEPCK

The synthesis of glucose by the liver is critical when there is a paucity of carbohydrates in the diet. This is particularly crucial for tissues such as the brain and red blood cells, which can not use fatty acids as a fuel source. The breakdown of glycogen can only maintain euglycemia for a short period. Consequently, the net synthesis of glucose from carbon sources such as lactate, glycerol, and amino acids plays a key role in maintaining euglycemia during a prolonged fast. PEPCK is central to this process because it catalyzes the rate-controlling reaction of the gluconeogenic pathway by converting oxaloacetate into phosphoenolpyruvate. The abundance of the cytosolic form of this enzyme is mainly controlled by the rate of transcription of its gene (27). The PEPCK gene is activated by cAMP and glucocorticoids and suppressed by insulin and glucose (14, 28, 29). The inhibition of PEPCK by insulin is the most important physiological mechanism to inhibit the synthesis of this rate-controlling enzyme at the end of a fast. However, the metabolism of glucose is sufficient to override the combined stimulatory effects of dexamethasone and cAMP on PEPCK gene transcription. This effect by glucose can be insulin independent, provided that GK is expressed by some artificial means, and clearly demonstrates the potential strength of the GK-mediated, glucose-derived signal (14, 15).
Dysregulation of PEPCK is a contributing factor to the pathogenesis of both type 1 and type 2 diabetes mellitus (30, 31). Not surprisingly, transgenic mice overexpressing PEPCK in the liver display increased HGP (30, 32). These animals have fasting hyperglycemia, decreased glycogen storage, and glucose intolerance, suggesting insulin resistance (32). Further analysis reveals that PEPCK-overexpressing mice have higher blood glucose and insulin levels relative to control animals in both the fasted and fed states. Glucose 6-phosphatase (G6Pase) mRNA levels are enhanced, concomitant with a decrease in the levels of the insulin receptor substrate 2 protein (30). Thus, increasing hepatic glucoseogenesis by PEPCK overexpression leads to insulin resistance and symptoms resembling type 2 diabetes mellitus.

G6Pase

Also vital to glucose production is the enzyme G6Pase, which catalyzes the dephosphorylation of glucose 6-phosphate to free glucose as the terminal step in gluconeogenesis and glycogenolysis. This process is carried out in the lumen of the endoplasmic reticulum and requires several other proteins in addition to the catalytic subunit. These include transporters for glucose 6-phosphate, glucose, and inorganic phosphate (33). The end result of this reaction allows glucose to be transported out of the hepatocyte and into the circulation. The gene encoding the G6Pase catalytic subunit is stimulated by cAMP and repressed by insulin in a manner similar to that for PEPCK (34–36). In contrast to the PEPCK gene, the G6Pase gene is stimulated by glucose, an effect that is not well understood (37, 38). If the balance involving glucose phosphorylation and glucose 6-phosphate hydrolysis is perturbed, producing a high G6Pase to GK ratio, abnormal hepatic glucose metabolism results and is manifested physiologically as a metabolic disturbance resembling type 2 diabetes mellitus (39). Thus, a delicate balance exists where GK and G6Pase compete to control the direction of glucose flux and ultimately the metabolic signaling system that promotes the fasting-to-fed transition of the liver.

Obese rodents have increased G6Pase activity and exhibit hepatic insulin resistance when compared with lean controls (39). Direct evidence linking G6Pase to the development of insulin resistance was gained from experiments wherein the catalytic subunit of G6Pase was overexpressed via adeno viral vectors in primary hepatocyte cultures and in vivo in rats. In primary hepatocytes, G6Pase overexpression augments activity of this enzyme and is sufficient to increase glucose 6-phosphate hydrolysis (40). These cells also display decreased lactate production and glucose usage relative to cells treated with a control adenovirus. These studies demonstrate that G6Pase overexpression impairs HGU, while simultaneously enhancing HGP. In similar studies, adenovirus-mediated overexpression of G6Pase in the liver results in decreased glycogen storage, hyperinsulinemia, and glucose intolerance (39). This is a phenotype remarkably similar to type 2 diabetes mellitus and is yet another example of how the overexpression of one key enzyme can perturb the phenotype of the hepatocyte and overall glucose homeostasis. Thus, increased flux through gluconeogenesis, whether via PEPCK or G6Pase overexpression, results in decreased HGU and increased HGP. These modulations have deleterious effects on whole-animal glucose homeostasis and may explain, at least in part, the metabolic defects observed in both type 1 and type 2 diabetes.

**Peroxisomal Proliferator-Activated Receptor (PPAR)-γ Coactivator-1/Foxo1**

PPAR-γ coactivator-1α (PGC-1α), originally discovered as a protein induced by adaptive thermogenesis, interacts with the nuclear hormone receptor PPAR-γ to facilitate gene activation (41). PGC-1α promotes the expression of uncoupling protein 1, an inner mitochondrial membrane protein that dissociates fuel oxidation from ATP production, thereby generating heat. The activity of PGC-1α is dependent on a physical interaction with specific transcription factors via a leucine-rich (LxxLL) domain; it was subsequently shown that this motif mediates contact with several nuclear hormone receptors, including the estrogen and glucocorticoid receptors (42).

Recently, Spiegelman and colleagues (8) made the observation that hepatic PGC-1α is a coactivator of several gluconeogenic genes, including G6Pase and...
PEPCK. This coactivator protein is induced by cAMP and glucocorticoids in hepatocytes and is elevated in the livers of fasting and diabetic animals. Adenoviral overexpression of PGC-1α increases glucose production in primary hepatocytes and activates gluconeogenesis in vivo by increasing the transcription of key enzymes involved in glucose synthesis, such as PEPCK and G6Pase (8). Additionally, the cAMP response element binding protein, activated by phosphorylation in response to a variety of signals, regulates hepatic gluconeogenesis in part by up-regulating PGC-1α (43). Signals that represent the fasted state, including cAMP and glucocorticoids, promote glucose synthesis while inhibiting glucose utilization. Mice with a heterozygous deletion in cAMP response element binding protein exhibit fasting hypoglycemia that is corrected by hepatic overexpression of a PGC-1α-encoding adenovirus, but not by a control adenovirus. Thus, a pathological enhancement of PGC-1α, such as occurs during diabetes, may amplify the glucocorticoid and cAMP signal to the PEPCK gene, resulting in uncontrolled HGP (43). Furthermore, the transcription factor Foxo1, which is regulated by exclusion from the nucleus upon insulin signaling through phosphorylation by protein kinase B (Akt), also interacts with PGC-1α to stimulate gluconeogenesis (36). Indeed, when a constitutively active version of Foxo1 is overexpressed in mice, there is a corresponding increase in the expression of the PEPCK and G6Pase genes, resulting in elevated blood glucose concentrations (44). Conversely, when a Foxo1 dominant-negative mutant, generated by deletion of a carboxyl domain fragment, is infused into db/db mice, there is a significant lowering of blood glucose levels (45). These observations define Foxo1 as a critical intermediate in the insulin-mediated regulation of gluconeogenesis. Thus, the direction of glucose flux in the hepatocyte affects the organism as a whole, such that increasing flux through anabolic, glucose-producing pathways interferes with the ability of the liver to transit from the fasting to the fed state. As a result, the control mechanisms that maintain euglycemia, including those that regulate gene transcription, are interrupted. This makes the interaction between PGC-1α and transcription factors that are responsible for activating genes involved in glucose production, such as Foxo1, potentially novel therapeutic targets for diabetes drug design.

Glutamine Fructose-6-Phosphate Amidotransferase

So far the discussion has been restricted to manipulations that increase HGP and their roles in contributing to the disruption of the fasted-to-fed phenotypic switch in the hepatocyte. Several different pathways in the hepatocyte contribute to the breakdown and storage of glucose, including glycolysis, the hexose monophosphate shunt, and glycogen and hexosamine biosynthesis. As we will discuss below, the activation of glucose-utilizing pathways usually promotes the hepatic fasting-to-fed transition via a poorly understood signaling system (4, 46, 47), which prevents the onset of type 1 or type 2 diabetes mellitus, or both. By contrast, the hexosamine biosynthetic pathway, which involves glucose catabolism, can actually lead to insulin resistance (48). Marshall and co-workers (49) first characterized this pathway as a mediator of glucose-induced desensitization of the insulin-stimulated adipocyte glucose transport system. The rate-controlling enzyme is glutamine-fructose-6-phosphate amidotransferase (GFAT). This enzyme catalyzes the reaction: fructose-6-phosphate + glutamine → glucosamine 6-phosphate + glutamate. The final products of the pathway are nucleotide hexosamines, such as uridine diphosphate-4-acetyl glucosamine (GlcNAc), that are substrates for O-linked GlcNAc transferase. O-linked GlcNAc transferase catalyzes the glycosylation of serine and threonine residues for a number of nuclear and cytosolic proteins, including glycogen synthase, c-Myc, and RNA polymerase II (50–53). This mechanism may allow cells to sense fuel availability and thus coordinately regulate their metabolism according to nutrient status.

Nutrient excess can promote insulin resistance via accumulation of hexosamine biosynthetic pathway end products. Several studies have confirmed the role of GFAT in establishing insulin resistance in multiple tissues, including the liver. Transgenic mouse models established by McClain and colleagues (54–56) illustrate that moderate GFAT overactivity results in a phenotype resembling type 2 diabetes. Further, infusing glucosamine into animals leads to a marked accumulation of hexosamine biosynthetic pathway end products that precedes the onset of insulin resistance (57). To our knowledge, these are the only known examples whereby a modest enhancement in the levels of a rate-controlling enzyme in a catabolic, glucose-utilizing pathway, or infusing the end products of this pathway, increase the likelihood of developing insulin resistance.

HEPATIC ENZYME ELEVATIONS AMELIORATING OR PREVENTING DIABETES

GK

The liver removes a substantial portion of glucose from the blood and thus is specially suited to metabolize large amounts of carbohydrates (58). The breakdown of glucose proceeds with phosphorylation of the hexose and its subsequent metabolism through various pathways (Fig. 2). GK (hexokinase IV) is the major glucose-phosphorylating enzyme in the liver; its abundance is regulated at a variety of levels, including transcriptionally by insulin and glucagon, and post-translationally by the GK regulatory protein (GKRP) (59, 60). Insulin is required for GK expression and may therefore be viewed as permissive for the ability of
glucose to impact hepatic transcriptional patterns. Thus, if GK activity is enhanced by alternative means, such as with plasmid transfection, adenoviral transduction, or transgenic overexpression, insulin is no longer required to initiate the metabolic reprogramming that occurs during the fasted-to-fed transition (4, 13, 14).

This principle can be illustrated in hepatoma cells, which in contrast to primary hepatocytes, typically lack GK activity. When hepatoma cells are stimulated with high levels of glucose (15–30 mM), there are no appreciable effects on glucose flux and utilization as measured by lactate production in the cell culture media or glycogen accumulation in the cell (17). Furthermore, glucose-responsive genes, such as L-type pyruvate kinase (L-PK) and PEPCK, are unaffected by high-glucose treatment in the absence of GK. Thus, these cells remain, metabolically and phenotypically, in the fasted state. However, when GK is expressed in hepatoma cells, glucose metabolism generates signals that stimulate or repress specific genes in a coordinated manner (14, 38). Conversely, blocking GK activity with mannoheptulose or glucosamine, competitively inhibiting of mammalian hexokinases, effectively reduces the ability of glucose to initiate this phenotypic switch (14, 61). One may argue that a lack of the glucose transporter 2 would possibly limit glucose flux into and out of the cells, even if GK was present. However, in the studies we present in this review, this does not seem to be the case because overexpression of GK is sufficient to restore glucose flux and gene expression patterns to those resembling the fed state (4, 17, 62).

GK activity is highly correlated with blood glucose concentration and is thus considered to be a hepatic “glucose-sensor” (63). Thus, extinguishing the signal provided by GK-derived glucose metabolism would theoretically impede the ability of the hepatocyte to undergo the fasting-to-fed transition. Indeed, liver-specific GK knockout mice exhibit a 40% increase in blood glucose concentrations (64), presumably due to a lack of glucose signaling and therefore an inability to initiate the correct metabolic reprogramming.

Hepatic GK mRNA and protein levels are very low in both human and rodent diabetes; insulin treatment rapidly restores GK activity to the hepatocyte (65). Because of these observations, hepatic replacement of this enzyme provides a possible therapeutic strategy to treat diabetes, and this idea has received considerable attention experimentally. Ferre et al. (4) generated transgenic mice wherein a portion of the PEPCK promoter was used to drive liver-specific GK expression. These mice have a 2-fold increase in GK activity relative to control animals, which results in increased hepatic glycogen storage. This modest GK overexpression in the liver protects against streptozotocin (STZ)-induced diabetes. STZ destroys pancreatic β-cells, which normally secrete insulin to prevent hyperglycemia. Insulin deficiency is a hallmark of type 1 diabetes, and because insulin is markedly reduced or absent in the STZ-animal, the fasted-to-fed transition of the liver is impaired. However, in GK-expressing transgenic mice treated with STZ, the phenotypic switch promoting HGU and decreasing HGP is carried out solely based on signals derived from glucose metabolism. This metabolic reprogramming by the glucose-derived signals allows the fed pattern of liver gene expression to be established in the absence of insulin. For example, the expression of the PFK2 and L-PK genes are increased, whereas expression of the PEPCK, CPT I, and CPT II genes are decreased (4). This reflects a switch from glucose production to glucose uptake and utilization that promotes the lowering of serum glucose, triglycerides, free fatty acids, and ketone bodies in GK-overexpressing transgenics relative to control mice (4). This study demonstrates that a modest augmentation of GK activity in the liver enhances glucose metabolism and promotes overall glucose homeostasis.

In a different set of studies, involving adenoviral GK infusion into the liver of normal rats, an approximate 5-fold increase in glycogen storage is achieved in the fasted state (66). These authors noted that relatively low (~3-fold) overexpression of GK does not significantly alter the plasma levels of triglycerides, free fatty acids, glucose, or insulin in these rats. However, this moderate increase in GK activity is sufficient to promote the fed patterns of gene expression in the fasting state of these animals (67), and although not tested, one would predict improved glucose tolerance. In contrast, a 6-fold overexpression of GK increases circulating triglycerides approximately 3-fold and free fatty acids about 4-fold. Although this level of GK activity did promote a 38% decrease in blood glucose levels concomitant with a 3-fold decrease in circulating insulin levels, it also raised the concern that GK overexpression leads to hyperlipidemia (66).

In other models of elevated GK activity, dyslipidemia is not observed. For example, overexpression of the human GK gene in transgenic mice using a liver-specific apolipoprotein A-I gene enhancer does not promote increased plasma triglycerides, but does improve glucose tolerance (68). Furthermore, transgenic mice with an extra GK gene locus display increased hepatic glucose metabolism, are protected from diet-induced diabetes, and do not develop hyperlipidemia (62, 69). One common feature of GK-overexpressing animals that do not display dyslipidemia is a modest (2- to 3-fold) increase in GK activity. Clearly, increases of 6-fold and above, although protective against diabetes mellitus, are deleterious in terms of lipid accumulation (66, 70).

Taken together, these studies imply that increasing GK activity may be a rational intervention to treat both major forms of diabetes mellitus. The logical argument is that glucose metabolism provides signals that coordinately regulate hepatic gene transcription patterns (Fig. 1); these signals drive the hepatocyte toward the fed state by inducing the appropriate metabolic enzymes, leading to increases in glycolysis, glycogen...
storage, and fatty acid synthesis. Simultaneously, glucose and ketone production are inhibited. This phenotypic switch occurs in hepatocytes irrespective of islet hormones, provided that GK is present. However, the exact signals and the factors required have not been completely characterized. It is important to emphasize that the dissociation of glucose signaling from insulin signaling is likely to occur only in pathological conditions in which therapeutic interventions have been made, as these two effectors are closely associated under normal (nondiabetic) conditions.

**GKRP**

The GK gene is regulated transcriptionally by insulin, glucagon, and other hormones (59, 60). Another level of control is maintained through the actions of GKRP. This 68-kDa protein sequesters GK in the nucleus during the fasting state (71). Binding of GK to GKRP is favored when fructose 6-phosphate levels are high, whereas elevated fructose 1-phosphate levels promote the release of GK from GKRP (71, 72). Physiologically, a nuclear pool of GK provides a readily available source of the enzyme during the fasted-to-fed transition. Indeed, GK contains a nuclear export sequence that ensures its proper return to the cytosol after release from the GKRP (73, 74). The importance of this mechanism is apparent in mice that have the GKRP gene ablated (75, 76). Farrelly et al. (75) noted that GK activity is approximately 33% and 83% lower in mice heterozygous and homozygous, respectively, for the GKRP knockout when compared with control mice. GK mRNA levels remain essentially unchanged in these animals. In a different study, Grimsby and colleagues (76) noted a 16% and 41% decrease in GK activity in heterozygous and homozygous GKRP knockout mice, respectively, when compared with wild-type animals. Both sets of studies confirmed that plasma insulin levels remain normal in the mutant mice. However, GKRP-/- animals have a marked reduction in glucose clearance rates compared with wild-type controls (75, 76). Thus, it is noteworthy that the GKRP has at least three theoretical functions: 1) sequestering GK in the nucleus to prevent futile cycling (glucose → glucose → 6-phosphate → glucose) in the fasting state; 2) protecting GK against proteolytic degradation; and 3) maintaining a nuclear reserve of GK that can be quickly released after a meal.

With these functions in mind, it is not surprising that GKRP overexpression protects against the development of diet-induced diabetes (77). Mice infused with an adenovirus expressing the GKRP gene display improved glucose tolerance and lower fasting blood glucose and insulin levels when compared with animals receiving a control adenovirus (77). This decrease in blood glucose and insulin may be indicative of an increased sensitivity to insulin. One further explanation for these results is that increasing the abundance of the GKRP extends GK half-life by protecting the enzyme from degradation (77). This mechanism allows for the release of GK from a nuclear pool, where it is transported into the cytoplasm after a meal. Thus, promoting a GK reserve ensures that the signal afforded by glucose metabolism will be present to help promote the fasting-to-fed transition of the hepatocyte.

**Glycogen Targeting Subunits**

Numerous proteins that are modified by hormones and metabolites control the regulation of glycogen synthesis and breakdown (78–80). Glycogen degradation helps maintain euglycemia between meals, whereas glycogen synthesis helps remove glucose from the blood via storage in muscle, fat, and liver. The glucose used as a substrate for glycogen synthesis originates from two distinct sources. Glucose phosphorylation by GK facilitates direct entry into the glycogen synthase reaction and therefore is termed the "direct" pathway. Glucose derived from other precursors, such as lactate, glycerol, and amino acids, through gluconeogenesis is called the "indirect" pathway. Both sources of glucose, however, culminate in the production of glucose 6-phosphate, activation of glycogen synthase, and thus promotion of hepatic glycogen storage (81).

GK overexpression enhances glycogen storage via the direct pathway by increasing glucose phosphorylation in the hepatocyte (4, 66). An alternative method of promoting glycogen storage is to increase the abundance of the glycogen targeting subunits, which bind to both the glycogen particle and to the enzymes that regulate its synthesis and degradation. The targeting of protein phosphatase 1 (PP1) to glycogen appears to be one mechanism by which the enzymes of glycogen metabolism are regulated. For example, enhancing the availability of scaffolding proteins that target PP1 to the glycogen molecule increases the activity of glycogen synthase and decreases the activity of phosphorlyase, thereby enhancing hepatic glycogen accumulation. This enhancement ultimately arises from the glucose 6-phosphate that is produced by both the direct and indirect pathways of glycogen synthesis (47). There are several different isoforms of the glycogen targeting subunit proteins, and they are encoded by four different genes in the same family (79). PTG (protein targeting to glycogen) and PPPR6 are ubiquitously distributed; Gs is expressed primarily in liver; Gs/L is found predominately in muscle. These isoforms, although similar in that they all bind to glycogen and protein phosphatase-1, have different binding affinities for glycogen synthase, glycogen phosphorylase, and phosphorylase kinase. They also promote differential levels of glycogen synthesis when overexpressed in cultured hepatocytes (82).

Newgard and colleagues (79) have established a variant of the Gm isoform, termed GmΔC, which is generated by truncation of a 735-amino acid, sarcoplasmic reticulum-binding domain found in the COOH-terminal region of the protein. This mutated
protein exhibits the full capacity to respond to glycogenolytic signals, which is in contrast to Gλ or PTG (82). Indeed, maneuvers that increase the amount of glycogen storage in the liver are able to reverse glucose intolerance in high-fat fed rats (83). Importantly, this manipulation also functions successfully in a rodent model of type 1 diabetes by preventing both hyperglycemia and the other metabolic disturbances generated by STZ treatment (84). It is important to note that maintaining the ability of the hepatocyte to break down glycogen is critical to the success of this strategy (47, 84).

The data from GmΔC overexpression in STZ-treated animals are quite striking. First, glycogen storage decreases G6Pase mRNA levels, but not PEPCK or pyruvate carboxylase mRNA levels (47). Second, blood triglycerides, free fatty acids, and ketone bodies are all normalized in the GmΔC mice relative to control STZ-treated mice (84). Remarkably, the lowering of blood glucose and other parameters in STZ-injected animals treated with GmΔC occurs despite GK protein levels being undetectable by Western blot analysis (84). Finally, food intake is decreased in STZ-treated GmΔC-expressing mice relative to controls. STZ injection and thus insulin depletion cause hyperphagia, yet storage of liver glycogen in GmΔC-treated mice prevents it. The underlying mechanisms of these effects are unclear. However, it is of interest to note that we are not aware of any other data demonstrating that blood glucose levels are normalized in a type 1 model of diabetes in the absence of liver GK. Therefore, several explanations may be put forth based on the available data: 1) it is possible that precursors of glucose (e.g., lactate and glycerol), generated by glucose metabolism and fatty acid breakdown in extrahepatic tissues, are removed from the blood stream by the liver and subsequently stored as glycogen through the indirect pathway; 2) lowering the expression of the G6Pase gene, a phenotype observed when glycogen storage is enhanced by glycogen targeting subunit overexpression (47), prevents glucose export from the liver into the circulation; 3) hepatic glycogen storage promotes a satiety signal that prevents the hyperphagia that would otherwise exacerbate the hyperglycemia in an STZ-treated (and thus insulin-deficient) rodent. It is possible, even likely, that combinations of these possibilities account for the observations seen in GmΔC-overexpressing animals. Also, it is interesting that the protective mechanisms of GK and GmΔC, in the prevention of diabetes, may be different. Importantly, the common link between these two different treatments is increased HGU and possibly reduced hepatic glucose output, resulting in the lowering of blood glucose.

**6-Phosphofructo-2-Kinase/Fructose-2,6-Bisphosphatase (PFK2)**

The pace of glycolysis is set predominately by the enzyme 6-phosphofructo-1-kinase (PFK1), which catalyzes the reaction: fructose-6-phosphate + ATP → fructose-1,6-bisphosphate + ADP (58). This enzyme is allosterically regulated by ATP in a negative fashion and AMP and fructose-2,6-bisphosphate (F-2,6-P2) in a positive fashion. The enzyme PFK2 (or bifunctional enzyme) controls both the synthesis and degradation of F-2,6-P2, the most important allosteric regulator of PFK1 (85). PFK2 has two distinct active sites; the activity of each depends on the nutritional and hormonal state of the hepatocyte. For example, hormonal regulation by glucagon, through cAMP activation of the protein kinase A (PKA), promotes phosphorylation at serine 32 of PFK2 that renders the kinase portion of the bifunctional enzyme essentially inactive through conformational changes. Thus, the phosphatase domain predominates under these conditions, effectively reducing F-2,6-P2 levels and promoting gluconeogenesis while concomitantly decreasing flux through glycolysis. Lange and co-workers have taken advantage of this knowledge to generate an enzyme that has a high kinase to bisphosphatase activity ratio. By making S32A (removes the PKA phosphorylation site) and H258A (reduces bisphosphatase enzyme activity) mutations, a double-mutant enzyme is produced that is a much more potent generator of F-2,6-P2 than the wild-type enzyme (85).

The double-mutant PFK2 (DM PFK2), which constitutively increases F-2,6-P2 levels, effectively lowers blood glucose levels in both STZ-treated and genetically insulin-resistant mice (86, 87). In the STZ-treated mice, an approximate 30-fold increase in the concentration of F-2,6-P2 is generated by infusion of an adenovirus expressing the DM PFK2 vs. a control adenovirus (86). The subsequent increase in HGU results in a lowering of blood glucose from 22 mM (STZ-control) to 11 mM (STZ-DM PFK2) and coincides with comparable decreases in plasma free fatty acids and triglycerides (86). In genetically insulin-resistant mice, which have a phenotype that mimics type 2 diabetes, a similar correction of metabolic perturbations is observed. Blood glucose levels are normalized in the DM PFK2 treated-animals whereas control animals remain hyperglycemic. Also, there is lowering of serum lipids in the DM PFK2 mice relative to control mice. Another interesting observation is that increased levels of hepatic F-2,6-P2 down-regulate the expression of the G6Pase gene and concomitantly increase the expression of the GK gene (87). These changes promote the transition of the hepatocyte from the fasting to the fed state. The mechanism underlying these results remains undetermined at the present time. It should be noted that these effects are typically associated with insulin signaling. However, it is becoming increasingly clear that glucose metabolism, in this case by augmenting F-2,6-P2 levels, has effects that are equally potent.

**Sterol-Regulatory Element-Binding Protein (SREBP) 1-c**

As mentioned in the introduction and diagrammed in Fig. 1, insulin and glucose act in concert to influence
hepatic gene transcription patterns. One transcription factor that has been suggested as a mediator of the insulin signal is SREBP-1c (88, 89). The SREBP family is encoded by two genes that produce three proteins. The SREBP-1 gene, through alternative splicing and the use of alternative promoters, produces two distinct transcription factors, termed SREBP-1a and SREBP-1c (90–92). The SREBP-2 gene product is a single protein with approximately 50% homology when compared with the products of the SREBP-1 gene. Each of these three transcription factors shares a similar modular structure that consists of: 1) an amino-terminal basic helix-loop-helix leucine zipper domain; 2) a central hydrophobic fragment that contains transmembrane segments; and 3) a carboxy-terminal stretch of approximately 590 amino acids that has regulatory functions (91, 92). These proteins are inserted into the cytosolic face of the endoplasmic reticulum membrane. Specific signals can promote proteolytic cleavage and release of the mature, active basic helix-loop-helix-LZ-containing transcription factor, which is then transported into the nucleus to activate target genes (93).

There is evidence that SREBP 1-c is an insulin-mediated transcription factor that stimulates GK expression. Foretz and colleagues (94) demonstrated that a mutated, mature form of SREBP 1-c, which is directly imported into the nucleus, functions as a dominant-positive transcription factor and induces GK gene transcription without a need for insulin. Further, these investigators found that a dominant-negative form of the transcription factor, which dimerizes with the endogenous protein but does not bind DNA, prevents the insulin induction of the GK gene (94). Interestingly, and in contrast to the above study, Stockman and Towle (95) found that a dominant-positive form of SREBP 1-c, controlled by a tetracycline-inducible adenoviral vector, only induced GK mRNA levels to 2% of that seen with insulin treatment in primary hepatocytes, while simultaneously increasing the expression of FAS.

Nonetheless, insulin induces SREBP 1-c in hepatocytes and restores expression of this factor in STZ-treated mice (96, 97). Therefore, hepatic adenoviral replacement of SREBP 1-c was used to examine whether this factor could correct STZ-induced diabetes in mice (9). STZ treatment completely ablates the expression of GK, FAS, and the low-density lipoprotein receptor, whereas PEPCK is strongly up-regulated due to the lack of insulin signaling. In contrast, mice treated with an adenovirus expressing the dominant-positive form of SREBP 1-c display robust induction of the GK and FAS genes, whereas expression of the PEPCK gene is strongly repressed (9). These effects at the gene expression level correlate with an increase in liver glycogen and triglycerides in SREBP 1-c-overexpressing mice; blood glucose also decreased significantly in SREBP 1-c-treated mice relative to mice receiving a control adenovirus (9). These data are consistent with those obtained by Hanson and colleagues (98), who found that SREBP-1c overexpression in primary hepatocytes represses the cAMP stimulation of the PEPCK gene, whereas a dominant-negative form of the transcription factor promotes PEPCK mRNA accumulation. Therefore, it appears that the hypoglycemic effects of hepatic overexpression of SREBP 1-c arise by its ability to mimic the action(s) of insulin.

c-Myc

c-Myc is a transcription factor that is involved in many diverse cellular processes, including growth, differentiation, apoptosis, and metabolism (99, 100). This factor, a protooncogene, has been studied largely in the context of cancer biology and recently was suggested to have a role in contributing to the Warburg effect, whereby tumor cells increase their glycolytic rate to provide energy for various growth processes (101). c-Myc interacts with another basic helix-loop-helix factor, Max, and the resulting heterodimer binds to E-box elements (consensus = CACGTG) to activate gene transcription (102, 103). Carbohydrate response elements, first described in the glucose-responsive L-PK and S14 genes, contain sequences very similar to sites favored by Myc-Max heterodimers (12, 104).

Studies by Bosch and colleagues (5, 105, 106) have demonstrated that there is a connection between carbohydrate metabolism and c-Myc expression levels. A 3-fold hepatic overexpression of c-Myc, under control of the PEPCK promoter in mice, leads to increased hepatic glucose metabolism and improved glucose disposal (105). These animals display increases in both GK and L-PK mRNA; activity of these glycolytic enzymes is also enhanced relative to control animals (105). Further, this modest augmentation of c-Myc abundance protects against the development of STZ- and diet-induced diabetes (5, 106). STZ-treated mice with a 3-fold increase in c-Myc levels have an increased hepatic expression of the GK, PFK2, and L-PK genes, concomitant with a decrease in the expression of the PEPCK gene, all relative to STZ-treated control mice (5). Moreover, c-Myc overexpression restores the abundance of SREBP-1c in the hepatocyte (107).

Blood glucose levels in the c-Myc-expressing transgenic are normalized, presumably due to the ability of c-Myc to promote the fasting-to-fed transition of the liver. This genetic reprogramming promotes appropriate metabolic enzyme gene induction, with the end result of glucose removal from the blood via enhancement of hepatic glucose storage and utilization. Further, blood ketone levels are normalized in STZ-treated Myc overexpressing mice relative to control mice, probably because of the decrease in the expression of the CPT I, CPT II, and 3-hydroxy-3-methylglutaryl coenzyme A synthase genes (5). Moreover, these c-Myc-induced regulatory mechanisms also prevent obesity and development of insulin resistance (106). All of these changes take place in the absence of...
insulin action, demonstrating the ability of elevated c-Myc levels to coordinately regulate hepatic gene transcription patterns. Questions that remain unaddressed are whether c-Myc directly transactivates the GK, PFK2, or SREBP-1c genes, all three genes, or induces their expression via an indirect mechanism.

We have shown recently that reducing the abundance of c-Myc or interfering with Myc-Max DNA binding in hepatoma cells and primary hepatocytes decreases the glucose-stimulated expression of the L-PK and G6Pase genes (108). Reducing c-Myc levels by 50%, using a recombinant adenovirus expressing antisense c-myc mRNA, inhibits glucose-stimulated L-PK gene expression by approximately 60% and completely blocks the glucose induction of the G6Pase gene. In contrast, the glucose repression of hormone-activated PEPCK promoter activity is unaffected by the reduction in c-Myc abundance (108). We interpret these data to indicate that multiple glucose-signaling pathways exist in the hepatocyte and that there are c-Myc-dependent and -independent mechanisms of glucose-mediated transcriptional regulation. These findings corroborate those of Bosch and co-workers (5, 105–107) and suggest that c-Myc may be a key factor in the regulation of hepatic metabolic enzyme genes. An alternative view is that c-Myc is primarily involved in cell proliferation and contributes to the changes in gene expression in a manner independent of the normal insulin- and glucose-signaling pathways. However, the transcription factor network required for the glucose-mediated reprogramming of metabolic genes is still under investigation.

### Carbohydrate Response Element Binding Protein (ChREBP)

Recently, Uyeda and co-workers (109) discovered a novel hepatic transcription factor that participates in the regulation of the L-PK gene by glucose, which they named ChREBP. ChREBP is an 864-amino acid (~95 kDa) protein that contains a nuclear localization signal and a basic helix-loop-helix leucine zipper motif (110). This transcription factor also contains several phosphorylation sites for PKA as well as one recognized by the AMP-activated kinase. Indeed, nuclear import and export are controlled by glucose and cAMP in an antagonistic fashion that is dependent on protein phosphorylation.

ChREBP resides in the cytosol when Ser196, which is a site recognized by PKA near the nuclear localization signal, is phosphorylated upon cAMP stimulation (111). Glucose promotes dephosphorylation by activation of PP2A (112). This phosphatase is activated by xylulose 5-phosphate (Xu-5-P), a metabolite generated by the nonoxidative branch of the pentose phosphate pathway (113). Further, Ser626 and Thr666 are phosphorylated by PKA, modifications that prevent DNA binding; activation of PP2A by Xu-5-P removes the phosphoryl group and increases affinity of ChREBP for DNA (110). Thus, increased glucose flux and metabolism, possibly via a pentose phosphate pathway metabolite, promote the ability of at least one factor to regulate metabolic enzyme gene expression.

Based on these observations, we speculate that overexpressing glucose 6-phosphate dehydrogenase (G6PDH), the rate-controlling enzyme of the pentose phosphate pathway, may be able to generate a metabolic switch in the liver by driving flux through the pathway and generating Xu-5-P and perhaps other signaling metabolites (Fig. 2). Xu-5-P alleviates cAMP-stimulated PKA phosphorylation of ChREBP (and possibly other factors), thereby promoting increased activity of this regulator. Moreover, a logical hypothesis is that a dominant-positive form of ChREBP (containing S196A, T666A, and S626A mutations) would be sufficient to activate the phenotypic switch of the liver, similar to the mechanisms discussed throughout this review. One might predict, then, that overexpression of G6PDH, a gene normally induced by insulin (114), or overexpression of a dominant-positive ChREBP would be able to prevent STZ-induced diabetes, diet-induced diabetes, or both (Fig. 2). Further studies are required to confirm these hypotheses.

### CONCLUSION

The common link among the studies discussed herein is that molecular manipulations that alter hepatic glucose metabolism regulate the fasted-to-fed transition in hepatocytes in a hormone-independent manner. This metabolic reprogramming can result in a shift from HGP to HGU, or vice versa. These changes affect overall glucose homeostasis. Modulations that increase HGP either induce or exacerbate insulin resistance, leading to diabetes. Conversely, increasing glucose uptake, utilization, and storage in the liver protects against the development of hyperglycemia and overt diabetes. The reciprocal regulation of glucose producing and utilizing enzymes requires that multiple transcription factors, coactivators, and corepressors function in a coordinated fashion. Finally, the ability of glucose to modulate hepatic gene transcription patterns and the capability of individual metabolic pathways to influence overall glucose homeostasis provide new targets for therapeutic intervention and is a testament to the hormone-like control strength of metabolic signaling.

### Note Added in Proof

Since the submission of this manuscript, further studies supporting the ideas discussed here have become available. Lange and colleagues (116) demonstrated that increasing hepatic F-2,6-P2 levels promotes Akt phosphorylation independently of insulin action, providing a potential link between glucose metabolism...
and insulin signaling. Work by Montminy’s group has revealed two novel proteins that participate in controlling the hepatic fasted-to-fed transition. Hairy Enhancer of Split, a transcriptional repressor that is upregulated during the fasting state, prevents PPARγ-induced transcriptional activity, also promoting the fasting state phenotype (17). TRB3, which binds to nonphosphorylated Akt and thus prevents its phosphorylation, leads to hyperglycemia when overexpressed in mice by promoting HGP (118).

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REFERENCES

3. O’Brien RM, Granner DK 1996 Regulation of gene expression by insulin. Physiol Rev 76:1109–1161
13. Doiron B, Cuif MH, Kahn A, Diaz-Guerra MJ 1994 Role of our colleagues whose work was omitted due to space

34. van Schaftingen E, Gerin I 2002 The glucose-6-phosphatase system. Biochem J 362:513–522


55. Tang J, Neigh RL, Cooksey RC, McClain DA 2000 Transgenic mice with increased hexosamine flux specifically targeted to β-cells exhibit hyperinsulinemia and peripheral insulin resistance. Diabetes 49:1492–1499


67. Scott DK, Collier JJ, Doan TT, Bunnell AS, Daniels MC, Eckert DT, O’Doherty RM 2003 A modest glucokinase overexpression in the liver promotes fed expression levels of glycolytic and lipogenic enzyme genes in the fasted state without altering SREBP-1c expression. Mol Cell Biochem 254:327–337


74. Shiota C, Coffey J, Grimsby J, Grippio JF, Magnuson MA 1999 Nuclear import of hepatic glucokinase depends upon glucokinase regulatory protein, whereas export is due to a nuclear export signal sequence in glucokinase. J Biol Chem 274:37125–37130


90. Edwards PA, Tabor D, Kast HR, Venkateswaran A 2000 Regulation of gene expression by SREBP and SCAP. Biochim Biophys Acta 1529:103–113


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