Cell Metabolism
Review

Hormonal Regulation of Hepatic Glucose Production in Health and Disease

Hua V. Lin[1] and Domenico Accili[2,*]
[1]Merck Research Laboratories, Rahway, NJ 07065, USA
[2]Columbia University College of Physicians and Surgeons, New York, NY10032, USA
*Correspondence: da230@columbia.edu
DOI 10.1016/j.cmet.2011.06.003

We review mechanisms that regulate production of glucose by the liver, focusing on areas of budding consensus, and endeavoring to provide a candid assessment of lingering controversies. We also attempt to reconcile data from tracer studies in humans and large animals with the growing compilation of mouse knockouts that display changes in glucose production. A clinical hallmark of diabetes, excessive glucose production remains key to its treatment. Hence, we attempt to integrate emerging pathways into the broader goal to rejuvenate the staid antidiabetic pharmacopeia.

Introduction

Hepatic glucose production (HGP) is a key physiological process that becomes altered in diabetic patients (Bogardus et al., 1984) and represents the main target of the antihyperglycemic effect of biguanides (Stumvoll et al., 1995). The combination of tracer and spectroscopy methods in humans, selective catheterization of portal and hepatic veins in dogs during tracer studies, conditional knockouts and metabolic phenotyping in mice, and NMR-based flux analysis in perfused liver and primary hepatocytes has yielded a wealth of information on mechanisms controlling HGP. However, these investigations appear to occur in tangential, if not parallel, universes, leading to often-conflicting claims. The purpose of this review is to integrate this knowledge by critically analyzing the extent to which different pathways meet exacting genetic, cellular biological, and integrated physiological criteria across different experimental models.

Features of HGP In Vivo, in the Perfused Liver, and in Cultured Cells

Classic work by Exton and Park established that key features of HGP can be reproduced in *isolated* liver (Exton and Park, 1967). This work demonstrated that hormonal regulation of HGP is (1) rapid, occurring within seconds of exposing the organ to glucagon or insulin, (2) sensitive, as either hormone is effective at subpicomolar concentrations, and (3) independent of ongoing protein synthesis. The forgotten implications of these landmark observations are that putative physiologic regulators of HGP ought to be regulated at low hormone concentrations by posttranslational modifications of existing signaling complexes and by substrate flux, rather than by de novo gene transcription.

In the 1980s, work in rodents and in cultured hepatocytes conclusively demonstrated that, in addition to substrate flux, insulin and glucagon also regulate expression of the rate-limiting enzymes of glycogenolysis (the catalytic subunit of glucose-6-phosphatase, encoded by *G6pc*) and gluconeogenesis (the cytosolic phosphenolpyruvate carboxykinase, encoded by *Pck1*). This work emphasized the role of cAMP- and insulin-responsive transcription factors (O'Brien and Granner, 1996). The unintended consequence of this landmark research was that regulation of gene transcription and HGP are all too often conflated into a single process, with recurring interpretive errors.

Tracer studies in dogs have defined hormonal regulation of HGP in detail. As in the isolated rodent liver, HGP is exquisitely sensitive to glucagon and insulin. Glucagon sets the basal tone, but insulin trumps glucagon at any concentration—just as it does in vitro. Both hormones affect primarily glycogenolysis by reciprocal changes of glycogen synthase and glycogen phosphorylase and by modulating glycolysis through glucokinase, fructose-bisphosphatase and pyruvate kinase (see below) (Cherrington, 1999). Hormonal regulation of gluconeogenesis has proven difficult to demonstrate. Acute elevations of insulin in the physiologic range have a transient effect on gluconeogenesis (Ramnanan et al., 2010), whereas high insulin concentrations are required to bring about persistent changes (Edgerton et al., 2009). Inhibition of gluconeogenesis by insulin parallels its effect to lower free fatty acids (FFAs) and lactate, consistent with a bimodal mechanism: direct stimulation of glycolysis and glycogen synthesis, and indirect inhibition through decreased gluconeogenic precurors (Bergman and Ader, 2000). The Cherrington group also made a commendable attempt to link flux data with signaling events, as will be seen below (Ramnanan et al., 2010).

Physical exercise, by virtue of its combined actions on stress and glucoregulatory hormones, as well as tissue glycogen levels, is a potent regulator of HGP (Holloszy and Kohrt, 1996).

HGP and Diabetes

Unlike muscle and adipose insulin resistance, which antedate hyperglycemia by years and remain relatively stable throughout the course of the disease (Weyer et al., 1999), the rise of HGP occurs "late" in the natural history of diabetes, but appears to worsen progressively, and to become refractory to treatment (Monnier et al., 2007). In type 2 diabetes, HGP is higher in the postabsorptive state, and fails to be properly suppressed by insulin, resulting primarily from excessive gluconeogenesis, rather than glycogenolysis (Rizza, 2010). As HGP is inversely correlated to insulin levels (Bogardus et al., 1984), its increase probably reflects as much the plight of the β cell as it does a deterioration of hepatic insulin action.

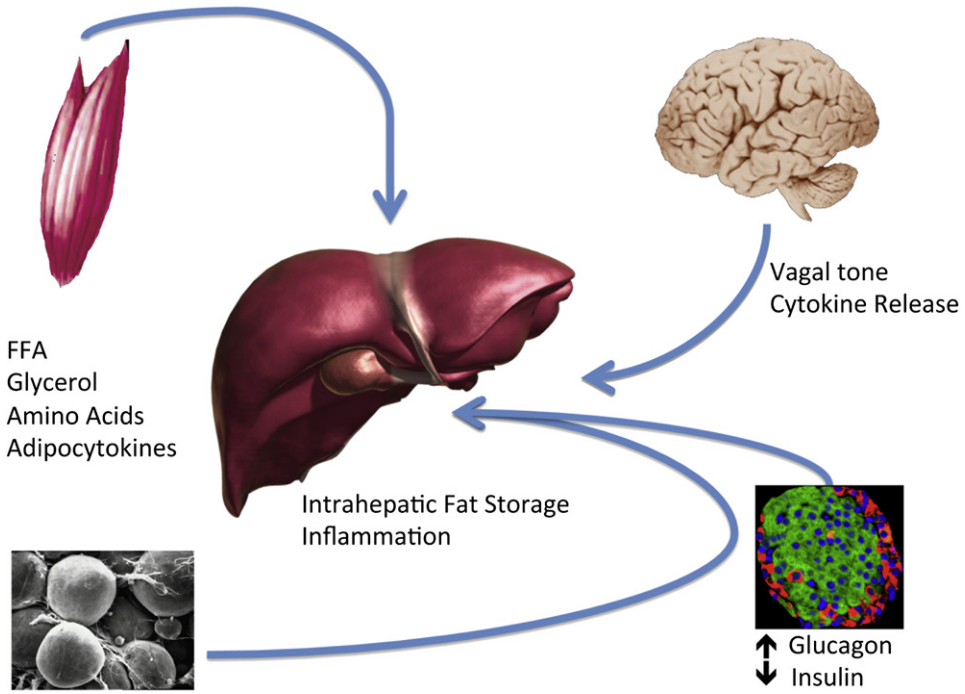

Figure 1. Direct and Ondirect control of HGP
The liver integrates cell-autonomous and cell-nonautonomous mechanisms to control glucose release into the bloodstream. The role of altered glucagon-to-insulin ratios in HGP of type 2 diabetics remains unsettled and of potential therapeutic import. Classic mechanisms of indirect control of HGP include release of gluconeogenic precursors from adipose tissue and muscle (FFAs, glycerol, amino acids), adipocytokines (leptin, adiponectin, resistin), neuronal control—possibly mediated through the vagus nerve. In addition, intrahepatic fat plays an important role in promoting HGP, possibly through accumulation of complex phospholipids. Finally, intrahepatic accumulation of resident macrophages has in recent years taken center stage as a potential mechanism of insulin resistance, leading to lipid accumulation and increased HGP.

Several factors contribute to elevated gluconeogenesis in diabetes: (1) increased supply of glucogenic precursors to the liver (glycerol, amino acids, FFAs), (2) increased liver lipid content, (3) cytokines and adipokines, (4) altered glucagon-to-insulin ratios, (5) in rodents, vagal control originating in the hypothalamus, and (6) decreased insulin receptor signaling in hepatocytes (Figure 1).

Inhibition of gluconeogenesis by insulin in humans remains disputed. The balance of the evidence is consistent with a small effect that requires high insulin concentrations, and is possibly secondary to decreased FFAs (Gastaldelli et al., 2001; Petersen et al., 1998). Equally controversial is the role of glucagon in the increased HGP of diabetes. Strong cases can be made for and against a dominant role of glucagon in driving HGP (Raju and Cryer, 2005). We would like to suggest that the effect of glucagon on HGP is secondary to insulin resistance for three reasons. First, insulin trumps glucagon at any concentration to inhibit HGP, making it unlikely that hyperglucagonemia itself would be sufficient to raise HGP, in the absence of insulin resistance. Second, somatostatin has a biphasic effect on HGP to initially inhibit it, then to stimulate it (Sherwin et al., 1976), indicating that glucagon may not be necessary for the development of hyperglycemia, and hence assigning a primary role to insulin. Third, glucagon trumps insulin in supressing hepatic de novo lipogenesis. Thus, even at concentrations that may not override the effect of insulin on HGP, glucagon ought to prevent liver lipid abnormalities. The fact that it doesn't provides further evidence that insulin resistance is the main driver of hepatic metabolic abnormalities in type 2 diabetes.

The roles of adipokines, liver fat, and neural control in driving HGP are reviewed below.

Regulation of HGP through Substrate Flux

Glycogen Synthase and Phosphorylase

The rapid onset of hormone action on HGP is likely independent of gene transcription. The opposing effects of insulin and glucagon on HGP pivot around their actions on glycogen synthase and phosphorylase. By activating the former, insulin favors glycogen deposition; glucagon activates the latter, resulting in glycogenolysis. It should be emphasized that increased glycogenolysis doesn't necessarily increase HGP, owing to glucose cycling (Petersen et al., 1998). Synthase activation by insulin involves phosphorylation at multiple sites and allosteric binding of glucose-6-phosphate (Roach, 2002). Phosphorylase is rate limiting for glycogenolysis and is oppositely regulated by covalent modifications, through PKA and phosphorylase kinase, and allosterically by its product glucose-6-phosphate. Demonstration that insulin inhibits phosphorylase by covalent modifications is elusive. In euglycemia, insulin paradoxically increases phosphorylase activity, shunting glucose to glycogen cycling. Phosphorylase inhibition by insulin is observed under hyperglycemic conditions, suggesting a role for elevated glucose-6-phosphate levels in this process (Petersen et al., 1998). Efforts to leverage

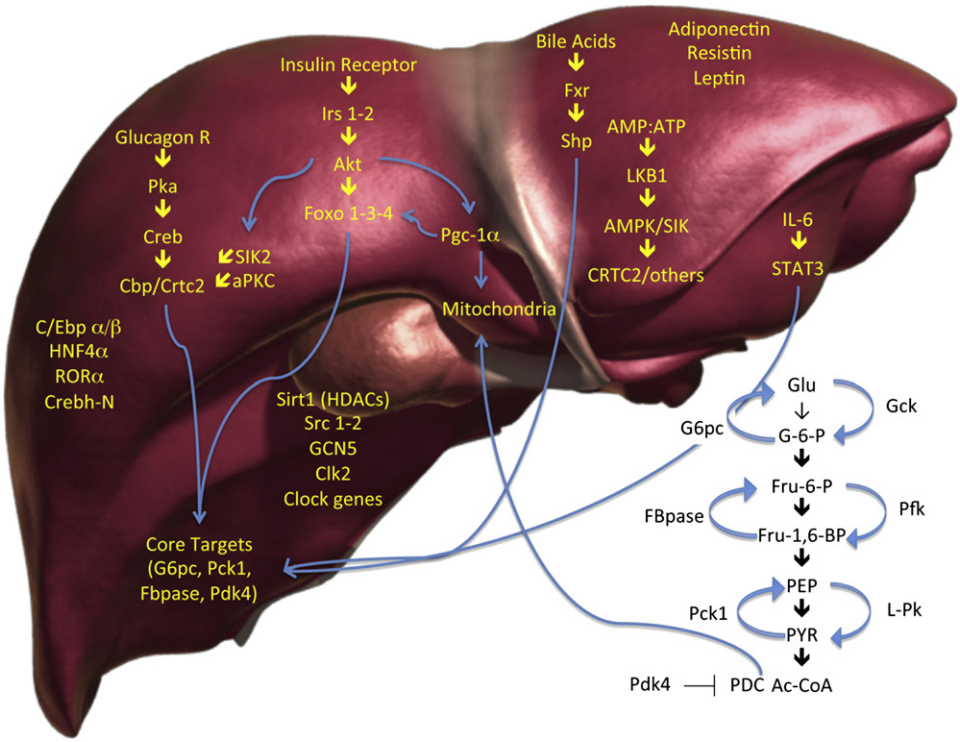

Figure 2. Hormonal and Nutrient Pathways of HGP Regulation in Hepatocytes

Some of the main signaling pathways reviewed in this article are summarized. Insulin, glucagon, and glucocorticoids remain the central regulators of HGP. Their transcriptional effects are mediated through the Akt/Foxo and possibly Crtc2 pathways. AMPK, by virtue of its energy-sensing role, is likely to participate in several of these processes, but its upstream regulators and downstream targets are elusive. Bile acids have profound effects on HGP through complex and redundant mechanisms. Cytokines released from a variety of sources (fat cells, circulating as well as resident macrophages) also affect HGP and are likely to impair insulin sensitivity. A second level of transcriptional integration is provided by cofactors such as Sirt1, Clock genes, Src1-3. Their hormonal regulation is unknown at present. But transcriptional effects account only for part of HGP regulation. Direct control over enzymatic flux through glycolysis and gluconeogenesis, summarized on the right, is likely to be the critical mechanism for rapid regulation of this process.

phosphorylase inhibition for diabetes treatment have floundered in recent years.

Fructose-1,6-Bisphosphatase

Fructose-1,6-bisphosphatase (FBP) catalyzes the penultimate step in gluconeogenesis, converting fructose-1,6-bisphosphate (F1,6BP) to fructose-6-phosphate (Figure 2). This step is necessary for the incorporation of three-carbon substrates into glucose (Pilkis and Claus, 1991) and makes FBP an attractive target for drug development, given that glycerol gluconeogenesis is increased in diabetes. Indeed, genetic mutations and pharmacological inhibition of FBP in rats and humans (Gumbiner et al., 2009) demonstrate its regulatory role in HGP. Feedback inhibition by fructose-2,6-bisphosphate (F2,6BP) and AMP reduces flux through FBP, while glucagon stimulation of cAMP/PKA rapidly reduces intracellular F2,6BP by favoring the phosphatase activity of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase and relieves FBP inhibition to drive gluconeogenesis (Pilkis and Claus, 1991). Accordingly, raising hepatic F2,6BP levels improves insulin sensitivity and lowers glycemia in mice, while reduction of F2,6BP levels has the opposite effect (Wu et al., 2006). In addition, FBP activation depletes its substrate F1,6BP—an allosteric activator of the glycolytic enzyme liver-type pyruvate kinase (PK). PK—the opposing enzyme to PCK1 in the phosphoenolpyruvate/pyruvate cycle—is also inhibited by glucagon and ATP (Pilkis and Claus, 1991). Thus, glucagon simultaneously activates FBP and inhibits PK by phosphorylation of both enzymes and by a coordinate increase in F1,6BP and decrease in F2,6BP, driving glucose formation. Moreover, AMP and ATP levels directly regulate gluconeogenic and glycolytic enzymes, thus affecting the balance between glucose storage/oxidation and production.

Pyruvate Dehydrogenase Kinase

The pyruvate dehydrogenase complex (PDC) converts pyruvate to acetyl-CoA, committing the substrate to cellular respiration. PDC is phosphorylated and inhibited by pyruvate dehydrogenase kinase (PDK) and dephosphorylated and activated by pyruvate dehydrogenase phosphatase. PDK is in turn activated by the products of PDC, acetyl-CoA, and NADH (Figure 2). Among the four PDK isoforms, PDK2 and PDK4 are induced by fasting and inhibited by insulin. PDK inhibits PDC during fasting, sparing pyruvate for gluconeogenesis. Accordingly, *Pdk4* knockout mice show fasting hypoglycemia, secondary to increased systemic glucose oxidation and decreased gluconeogenic substrate delivery to the liver (Jeoung et al., 2006). Pharmacological inhibitors of PDK reduce glycemia in diabetic rodents (Mayers et al., 2005), but the underlying mechanism of increasing glucose oxidation at the expense of fatty acid oxidation suggests a potential risk for hepatosteatosis.

Transcriptional Regulation of HGP by Insulin, cAMP, and Somatostatin

Forkhead-O Transcription Factors

The identification of the forkhead transcription factor *daf-16* as effector of insulin receptor signaling in *C. elegans* led to the testable hypothesis that its mammalian ortholog, FoxO1, was the long-sought hormone-regulated transcription factor that integrated cell surface receptor signaling with HGP (Accili and Arden, 2004). FoxO proteins meet the "Exton and Park" criteria for putative mediators of hormonal HGP. cAMP promotes FoxO nuclear retention and dephosphorylation, whereas insulin at low concentrations promptly inactivates FoxO by driving its nuclear exclusion via Akt-dependent phosphorylation (Nakae et al., 2001). The main FoxO target, *G6pc* is key to physiologic control of HGP. In dogs subjected to physiologic hyperinsulinemia, FoxO1 phosphorylation parallels inhibition of *G6pc* messenger RNA (mRNA) and suppression of glycogenolysis (Ramnanan et al., 2010). FoxO1 loss of function in liver reduces HGP by half by decreasing glycogenolysis, gluconeogenesis, and glucose cycling and results in neonatal and starvation-induced hypoglycemia (Matsumoto et al., 2007), whereas its gain of function prevents hormonal regulation of HGP (Nakae et al., 2002). The extent to which residual HGP in FoxO1 knockout mice reflects nontranscriptional effects of hormones on glucose flux, indirect regulation of HGP (e.g., through the central nervous system [CNS]), or additional hepatic transcription factors (e.g., CRTC2) is at present unknown. When all three *Foxo* genes (1, 3a, and 4) are ablated from mouse liver, fasting glycemia is extremely low (~30 mg/dl), but decreases in *G6pc* are moderate, indicating that additional target genes and mechanisms contribute to FoxO regulation of HGP (Haeusler et al., 2010) (Figure 2).

Insulin Signaling Pathways to HGP

The Irs/PI-3K/Akt/FoxO pathway is critical for insulin regulation of hepatic glucose metabolism (Dong et al., 2008). Additional serine/threonine kinases, such as atypical PKCs and salt-inducible kinases (SIK1 and 2), have been proposed to regulate HGP. But atypical PKC knockout mice have normal HGP (Matsumoto et al., 2003), amidst lingering controversy on PKC activation by insulin. The involvement of SIK1 and 2 is indirect: SIK1 is regulated transcriptionally (Koo et al., 2005), whereas SIK2 is regulated via Akt-dependent phosphorylation (Dentin et al., 2007) and can thus likely be subsumed under the Akt pathway. Signal transducer and activator of transcription 3 (STAT3) is phosphorylated in response to insulin, and its ablation increases HGP (Inoue et al., 2004a). However, this appears to be an indirect effect of insulin, as will be discussed in the section on liver/brain interactions.

Attempts to identify Akt modulators that affect HGP have been marred by confusing results. Tribbles homolog 3 (Trb3) was identified as an inhibitory pseudosubstrate of Akt, whose gain of function induced insulin resistance and raised glycemia in mice (Du et al., 2003). But loss-of-function data are generally inconsistent with a role of Trb3 in insulin action or HGP: whereas one report showed improved glucose tolerance after small interfering RNA-mediated Trb3 knockdown (Koo et al., 2004), Akt signaling and hepatic glucose metabolism were normal in different models of Trb3 loss of function in rodents (Okamoto et al., 2007). A similar fate befell adaptor protein APPL1: originally shown to prevent the interaction of Trb3 with Akt and increase HGP inhibition by insulin, its knockout had no effect on glucose tolerance and insulin sensitivity (Tan et al., 2010). In summary, it appears that the rise of HGP in diabetes cannot be explained by postreceptor mechanisms of insulin resistance and requires decreased insulin receptor number and/or activity as the sine qua non of reduced insulin signaling (Lauro et al., 1998).

Peroxisome Proliferator-Activated Receptor γ Coactivator-1α

Peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) is induced by fasting and promotes transcription of glucogenic enzymes and mitochondrial proteins (Yoon et al., 2001). FoxO1 is the obligate partner for the former, but not for the latter effect (Matsumoto et al., 2007). The FoxO1 requirement explains why knockout of *Pgc1α* has modest effects on HGP (Lin et al., 2004). Substrate flux analysis by MR spectroscopy shows that PGC-1α affects HGP by promoting mitochondrial biogenesis and oxidative metabolism (Burgess et al., 2006). The extent and relative role of direct regulation of PGC-1α by insulin is disputed: one report indicates that insulin decreases *Pgc1α* mRNA (Herzig et al., 2001), while another shows that it promotes PGC-1α phosphorylation (Li et al., 2007). The metabolic phenotype of liver-specific PGC-1α knockouts has not been reported to date.

cAMP Response Element-Binding Protein

Transcription factor cAMP response element-binding protein (CREB) belongs to a family of stress-activated DNA binding proteins with multifaceted functions. Hepatic CREB is phosphorylated in response to glucagon, catecholamines, and insulin (Koo et al., 2005) and binds to *cis*-acting cAMP response elements on target promoters. Phosphorylated CREB acts as a scaffold for coregulators such as CBP, p300, and CREB-regulated transcription coactivator 2 (CRTC2) that activate gluconeogenic genes (Koo et al., 2005). A dominant-negative CREB mutant causes fasting hypoglycemia that can be rescued by PGC-1α overexpression (Herzig et al., 2001). Acute CREB knockdown in liver reduced glycemia and improved insulin sensitivity in diabetic mice and rats (Erion et al., 2009a), but to date we lack a model of liver-specific CREB knockout that would allow us to parse its complex effects. The mechanism by which CREB affects HGP remains unclear, given that the competing hormones insulin and glucagon have similar effects on CREB phosphorylation.

CREB-Regulated Transcriptional Coactivator 2

CREB-regulated transcriptional coactivator 2 (CRTC2; also known as TORC2, not to be confused with the target of rapamycin complex 2) is a CREB coactivator that confers hormone regulation on HGP (Koo et al., 2005). During fasting, CRTC2 is dephosphorylated, allowing its nuclear translocation. Nuclear CRTC2 binds to CREB and recruits CBP and p300 to activate *Pck1*, *G6pc*, and *Pgc1α* transcription (Koo et al., 2005). Insulin triggers CRTC2 phosphorylation via SIK2, promoting its nuclear exclusion and proteosomal degradation (Dentin et al., 2007). Two CRTC2 knockout alleles have been made to test its role in vivo: a liver-specific knockout has no effect on HGP or glycemia (Le Lay et al., 2009), while a ubiquitous knockout lowers fasting glycemia by ~25% as well as *Pck1* and *G6pc* levels, together with a 2-fold reduction of glucose output from isolated hepatocytes, but HGP was not measured in vivo (Wang et al., 2010).

The reasons for this discrepancy are unclear and will have to be addressed if a consensus on the role of CRTC2 in HGP is to be reached.

Glucocorticoids

Glucocorticoids (GCs) drive HGP directly by transcriptional induction of *Pck1* and tyrosine aminotransferase (*Tat*), and indirectly through their actions in muscle and adipose tissue to promote amino acid and glycerol flux to the liver, and by decreasing insulin production in pancreatic β cells. Pharmacological inhibition of the enzyme required to generate bioactive corticosterone, 11β-hydroxysteroid dehydrogenase type 1 improves glucose control in type 2 diabetic patients (Rosenstock et al., 2010). However, regulation of glycogenolysis in primary hepatocytes doesn't require GC (Matsumoto et al., 2007), indicating that these hormones participate primarily in the regulation of HGP by stress, rather than by fasting and feeding.

GC action on *Pck1* and *Tat* transcription is mediated through ligand-induced binding of the nuclear glucocorticoid receptor (GR, encoded by *Nr3c1*) to GC response elements. Liver-specific GR knockout mice are euglycemic in physiologic conditions but are prone to hypoglycemia during starvation (Opherk et al., 2004). Knockdown of liver and adipose GR reduced HGP and glycemia in diabetic rodents, as did a liver-selective synthetic GR antagonist (Watts et al., 2005). These studies indicate that hepatic GC/GR signaling is essential for the stress-induced glycemic response. Efforts to leverage the eminent pharmacological tractability of GCs to modulate HGP are limited by potential side effects of GC blockade on the hypothalamic-pituitary-adrenal axis, and by lingering uncertainty on the contribution of GC excess to the pathogenesis of human diabetes.

NR4As

Transcription of the three orphan nuclear receptors of the NR4A family (Nurr77, Nurr1, and NOR-1, encoded by *Nr4a1*, *2*, and *3*) is rapidly induced by glucagon or fasting in liver (Pei et al., 2006). Overexpression of each NR4A isoform in cultured hepatocytes activates *G6pc*, *Fbp*, and enolase. Accordingly, a pan-NR4A dominant negative mutant lowered HGP, while Nurr77 overexpression in liver increased it (Pei et al., 2006). But whole-body Nurr77 knockout also increased HGP (Chao et al., 2009). It should be noted that glucagon promotes HGP without stimulating new protein synthesis (Exton and Park, 1967), making it unlikely that NR4As—whose induction requires de novo synthesis—are physiologic mediators of glucagon's effects on HGP.

Adenosine 5′-Monophosphate-Activated Protein Kinase

Adenosine 5′-monophosphate-activated protein kinase (AMPK) is a cellular sensor of energy levels. Activated by AMP- or Ca^{2+}-dependent phosphorylation through LKB1 and CaMKKβ, respectively, AMPK generates ATP by increasing fatty acid oxidation and reducing ATP hydrolysis through decreased lipogenesis and glucogenesis (Zhang et al., 2009). Glucagon can also activate AMPK, but it fails to do so in liver-specific *Pck1* knockout mice (Berglund et al., 2009), suggesting that its effects on AMPK are mediated by cellular ATP depletion through gluconeogenesis. Whole-body knockout of AMPKα2—the predominant catalytic subunit in liver—did not affect HGP, whereas liver-specific AMPKα2 knockout caused a modest increase in basal HGP (Andreelli et al., 2006). The multiplicity of AMPK functions complicates the dissection of its upstream regulators and downstream targets.

Upstream. AMPK activators, 5-aminoimidazole-4-carboxamide riboside (AICAR), metformin, A-769662, and adiponectin suppress HGP (Zhang et al., 2009). However, these effects are preserved in mice lacking AMPK (Foretz et al., 2010), indicating that they are mediated through different mechanisms, e.g., cellular respiration. Presently, it's unknown whether the effect of the adipokine resistin on HGP is mediated through its ability to regulate AMPK. Inactivation of the AMPK kinase LKB1 increases glycemia and causes nuclear accumulation of CRTC2 (Shaw et al., 2005), but HGP was not measured in this study, and individual contributions of LKB1 substrates—AMPK, SIK1-2, and MAP/microtubule affinity-regulating kinase 2 (MARK2)—to this phenotype haven't been determined.

Downstream. Despite the important role of FoxO in HGP, its regulation by AMPK has not been examined extensively; in vitro data suggest that AMPK promotes FoxO3a activity (Greer et al., 2007). More efforts have gone into demonstrating the role of AMPK in CRTC2 phosphorylation. The latter can be blocked by AMPK and related kinases (SIK1-2, MARK2) (Koo et al., 2005) and becomes impaired when AMPKα1 and α2 are ablated in liver. The meaning of this observation is mysterious, as these mice have normal HGP (Foretz et al., 2010). The contribution of additional AMPK substrates to HGP is thus far based on in vitro data and awaits experimental confirmation in vivo. Collectively, these data suggest that AMPK and related kinases can restrain gluconeogenesis during extreme energy deficit or diabetes, but not under physiological conditions.

Regulation of HGP by Bile Acid Signaling

Farnesoid X Receptor

Bile acids (BAs) inhibit HGP, and BA sequestrants decrease glycemia and improve dyslipidemia in type 2 diabetics. BAs bind nuclear receptor farnesoid X receptor (FXR) and lower glucose by hepatic and extra-hepatic mechanisms. The BA cholic acid inhibits *Pck1* and *G6pc via* FXR-dependent induction of small heterodimer partner (SHP) (Ma et al., 2006). Phenotyping of FXR knockouts has yielded different but not irreconcilable results, with one study showing transient fasting hypoglycemia due to defective glycogen turnover (Cariou et al., 2005), and another one showing hyperglycemia secondary to hepatic and muscle insulin resistance (Ma et al., 2006). These observations are likely to reflect multiple glucoregulatory pathways affected by FXR deficiency, such as BA biosynthesis, which can potentially affect incretin secretion (Thomas et al., 2009), and FGF15/19 regulation as discussed below.

The Corepressor SHP

The FXR target gene *Shp* (*Nr0b2*) encodes an atypical orphan nuclear receptor that lacks a DNA binding domain and represses expression of genes implicated in HGP, including *G6pc*, *Pck1*, and *Pdk4* (Kim et al., 2008). *Shp* knockout mice show modest fed and fasted hyperglycemia and resistance to BA inhibition of *Pck1* and *G6pc* (Ma et al., 2006). Conversely, SHP overexpression in liver reduced *Pck1* and *G6pc* expression and lowered glycemia in diabetic mice (Kim et al., 2008).

Fibroblast Growth Factor 15/19

Postprandial release of BA activates expression of FGF15 (19 in rodents) in the small intestine through FXR (Inagaki et al., 2005).

FGF15 inhibits BA synthesis and promotes hepatic glycogen synthesis. Interestingly, these actions appear to be mediated by ERK rather than Akt (Kir et al., 2011). However, FGF15 can also activate Akt and inhibit *Pck1* through the canonical FoxO1 pathway (Shin and Osborne, 2009). The insulin-like actions of FGF15/19 provide a potential alternative pathway to control HGP.

Orphan Regulators of HGP

CCAAT/Enhancer-Binding Protein α and β

Ablation of transcription factor CCAAT/enhancer-binding protein α (C/EBPα) causes lethal neonatal hypoglycemia due to delayed onset of *G6pc* and *Pck1* expression (Wang et al., 1995). But in the adult animal, data are discordant, with some loss-of-function studies showing significant hypoglycemia and liver damage (Lee et al., 1997), while others showed no effect on HGP (Inoue et al., 2004b). Regardless of this discrepancy, there is no evidence of C/EBPα regulation by hormone signaling. It's worth noting that C/EBPα coordinately controls glycogen synthase, *Pck1*, and *G6pc*, providing a potential mechanism for hepatic autoregulation. C/EBPβ deletion in mice also causes hypoglycemia in neonates and in fasted adults. This phenotype illustrates the difficulties of extrapolating from effects on gene expression to effects on HGP. In fact, while C/EBPβ gain-of-function activates *Pck1* and *G6pc* in hepatocytes, its loss of function lowers HGP by decreasing cAMP production (Croniger et al., 2001), and not by affecting gene expression (Figure 2).

Steroid Receptor Coactivator Family

Histone acetyltransferases steroid receptor coactivator 1 (SRC-1), 2, and 3 coactivate several nuclear receptors and transcription factors. Hepatic expression of SRC-1 and SRC-3, but not SRC-2, is induced by fasting. Despite apparent functional promiscuity in cell culture experiments, different SRC knockout mice have distinct phenotypes. Ubiquitous and liver-specific *Src1* knockout mice are hypoglycemic due to reduced HGP, associated with decreased expression of *Pck1*, *Fbp*, and pyruvate carboxylase, but not *G6pc* (Louet et al., 2010). Mechanistic studies revealed that SRC-1 coactivates C/EBPα and PGC-1α and is required for *Cebpα* and *Foxo1* expression in liver (Louet et al., 2010), consistent with a role in controlling gluconeogenesis during the fed-to-fasting transition. SRC-2 regulates *G6pc* expression by coactivating retinoid-related orphan receptor α, and hepatic SRC-2 loss of function led to fasting hypoglycemia, owing to low *G6pc* expression, and potential secondary effects of hepatic BA accumulation due to defective secretion into the gut (Chopra et al., 2011). The role of SRC-3 in HGP has not been determined. To establish that these interesting observations have physiological relevance, it will be necessary to study whether hormones and nutrients regulate the activity of these coactivators.

Sirtuin-1

The NAD^+-dependent protein deacetylase Sirt1 can activate HGP by deacetylating its substrates PGC-1α, FoxO1, STAT3, and FXR (Schwer and Verdin, 2008). Changes of hepatic glucose metabolism in liver-specific Sirt1 knockouts are unremarkable (Chen et al., 2008), but this might reflect opposing effects of other Sirt1-dependent processes, such as CRTC2 degradation (Liu et al., 2008), SHP induction (Wei et al., 2011), and AMPK activation (Hou et al., 2008). In fact, Sirt1 ablation lowered HGP in insulin-resistant mice lacking both Irs1 and Irs2, restoring the postprandial suppression of *Pgc1α* and *Pck1*. And acute knockdown of Sirt1 in liver of rodents (Erion et al., 2009b) reduced fasting glycemia, decreased *Pck1*, *G6pc*, and *Fbp*, and increased glucokinase. Conversely, Sirt1 overexpression in liver increased gluconeogenic gene expression during fasting (Rodgers and Puigserver, 2007), but ubiquitous overexpression reduced fasting glycemia and improved glucose tolerance in obese mice, despite constitutive deacetylation of PGC-1α and FoxO1 in liver (Banks et al., 2008), owing possibly to decreased hepatosteatosis and increased adiponectin levels. Under conditions of nutrient excess, these indirect pathways might trump Sirt1's direct gluconeogenic effects.

Circadian Pacemakers

Circadian oscillations in glucose and lipid metabolism are well documented and disruptions of such temporal regulation are associated with metabolic diseases. Core clock components regulate rhythmicity and amplitude of HGP, including Clock, Bmal1, Period2, Cryptochrome (Cry) 1 and 2, and Rev-Erbα (Bass and Takahashi, 2010). The liver clock is regulated by cellular energy sensors such as AMPK, which modulates Cry1 degradation (Lamia et al., 2009), and NAD^+, which regulates Sirt1- and poly [ADP-ribose] polymerase-1-dependent post-translational modifications of clock components (Nakahata et al., 2008). PGC-1α and GR (So et al., 2009) can also modulate components of the liver clock. Additional work is necessary to dissect the significance of these pathways in diabetes pathogenesis and treatment.

Direct versus Indirect Mechanisms

Central Nervous System Effects on HGP in Rodents

In rodents, the direct effects of insulin in liver are necessary, but not sufficient to inhibit HGP (Okamoto et al., 2005). Insulin receptor signaling in hypothalamic neurons affects HGP by activating K_{ATP} channels and suppressing flux through G6PC, but not PCK1 (Obici and Rossetti, 2003). The site of insulin action includes orexigenic (appetite-promoting) NPY/AgRP neurons (Könner et al., 2007) and anorexigenic POMC neurons (Lin et al., 2010). The mechanism may also involve activation of STAT3 via IL-6 signaling (Inoue et al., 2006), providing a potential link with inflammatory changes that are associated with insulin resistance.

Whether neuronal control of HGP is unique to rodents remains disputed. HGP rates in rodents are ~10-fold higher than humans, indicating that potential CNS effects might go undetected in human studies. Direct delivery of insulin to the CNS of dogs doesn't affect HGP, raising the possibility that the "CNS effect" on HGP reflect a failure to properly replace basal portal insulin levels during glucose clamps (Edgerton et al., 2006). However, it should be noted that insulin fails to suppress HGP in mice lacking peripheral but not hepatic insulin receptors. In these mice, the portohepatic insulin gradient is probably ablated by the lack of receptor-mediated insulin clearance in tissues exposed to the systemic circulation, and portal insulin levels should suffice to inhibit HGP—but don't (Okamoto et al., 2005).

Free Fatty Acids, Adipokines, and HGP

FFA uptake into the liver is thought to impair HGP regulation by insulin (Lewis et al., 1997). However, the rise of FFAs in insulin

resistance antedates fasting hyperglycemia—the hallmark of increased HGP. And the onset of hyperglycemia is not associated with worsening FFA profiles. Furthermore, manipulations of circulating F elicit similar changes in gluconeogenesis in type 2 diabetics and nondiabetic subjects (Boden et al., 2001). In mice lacking hepatocyte insulin receptors, acute lowering of FFAs fails to suppress HGP (Fisher and Kahn, 2003). Therefore, FFAs likely contribute to render HGP refractory to insulin but are unlikely to be the inciting factor. Adding to the complexity of this signaling mechanism, hypothalamic sensing of circulating FFAs regulates HGP, and may counteract the direct effect of FFAs on hepatocytes (Lam et al., 2005). Whether FFAs are the bellwether of other adipocyte secretory products that impair insulin action on HGP remains controversial. In vivo regulation of leptin and adiponectin by insulin requires prolonged incubations. Thus, neither is likely to contribute to the rapid hormonal regulation of HGP, but they might contribute to setting a basal HGP tone.

Renal and Intestinal Gluconeogenesis

Renal epithelial cells produce glucose through a process regulated by both insulin and pH. Cultured renal epithelial cells lose the ability to respond to insulin, which can be restored by reconstituting FoxO1 (Nakae et al., 2001). The small intestine also expresses *G6pc* and makes a contribution to systemic glucose production that may become more significant in starvation and diabetes. Intestinal gluconeogenesis has been implicated in nutrient sensing in the portal vein (Troy et al., 2008). These observations need to be interpreted with caution because of the technical challenges in accurately measuring glucose production in tissues with highly active glucose utilization such as kidney and intestine.

Controversial Areas

Flux Control versus Gene Transcription

As indicated, a creative tension exists between the physiologists, who never fail to remind us that flux control trumps transcriptional control of HGP, and the cell/molecular biologists, who have been tenaciously chasing hormone-regulated transcription factors as the holy grail of HGP. Based on the data reviewed here, the truth appears to lie somewhere in between: in vivo, it takes ~30 min to detect effects on glycogenolysis and gluconeogenesis, and while the latter are unrelated to changes in *Pck1* mRNA—let alone protein—the former show a striking correlation with *G6pc* mRNA and FoxO1 phosphorylation (Ramnanan et al., 2010). Hence, we propose that flux control is important in the first 30 min but that gene transcription kicks in earlier than formerly surmised.

How Many Genes Mediate Hormonal Effects on HGP?

HGP is a complex and genetically heterogeneous process that cannot be subsumed under a single mechanism. Nonetheless, as illustrated by the litany of "buts," "unclears," and "unknowns" that graces this overview, most of the genes proposed to play a role in this process fail one or more tests of physiologic relevance. Among the reasons for this discrepancy are the following: (1) Obliviousness to—and, for younger researchers, unawareness of—the "Exton and Park" criteria. (2) Variations of mRNAs encoding glucogenic enzymes, let alone changes in reporter gene activities in hepatoma cells, do not portend effects on HGP—the latter should be tested directly. (3) Genetic ablation experiments resulting in fasting hypoglycemia don't necessarily indicate a physiologic role in *hormonal* regulation of HGP, given the latter's redundancy. (4) Gain-of-function experiments with transcription factors and coactivators should be interpreted cautiously, as they are especially prone to artifacts. (5) Physiologically relevant conclusions on the role of any given gene product should be supported by both acute and chronic manipulations in vivo. Acute transduction of the liver with DNA- or RNA-based reagents by direct delivery, while expeditious, often results in hepatocyte damage, itself a regulator of HGP. As a result, interpretation of such data is problematic. (6) Conversely, compensatory mechanisms (e.g., glucose cycling) may obfuscate the interpretation of gene knockouts. A useful approach in this regard is to study induction of HGP at birth, as it first acquires hormone responsiveness (Girard et al., 1992). (7) Many genes affect HGP indirectly, through effects on hepatocyte function or intercellular communication that are not involved in physiologic hormonal or nutrient regulation; to determine their relevance to physiologic conditions and disease states, it's helpful to study their posttranslational regulation in response to insulin and glucagon.

Gluconeogenesis and Pck1

Recent studies have led to a reassessment of the role of PCK1 as rate-limiting enzyme in gluconeogenesis. Liver-specific *Pck1* knockouts show a blockade of lactate and amino acid-derived gluconeogenesis but maintain normal fasting glycemia, possibly owing to increased gluconeogenesis from glycerol and reduced glucose utilization (She et al., 2003). Ex vivo studies in mouse livers with various levels of *Pck1* ablation show a tight correlation between PCK1 activity and TCA cycle flux, but weak control by PCK1 over gluconeogenic capacity (Burgess et al., 2007). The level of HGP regulation exerted through *Pck1* transcription in vivo is also modest, as gluconeogenesis can be suppressed without changes in *Pck1* expression in dogs (Ramnanan et al., 2010), and liver biopsies from patients with type 2 diabetes fail to demonstrate changes in *Pck1* (Samuel et al., 2009). In vitro, PCK1 can be inhibited by acetylation independent of changes in protein levels (Lin et al., 2009), but the physiological significance of this finding in liver metabolism hasn't been determined.

Therapeutic Implications

How Does Metformin Work?

Several explanations for the effect of metformin to decrease HGP have been set forth. The original hypothesis, that it does so through activation of AMPK, has not been borne out by genetic ablation of AMPK or its kinase LKB1, which, if anything, sensitizes to metformin action on plasma glucose levels (Foretz et al., 2010). Another study found that metformin was unable to lower glycemia in liver LKB1-deficient mice, but this study didn't measure HGP (Shaw et al., 2005). Metformin reduces cellular respiration in hepatocytes by inhibiting mitochondrial respiratory chain complex I (El-Mir et al., 2000) and might thus blunt gluconeogenesis by reducing intracellular ATP. Interestingly, thiazolidinediones and berberine are also mild inhibitors of respiratory chain complex I (Turner et al., 2008). Other potential mediators include elevations in AMP/ATP or NAD^+/NADH ratios or reactive nitrogen species (Fujita et al., 2010). The alternative explanation that metformin decreases HGP via CBP phosphorylation by PKCλ, leading to decreased CREB/CRTC2 complex formation (He et al., 2009), is unlikely considering the lack of effect on

HGP of CRTC2 (Le Lay et al., 2009) or PKCλ knockouts (Matsumoto et al., 2003). Metformin has been shown to decrease mRNA levels of transcription factor KLF-15, whose targets include *G6pc* and *Pck1* (Takashima et al., 2010), but the signaling pathways by which metformin regulates gene expression are unknown. In sum, it's unlikely that metformin has an obligate target. It's more likely to act by lowering ATP levels, with pleiotropic consequences. The role of metformin transporters and their complex genetic variation in determining metformin sensitivity and failure remains underscrutinized.

Future Prospects

Is HGP inhibition the right approach to diabetes treatment? Arguably, increased HGP is a "late" defect in disease progression, and it's conceivable that preventive treatments (for example, increasing glucose disposal or enhancing β cell function) will offset the need to intervene on this aspect of liver dysfunction in diabetes. On the other hand, other pathogenetic mechanisms (increased liver fat content and secretion of VLDL-rich lipoproteins) will continue to require targeting the liver in diabetes (Kim-Muller and Accili, 2011). Thus, we are unlikely to write off the liver as a site of action of antidiabetic medications any time soon. Among the mechanisms that have been explored to sensitize the liver to insulin, the key obstacle has been the potential for hypoglycemia, an inevitable concern with drugs that concurrently inhibit HGP and enhance glucose disposal.

As glucagon levels are inappropriately elevated in diabetes, inhibition of the glucagon receptor pathway is an attractive therapeutic path. Acute inactivation of the glucagon receptor (GcgR) lowers glucose in diabetic mice, while GcgR knockout mice are hypoglycemic and resistant to STZ-induced diabetes (Conarello et al., 2007). Inhibitors of glucagon/GcgR action have shown promise in diabetes treatment, but their long-term impact on lipid and amino acid metabolism is yet unknown.

Sirt1 activators lower glycemia in diabetic rodents but are tainted by controversies over their bona fide Sirt1 activation and in vivo efficacy (Pacholec et al., 2010). Activators of the xenobiotic receptor, constitutive androstane receptor (CAR), lower hyperglycemia and body weight in mice (Dong et al., 2009), but the role of CAR in drug metabolism and thyroid hormone metabolism may hamper their utility as antidiabetic compounds. Inhibition of PDK4 or FBPase has a long and checkered history dating back to dichloroacetate. It remains challenging to fine-tune glucose flux without causing irreversible hypoglycemia or contributing to lactic acidosis. Buoyed by new injection devices and a broader acceptance of parenteral treatments, peptide mimetics of insulin action, or "selective insulin sensitizers" deserve scrutiny (Kim-Muller and Accili, 2011).

Conclusions

Key biochemical, cellular, and integrated physiological mechanisms by which hormones regulate HGP have been clarified. But few players thus identified are tractable drug targets. Therefore, given its efficacy, tolerability and inexpensiveness, metformin is likely to remain the mainstay of therapy, despite its limitations. New antidiabetic agents should therefore either reduce HGP by a mechanism distinct from metformin's, or target different aspects of diabetes pathophysiology (impaired glucose uptake, excessive lipolysis, increased tissue fat content, β cell dysfunction, elevated atherogenic lipoproteins), or improve safety vis-à-vis lactic acidosis or use in renal failure, or outperform metformin's durability. The studies reviewed here will help chart the way ahead.

ACKNOWLEDGMENTS

Supported by grants from the National Institutes of Health, the Russell Berrie Foundation, and the Brehm Coalition. We thank members of the Accili laboratory and colleagues at Merck Research Laboratories for insightful discussions and critical reading of the manuscript. We regret that we are unable to credit all contributors to this field.

REFERENCES

Accili, D., and Arden, K.C. (2004). FoxOs at the crossroads of cellular metabolism, differentiation, and transformation. Cell *117*, 421–426.

Andreelli, F., Foretz, M., Knauf, C., Cani, P.D., Perrin, C., Iglesias, M.A., Pillot, B., Bado, A., Tronche, F., Mithieux, G., et al. (2006). Liver adenosine monophosphate-activated kinase-alpha2 catalytic subunit is a key target for the control of hepatic glucose production by adiponectin and leptin but not insulin. Endocrinology *147*, 2432–2441.

Banks, A.S., Kon, N., Knight, C., Matsumoto, M., Gutiérrez-Juárez, R., Rossetti, L., Gu, W., and Accili, D. (2008). SirT1 gain of function increases energy efficiency and prevents diabetes in mice. Cell Metab. *8*, 333–341.

Bass, J., and Takahashi, J.S. (2010). Circadian integration of metabolism and energetics. Science *330*, 1349–1354.

Berglund, E.D., Lee-Young, R.S., Lustig, D.G., Lynes, S.E., Donahue, E.P., Camacho, R.C., Meredith, M.E., Magnuson, M.A., Charron, M.J., and Wasserman, D.H. (2009). Hepatic energy state is regulated by glucagon receptor signaling in mice. J. Clin. Invest. *119*, 2412–2422.

Bergman, R.N., and Ader, M. (2000). Free fatty acids and pathogenesis of type 2 diabetes mellitus. Trends Endocrinol. Metab. *11*, 351–356.

Boden, G., Chen, X., Capulong, E., and Mozzoli, M. (2001). Effects of free fatty acids on gluconeogenesis and autoregulation of glucose production in type 2 diabetes. Diabetes *50*, 810–816.

Bogardus, C., Lillioja, S., Howard, B.V., Reaven, G., and Mott, D. (1984). Relationships between insulin secretion, insulin action, and fasting plasma glucose concentration in nondiabetic and noninsulin-dependent diabetic subjects. J. Clin. Invest. *74*, 1238–1246.

Burgess, S.C., Leone, T.C., Wende, A.R., Croce, M.A., Chen, Z., Sherry, A.D., Malloy, C.R., and Finck, B.N. (2006). Diminished hepatic gluconeogenesis via defects in tricarboxylic acid cycle flux in peroxisome proliferator-activated receptor gamma coactivator-1alpha (PGC-1alpha)-deficient mice. J. Biol. Chem. *281*, 19000–19008.

Burgess, S.C., He, T., Yan, Z., Lindner, J., Sherry, A.D., Malloy, C.R., Browning, J.D., and Magnuson, M.A. (2007). Cytosolic phosphoenolpyruvate carboxykinase does not solely control the rate of hepatic gluconeogenesis in the intact mouse liver. Cell Metab. *5*, 313–320.

Cariou, B., van Harmelen, K., Duran-Sandoval, D., van Dijk, T., Grefhorst, A., Bouchaert, E., Fruchart, J.C., Gonzalez, F.J., Kuipers, F., and Staels, B. (2005). Transient impairment of the adaptive response to fasting in FXR-deficient mice. FEBS Lett. *579*, 4076–4080.

Chao, L.C., Wroblewski, K., Zhang, Z., Pei, L., Vergnes, L., Ilkayeva, O.R., Ding, S.Y., Reue, K., Watt, M.J., Newgard, C.B., et al. (2009). Insulin resistance and altered systemic glucose metabolism in mice lacking Nur77. Diabetes *58*, 2788–2796.

Chen, D., Bruno, J., Easlon, E., Lin, S.J., Cheng, H.L., Alt, F.W., and Guarente, L. (2008). Tissue-specific regulation of SIRT1 by calorie restriction. Genes Dev. *22*, 1753–1757.

Cherrington, A.D. (1999). Banting Lecture 1997. Control of glucose uptake and release by the liver in vivo. Diabetes *48*, 1198–1214.

Chopra, A.R., Kommagani, R., Saha, P., Louet, J.F., Salazar, C., Song, J., Jeong, J., Finegold, M., Viollet, B., DeMayo, F., et al. (2011). Cellular energy depletion resets whole-body energy by promoting coactivator-mediated dietary fuel absorption. Cell Metab. *13*, 35–43.

Conarello, S.L., Jiang, G., Mu, J., Li, Z., Woods, J., Zycband, E., Ronan, J., Liu, F., Roy, R.S., Zhu, L., et al. (2007). Glucagon receptor knockout mice are resistant to diet-induced obesity and streptozotocin-mediated beta cell loss and hyperglycaemia. Diabetologia *50*, 142–150.

Croniger, C.M., Millward, C., Yang, J., Kawai, Y., Arinze, I.J., Liu, S., Harada-Shiba, M., Chakravarty, K., Friedman, J.E., Poli, V., and Hanson, R.W. (2001). Mice with a deletion in the gene for CCAAT/enhancer-binding protein beta have an attenuated response to cAMP and impaired carbohydrate metabolism. J. Biol. Chem. *276*, 629–638.

Dentin, R., Liu, Y., Koo, S.H., Hedrick, S., Vargas, T., Heredia, J., Yates, J., 3rd, and Montminy, M. (2007). Insulin modulates gluconeogenesis by inhibition of the coactivator TORC2. Nature *449*, 366–369.

Dong, X.C., Copps, K.D., Guo, S., Li, Y., Kollipara, R., DePinho, R.A., and White, M.F. (2008). Inactivation of hepatic Foxo1 by insulin signaling is required for adaptive nutrient homeostasis and endocrine growth regulation. Cell Metab. *8*, 65–76.

Dong, B., Saha, P.K., Huang, W., Chen, W., Abu-Elheiga, L.A., Wakil, S.J., Stevens, R.D., Ilkayeva, O., Newgard, C.B., Chan, L., and Moore, D.D. (2009). Activation of nuclear receptor CAR ameliorates diabetes and fatty liver disease. Proc. Natl. Acad. Sci. USA *106*, 18831–18836.

Du, K., Herzig, S., Kulkarni, R.N., and Montminy, M. (2003). TRB3: a tribbles homolog that inhibits Akt/PKB activation by insulin in liver. Science *300*, 1574–1577.

Edgerton, D.S., Lautz, M., Scott, M., Everett, C.A., Stettler, K.M., Neal, D.W., Chu, C.A., and Cherrington, A.D. (2006). Insulin's direct effects on the liver dominate the control of hepatic glucose production. J. Clin. Invest. *116*, 521–527.

Edgerton, D.S., Ramnanan, C.J., Grueter, C.A., Johnson, K.M., Lautz, M., Neal, D.W., Williams, P.E., and Cherrington, A.D. (2009). Effects of insulin on the metabolic control of hepatic gluconeogenesis in vivo. Diabetes *58*, 2766–2775.

El-Mir, M.Y., Nogueira, V., Fontaine, E., Avéret, N., Rigoulet, M., and Leverve, X. (2000). Dimethylbiguanide inhibits cell respiration via an indirect effect targeted on the respiratory chain complex I. J. Biol. Chem. *275*, 223–228.

Erion, D.M., Ignatova, I.D., Yonemitsu, S., Nagai, Y., Chatterjee, P., Weismann, D., Hsiao, J.J., Zhang, D., Iwasaki, T., Stark, R., et al. (2009a). Prevention of hepatic steatosis and hepatic insulin resistance by knockdown of cAMP response element-binding protein. Cell Metab. *10*, 499–506.

Erion, D.M., Yonemitsu, S., Nie, Y., Nagai, Y., Gillum, M.P., Hsiao, J.J., Iwasaki, T., Stark, R., Weismann, D., Yu, X.X., et al. (2009b). SirT1 knockdown in liver decreases basal hepatic glucose production and increases hepatic insulin responsiveness in diabetic rats. Proc. Natl. Acad. Sci. USA *106*, 11288–11293.

Exton, J.H., and Park, C.R. (1967). Control of gluconeogenesis in liver. I. General features of gluconeogenesis in the perfused livers of rats. J. Biol. Chem. *242*, 2622–2636.

Fisher, S.J., and Kahn, C.R. (2003). Insulin signaling is required for insulin's direct and indirect action on hepatic glucose production. J. Clin. Invest. *111*, 463–468.

Foretz, M., Hébrard, S., Leclerc, J., Zarrinpashneh, E., Soty, M., Mithieux, G., Sakamoto, K., Andreelli, F., and Viollet, B. (2010). Metformin inhibits hepatic gluconeogenesis in mice independently of the LKB1/AMPK pathway via a decrease in hepatic energy state. J. Clin. Invest. *120*, 2355–2369.

Fujita, Y., Hosokawa, M., Fujimoto, S., Mukai, E., Abudukadier, A., Obara, A., Ogura, M., Nakamura, Y., Toyoda, K., Nagashima, K., et al. (2010). Metformin suppresses hepatic gluconeogenesis and lowers fasting blood glucose levels through reactive nitrogen species in mice. Diabetologia *53*, 1472–1481.

Gastaldelli, A., Toschi, E., Pettiti, M., Frascerra, S., Quiñones-Galvan, A., Sironi, A.M., Natali, A., and Ferrannini, E. (2001). Effect of physiological hyperinsulinemia on gluconeogenesis in nondiabetic subjects and in type 2 diabetic patients. Diabetes *50*, 1807–1812.

Girard, J., Ferré, P., Pégorier, J.P., and Duée, P.H. (1992). Adaptations of glucose and fatty acid metabolism during perinatal period and suckling-weaning transition. Physiol. Rev. *72*, 507–562.

Greer, E.L., Oskoui, P.R., Banko, M.R., Maniar, J.M., Gygi, M.P., Gygi, S.P., and Brunet, A. (2007). The energy sensor AMP-activated protein kinase directly regulates the mammalian FOXO3 transcription factor. J. Biol. Chem. *282*, 30107–30119.

Gumbiner, B., van Poelje, P., Bullough, D., Watling, S., Milad, M., Stern, T., Foyt, H., and Erion, M. (2009). Pronounced glucose (G) reduction in poorly controlled T2DM with MB07803, a novel fructose-1,6-bisphosphatase inhibitor (FBPasel) with reduced potential for acid-base disturbance vs the 1st generation FBPasel CS-917. Diabetes *58* (*S1*), A11-LB.

Haeusler, R.A., Kaestner, K.H., and Accili, D. (2010). FoxOs function synergistically to promote glucose production. J. Biol. Chem. *285*, 35245–35248.

He, L., Sabet, A., Djedjos, S., Miller, R., Sun, X., Hussain, M.A., Radovick, S., and Wondisford, F.E. (2009). Metformin and insulin suppress hepatic gluconeogenesis through phosphorylation of CREB binding protein. Cell *137*, 635–646.

Herzig, S., Long, F., Jhala, U.S., Hedrick, S., Quinn, R., Bauer, A., Rudolph, D., Schutz, G., Yoon, C., Puigserver, P., et al. (2001). CREB regulates hepatic gluconeogenesis through the coactivator PGC-1. Nature *413*, 179–183.

Holloszy, J.O., and Kohrt, W.M. (1996). Regulation of carbohydrate and fat metabolism during and after exercise. Annu. Rev. Nutr. *16*, 121–138.

Hou, X., Xu, S., Maitland-Toolan, K.A., Sato, K., Jiang, B., Ido, Y., Lan, F., Walsh, K., Wierzbicki, M., Verbeuren, T.J., et al. (2008). SIRT1 regulates hepatocyte lipid metabolism through activating AMP-activated protein kinase. J. Biol. Chem. *283*, 20015–20026.

Inagaki, T., Choi, M., Moschetta, A., Peng, L., Cummins, C.L., McDonald, J.G., Luo, G., Jones, S.A., Goodwin, B., Richardson, J.A., et al. (2005). Fibroblast growth factor 15 functions as an enterohepatic signal to regulate bile acid homeostasis. Cell Metab. *2*, 217–225.

Inoue, H., Ogawa, W., Ozaki, M., Haga, S., Matsumoto, M., Furukawa, K., Hashimoto, N., Kido, Y., Mori, T., Sakaue, H., et al. (2004a). Role of STAT-3 in regulation of hepatic gluconeogenic genes and carbohydrate metabolism in vivo. Nat. Med. *10*, 168–174.

Inoue, Y., Inoue, J., Lambert, G., Yim, S.H., and Gonzalez, F.J. (2004b). Disruption of hepatic C/EBPalpha results in impaired glucose tolerance and age-dependent hepatosteatosis. J. Biol. Chem. *279*, 44740–44748.

Inoue, H., Ogawa, W., Asakawa, A., Okamoto, Y., Nishizawa, A., Matsumoto, M., Teshigawara, K., Matsuki, Y., Watanabe, E., Hiramatsu, R., et al. (2006). Role of hepatic STAT3 in brain-insulin action on hepatic glucose production. Cell Metab. *3*, 267–275.

Jeoung, N.H., Wu, P., Joshi, M.A., Jaskiewicz, J., Bock, C.B., Depaoli-Roach, A.A., and Harris, R.A. (2006). Role of pyruvate dehydrogenase kinase isoenzyme 4 (PDHK4) in glucose homoeostasis during starvation. Biochem. J. *397*, 417–425.

Kim, Y.D., Park, K.G., Lee, Y.S., Park, Y.Y., Kim, D.K., Nedumaran, B., Jang, W.G., Cho, W.J., Ha, J., Lee, I.K., et al. (2008). Metformin inhibits hepatic gluconeogenesis through AMP-activated protein kinase-dependent regulation of the orphan nuclear receptor SHP. Diabetes *57*, 306–314.

Kim-Muller, J.Y., and Accili, D. (2011). Cell biology. Selective insulin sensitizers. Science *331*, 1529–1531.

Kir, S., Beddow, S.A., Samuel, V.T., Miller, P., Previs, S.F., Suino-Powell, K., Xu, H.E., Shulman, G.I., Kliewer, S.A., and Mangelsdorf, D.J. (2011). FGF19 as a postprandial, insulin-independent activator of hepatic protein and glycogen synthesis. Science *331*, 1621–1624.

Könner, A.C., Janoschek, R., Plum, L., Jordan, S.D., Rother, E., Ma, X., Xu, C., Enriori, P., Hampel, B., Barsh, G.S., et al. (2007). Insulin action in AgRP-expressing neurons is required for suppression of hepatic glucose production. Cell Metab. *5*, 438–449.

Koo, S.H., Satoh, H., Herzig, S., Lee, C.H., Hedrick, S., Kulkarni, R., Evans, R.M., Olefsky, J., and Montminy, M. (2004). PGC-1 promotes insulin resistance in liver through PPAR-alpha-dependent induction of TRB-3. Nat. Med. *10*, 530–534.

Koo, S.H., Flechner, L., Qi, L., Zhang, X., Screaton, R.A., Jeffries, S., Hedrick, S., Xu, W., Boussouar, F., Brindle, P., et al. (2005). The CREB coactivator TORC2 is a key regulator of fasting glucose metabolism. Nature *437*, 1109–1111.

Lam, T.K., Pocai, A., Gutierrez-Juarez, R., Obici, S., Bryan, J., Aguilar-Bryan, L., Schwartz, G.J., and Rossetti, L. (2005). Hypothalamic sensing of circulating fatty acids is required for glucose homeostasis. Nat. Med. *11*, 320–327.

Lamia, K.A., Sachdeva, U.M., DiTacchio, L., Williams, E.C., Alvarez, J.G., Egan, D.F., Vasquez, D.S., Juguilon, H., Panda, S., Shaw, R.J., et al. (2009). AMPK regulates the circadian clock by cryptochrome phosphorylation and degradation. Science *326*, 437–440.

Lauro, D., Kido, Y., Castle, A.L., Zarnowski, M.J., Hayashi, H., Ebina, Y., and Accili, D. (1998). Impaired glucose tolerance in mice with a targeted impairment of insulin action in muscle and adipose tissue. Nat. Genet. *20*, 294–298.

Le Lay, J., Tuteja, G., White, P., Dhir, R., Ahima, R., and Kaestner, K.H. (2009). CRTC2 (TORC2) contributes to the transcriptional response to fasting in the liver but is not required for the maintenance of glucose homeostasis. Cell Metab. *10*, 55–62.

Lee, Y.H., Sauer, B., Johnson, P.F., and Gonzalez, F.J. (1997). Disruption of the c/ebp alpha gene in adult mouse liver. Mol. Cell. Biol. *17*, 6014–6022.

Lewis, G.F., Vranic, M., Harley, P., and Giacca, A. (1997). Fatty acids mediate the acute extrahepatic effects of insulin on hepatic glucose production in humans. Diabetes *46*, 1111–1119.

Li, X., Monks, B., Ge, Q., and Birnbaum, M.J. (2007). Akt/PKB regulates hepatic metabolism by directly inhibiting PGC-1alpha transcription coactivator. Nature *447*, 1012–1016.

Lin, J., Wu, P.H., Tarr, P.T., Lindenberg, K.S., St-Pierre, J., Zhang, C.Y., Mootha, V.K., Jäger, S., Vianna, C.R., Reznick, R.M., et al. (2004). Defects in adaptive energy metabolism with CNS-linked hyperactivity in PGC-1alpha null mice. Cell *119*, 121–135.

Lin, Y.Y., Lu, J.Y., Zhang, J., Walter, W., Dang, W., Wan, J., Tao, S.C., Qian, J., Zhao, Y., Boeke, J.D., et al. (2009). Protein acetylation microarray reveals that NuA4 controls key metabolic target regulating gluconeogenesis. Cell *136*, 1073–1084.

Lin, H.V., Plum, L., Ono, H., Gutiérrez-Juárez, R., Shanabrough, M., Borok, E., Horvath, T.L., Rossetti, L., and Accili, D. (2010). Divergent regulation of energy expenditure and hepatic glucose production by insulin receptor in agouti-related protein and POMC neurons. Diabetes *59*, 337–346.

Liu, Y., Dentin, R., Chen, D., Hedrick, S., Ravnskjaer, K., Schenk, S., Milne, J., Meyers, D.J., Cole, P., Yates, J., 3rd., et al. (2008). A fasting inducible switch modulates gluconeogenesis via activator/coactivator exchange. Nature *456*, 269–273.

Louet, J.F., Chopra, A.R., Sagen, J.V., An, J., York, B., Tannour-Louet, M., Saha, P.K., Stevens, R.D., Wenner, B.R., Ilkayeva, O.R., et al. (2010). The coactivator SRC-1 is an essential coordinator of hepatic glucose production. Cell Metab. *12*, 606–618.

Ma, K., Saha, P.K., Chan, L., and Moore, D.D. (2006). Farnesoid X receptor is essential for normal glucose homeostasis. J. Clin. Invest. *116*, 1102–1109.

Matsumoto, M., Ogawa, W., Akimoto, K., Inoue, H., Miyake, K., Furukawa, K., Hayashi, Y., Iguchi, H., Matsuki, Y., Hiramatsu, R., et al. (2003). PKClambda in liver mediates insulin-induced SREBP-1c expression and determines both hepatic lipid content and overall insulin sensitivity. J. Clin. Invest. *112*, 935–944.

Matsumoto, M., Pocai, A., Rossetti, L., Depinho, R.A., and Accili, D. (2007). Impaired regulation of hepatic glucose production in mice lacking the forkhead transcription factor Foxo1 in liver. Cell Metab. *6*, 208–216.

Mayers, R.M., Leighton, B., and Kilgour, E. (2005). PDH kinase inhibitors: a novel therapy for Type II diabetes? Biochem. Soc. Trans. *33*, 367–370.

Monnier, L., Colette, C., Dunseath, G.J., and Owens, D.R. (2007). The loss of postprandial glycemic control precedes stepwise deterioration of fasting with worsening diabetes. Diabetes Care *30*, 263–269.

Nakae, J., Kitamura, T., Silver, D.L., and Accili, D. (2001). The forkhead transcription factor Foxo1 (Fkhr) confers insulin sensitivity onto glucose-6-phosphatase expression. J. Clin. Invest. *108*, 1359–1367.

Nakae, J., Biggs, W.H., 3rd, Kitamura, T., Cavenee, W.K., Wright, C.V., Arden, K.C., and Accili, D. (2002). Regulation of insulin action and pancreatic beta-cell function by mutated alleles of the gene encoding forkhead transcription factor Foxo1. Nat. Genet. *32*, 245–253.

Nakahata, Y., Kaluzova, M., Grimaldi, B., Sahar, S., Hirayama, J., Chen, D., Guarente, L.P., and Sassone-Corsi, P. (2008). The NAD+-dependent deacetylase SIRT1 modulates CLOCK-mediated chromatin remodeling and circadian control. Cell *134*, 329–340.

O'Brien, R.M., and Granner, D.K. (1996). Regulation of gene expression by insulin. Physiol. Rev. *76*, 1109–1161.

Obici, S., and Rossetti, L. (2003). Minireview: nutrient sensing and the regulation of insulin action and energy balance. Endocrinology *144*, 5172–5178.

Okamoto, H., Obici, S., Accili, D., and Rossetti, L. (2005). Restoration of liver insulin signaling in Insr knockout mice fails to normalize hepatic insulin action. J. Clin. Invest. *115*, 1314–1322.

Okamoto, H., Latres, E., Liu, R., Thabet, K., Murphy, A., Valenzeula, D., Yancopoulos, G.D., Stitt, T.N., Glass, D.J., and Sleeman, M.W. (2007). Genetic deletion of Trb3, the mammalian Drosophila tribbles homolog, displays normal hepatic insulin signaling and glucose homeostasis. Diabetes *56*, 1350–1356.

Opherk, C., Tronche, F., Kellendonk, C., Kohlmüller, D., Schulze, A., Schmid, W., and Schütz, G. (2004). Inactivation of the glucocorticoid receptor in hepatocytes leads to fasting hypoglycemia and ameliorates hyperglycemia in streptozotocin-induced diabetes mellitus. Mol. Endocrinol. *18*, 1346–1353.

Pacholec, M., Bleasdale, J.E., Chrunyk, B., Cunningham, D., Flynn, D., Garofalo, R.S., Griffith, D., Griffor, M., Loulakis, P., Pabst, B., et al. (2010). SRT1720, SRT2183, SRT1460, and resveratrol are not direct activators of SIRT1. J. Biol. Chem. *285*, 8340–8351.

Pei, L., Waki, H., Vaitheesvaran, B., Wilpitz, D.C., Kurland, I.J., and Tontonoz, P. (2006). NR4A orphan nuclear receptors are transcriptional regulators of hepatic glucose metabolism. Nat. Med. *12*, 1048–1055.

Petersen, K.F., Laurent, D., Rothman, D.L., Cline, G.W., and Shulman, G.I. (1998). Mechanism by which glucose and insulin inhibit net hepatic glycogenolysis in humans. J. Clin. Invest. *101*, 1203–1209.

Pilkis, S.J., and Claus, T.H. (1991). Hepatic gluconeogenesis/glycolysis: regulation and structure/function relationships of substrate cycle enzymes. Annu. Rev. Nutr. *11*, 465–515.

Raju, B., and Cryer, P.E. (2005). Maintenance of the postabsorptive plasma glucose concentration: insulin or insulin plus glucagon? Am. J. Physiol. Endocrinol. Metab. *289*, E181–E186.

Ramnanan, C.J., Edgerton, D.S., Rivera, N., Irimia-Dominguez, J., Farmer, B., Neal, D.W., Lautz, M., Donahue, E.P., Meyer, C.M., Roach, P.J., and Cherrington, A.D. (2010). Molecular characterization of insulin-mediated suppression of hepatic glucose production in vivo. Diabetes *59*, 1302–1311.

Rizza, R.A. (2010). Pathogenesis of fasting and postprandial hyperglycemia in type 2 diabetes: implications for therapy. Diabetes *59*, 2697–2707.

Roach, P.J. (2002). Glycogen and its metabolism. Curr. Mol. Med. *2*, 101–120.

Rodgers, J.T., and Puigserver, P. (2007). Fasting-dependent glucose and lipid metabolic response through hepatic sirtuin 1. Proc. Natl. Acad. Sci. USA *104*, 12861–12866.

Rosenstock, J., Banarer, S., Fonseca, V.A., Inzucchi, S.E., Sun, W., Yao, W., Hollis, G., Flores, R., Levy, R., Williams, W.V., et al; INCB13739-202 Principal Investigators. (2010). The 11-beta-hydroxysteroid dehydrogenase type 1 inhibitor INCB13739 improves hyperglycemia in patients with type 2 diabetes inadequately controlled by metformin monotherapy. Diabetes Care *33*, 1516–1522.

Samuel, V.T., Beddow, S.A., Iwasaki, T., Zhang, X.M., Chu, X., Still, C.D., Gerhard, G.S., and Shulman, G.I. (2009). Fasting hyperglycemia is not associated with increased expression of PEPCK or G6Pc in patients with Type 2 Diabetes. Proc. Natl. Acad. Sci. USA *106*, 12121–12126.

Schwer, B., and Verdin, E. (2008). Conserved metabolic regulatory functions of sirtuins. Cell Metab. *7*, 104–112.

Shaw, R.J., Lamia, K.A., Vasquez, D., Koo, S.H., Bardeesy, N., Depinho, R.A., Montminy, M., and Cantley, L.C. (2005). The kinase LKB1 mediates glucose homeostasis in liver and therapeutic effects of metformin. Science *310*, 1642–1646.

She, P., Burgess, S.C., Shiota, M., Flakoll, P., Donahue, E.P., Malloy, C.R., Sherry, A.D., and Magnuson, M.A. (2003). Mechanisms by which liver-specific

PEPCK knockout mice preserve euglycemia during starvation. Diabetes *52*, 1649–1654.

Sherwin, R.S., Fisher, M., Hendler, R., and Felig, P. (1976). Hyperglucagonemia and blood glucose regulation in normal, obese and diabetic subjects. N. Engl. J. Med. *294*, 455–461.

Shin, D.J., and Osborne, T.F. (2009). FGF15/FGFR4 integrates growth factor signaling with hepatic bile acid metabolism and insulin action. J. Biol. Chem. *284*, 11110–11120.

So, A.Y., Bernal, T.U., Pillsbury, M.L., Yamamoto, K.R., and Feldman, B.J. (2009). Glucocorticoid regulation of the circadian clock modulates glucose homeostasis. Proc. Natl. Acad. Sci. USA *106*, 17582–17587.

Stumvoll, M., Nurjhan, N., Perriello, G., Dailey, G., and Gerich, J.E. (1995). Metabolic effects of metformin in non-insulin-dependent diabetes mellitus. N. Engl. J. Med. *333*, 550–554.

Takashima, M., Ogawa, W., Hayashi, K., Inoue, H., Kinoshita, S., Okamoto, Y., Sakaue, H., Wataoka, Y., Emi, A., Senga, Y., et al. (2010). Role of KLF15 in regulation of hepatic gluconeogenesis and metformin action. Diabetes *59*, 1608–1615.

Tan, Y., You, H., Coffey, F.J., Wiest, D.L., and Testa, J.R. (2010). Appl1 is dispensable for Akt signaling in vivo and mouse T-cell development. Genesis *48*, 531–539.

Thomas, C., Gioiello, A., Noriega, L., Strehle, A., Oury, J., Rizzo, G., Macchiarulo, A., Yamamoto, H., Mataki, C., Pruzanski, M., et al. (2009). TGR5-mediated bile acid sensing controls glucose homeostasis. Cell Metab. *10*, 167–177.

Troy, S., Soty, M., Ribeiro, L., Laval, L., Migrenne, S., Fioramonti, X., Pillot, B., Fauveau, V., Aubert, R., Viollet, B., et al. (2008). Intestinal gluconeogenesis is a key factor for early metabolic changes after gastric bypass but not after gastric lap-band in mice. Cell Metab. *8*, 201–211.

Turner, N., Li, J.Y., Gosby, A., To, S.W., Cheng, Z., Miyoshi, H., Taketo, M.M., Cooney, G.J., Kraegen, E.W., James, D.E., et al. (2008). Berberine and its more biologically available derivative, dihydroberberine, inhibit mitochondrial respiratory complex I: a mechanism for the action of berberine to activate AMP-activated protein kinase and improve insulin action. Diabetes *57*, 1414–1418.

Wang, N.D., Finegold, M.J., Bradley, A., Ou, C.N., Abdelsayed, S.V., Wilde, M.D., Taylor, L.R., Wilson, D.R., and Darlington, G.J. (1995). Impaired energy homeostasis in C/EBP alpha knockout mice. Science *269*, 1108–1112.

Wang, Y., Inoue, H., Ravnskjaer, K., Viste, K., Miller, N., Liu, Y., Hedrick, S., Vera, L., and Montminy, M. (2010). Targeted disruption of the CREB coactivator Crtc2 increases insulin sensitivity. Proc. Natl. Acad. Sci. USA *107*, 3087–3092.

Watts, L.M., Manchem, V.P., Leedom, T.A., Rivard, A.L., McKay, R.A., Bao, D., Neroladakis, T., Monia, B.P., Bodenmiller, D.M., Cao, J.X., et al. (2005). Reduction of hepatic and adipose tissue glucocorticoid receptor expression with antisense oligonucleotides improves hyperglycemia and hyperlipidemia in diabetic rodents without causing systemic glucocorticoid antagonism. Diabetes *54*, 1846–1853.

Wei, D., Tao, R., Zhang, Y., White, M.F., and Dong, X.C. (2011). Feedback regulation of hepatic gluconeogenesis through modulation of SHP/Nr0b2 gene expression by Sirt1 and FoxO1. Am. J. Physiol. Endocrinol. Metab. *300*, E312–E320.

Weyer, C., Bogardus, C., Mott, D.M., and Pratley, R.E. (1999). The natural history of insulin secretory dysfunction and insulin resistance in the pathogenesis of type 2 diabetes mellitus. J. Clin. Invest. *104*, 787–794.

Wu, C., Khan, S.A., Peng, L.J., Li, H., Carmella, S.G., and Lange, A.J. (2006). Perturbation of glucose flux in the liver by decreasing F26P2 levels causes hepatic insulin resistance and hyperglycemia. Am. J. Physiol. Endocrinol. Metab. *291*, E536–E543.

Yoon, J.C., Puigserver, P., Chen, G., Donovan, J., Wu, Z., Rhee, J., Adelmant, G., Stafford, J., Kahn, C.R., Granner, D.K., et al. (2001). Control of hepatic gluconeogenesis through the transcriptional coactivator PGC-1. Nature *413*, 131–138.

Zhang, B.B., Zhou, G., and Li, C. (2009). AMPK: an emerging drug target for diabetes and the metabolic syndrome. Cell Metab. *9*, 407–416.