

Role of the liver in the control of carbohydrate and lipid homeostasis

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SUMMARY

The liver plays a unique role in controlling carbohydrate metabolism by maintaining glucose concentrations in a normal range over both short and long periods of times. In type 2 diabetes, alterations in hepatic glucose metabolism are observed, i.e. increased post-absorptive glucose production and impaired suppression of glucose production together with diminished glucose uptake following carbohydrate ingestion. The simultaneous overproduction of glucose and fatty acids in liver further stimulates the secretion of insulin by the pancreatic β cells, and elicits further peripheral insulin resistance thereby establishing a vicious circle. The present review will focus on some of the genetically-altered mouse models that have helped identify enzymes or transcription factors that are essential for maintaining either glucose or lipid homeostasis in liver. Among these mouse models, we will discuss transgenic mice overexpressing key gluconeogenic enzymes (PEPCK, G6Pase) or transcription factors (Foxo1, Pgc1- ζ) that control *de novo* glucose synthesis. In addition, since the possibility of controlling hepatic glucose utilization as a treatment of type 2 diabetes has been explored we will review some of the strategies proved to be valuable for improving the hyperglycemic phenotype.

Key-words: Liver · Glucose metabolism · Lipid synthesis · Glucose and insulin signaling · Transgenic and knockout · Mouse models · Gene regulation.

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RÉSUMÉ

Rôle du foie dans le contrôle de l'homéostasie glucidique et lipidique

Le foie joue un rôle essentiel dans le contrôle de l'homéostasie glucidique en permettant le maintien d'une glycémie normale à la fois à court et à long terme. Au cours du diabète de type 2, des altérations du métabolisme glucidique sont observées, avec en particulier une augmentation de la production hépatique de glucose concomitante d'une diminution de l'utilisation du glucose suivant l'absorption d'un régime riche en glucides. L'augmentation simultanée de la production hépatique de glucose et de la synthèse des acides gras entraîne une stimulation exacerbée de la sécrétion d'insuline par les cellules β du pancréas, le tout aggravant la résistance à l'insuline périphérique. Le but de cette revue est de présenter et de discuter certains des modèles de souris génétiquement modifiées ayant apporté des éléments de réponse importants sur le rôle d'enzymes hépatiques ou de facteurs de transcription impliqués dans le contrôle de l'homéostasie glucidique et lipidique. Parmi ces modèles animaux, nous discuterons en particulier, des modèles *in vivo* de sur-expression d'enzymes clefs de la néoglucogenèse (PEPCK, G6Pase) ou de facteurs de transcription (Foxo1, Pgc1- ζ). De plus, nous discuterons les stratégies thérapeutiques potentielles destinées, *via* une augmentation de l'utilisation de glucose, à corriger l'hyperglycémie du diabétique.

Mots-clés : Foie · Métabolisme du glucose · Synthèse des lipides · Signal glucose et insuline · Modèles de souris transgéniques et invalidées · Régulation de l'expression des gènes.

While it serves a variety of functions, the liver plays a unique role in controlling carbohydrate metabolism by maintaining glucose concentrations in a normal range over both short and long periods of times. Hepatocytes express dozens of enzymes that are alternatively turned on or off depending on whether blood glucose levels are either rising or falling out of the normal range. In the post-absorptive state (overnight fast), hepatic glucose production (HGP) ensures a sufficient supply of glucose to the central nervous system and at the same time it regulates fasting plasma glucose concentrations. In the post-prandial period, the liver takes up a portion of ingested carbohydrates to restore glycogen stores. In addition, when glucose concentrations are elevated, the liver has the ability to synthesize lipids *de novo* through the lipogenic pathway. This net hepatic glucose uptake, which results from simultaneous suppression of glucose-producing pathways and stimulation of both hepatic glucose uptake and anabolic pathways of glucose disposal, restricts post-prandial increases in plasma glucose concentrations. In type 2 diabetes, alterations in hepatic glucose metabolism are observed, i.e. increased post-absorptive glucose production and impaired suppression of glucose production together with diminished splanchnic glucose uptake following carbohydrate ingestion [1]. In addition, the simultaneous overproduction of glucose and fatty acids in liver further stimulates the secretion of insulin by the pancreatic β cells, and exacerbates peripheral insulin resistance thereby establishing a vicious circle [2].

The key role of the liver in controlling both carbohydrate and lipid homeostasis *in vivo* has been confirmed by recently generated transgenic and knockout models. The

present review will therefore focus on some of genetically-altered mouse models that have helped understand what metabolic pathways, enzymes or transcription factors are essential for maintaining either carbohydrate or lipid homeostasis in liver. This discussion will not provide an exhaustive list of all the recent published mouse models, but will rather focus on the metabolic alterations that could be determinant in the development of hepatic insulin resistance or type 2 diabetes.

Regulation of hepatic glucose production

The net glucose release is the result of two simultaneous ongoing pathways that are tightly regulated. Indeed, liver produces glucose by breaking down glycogen (glycogenolysis) and by *de novo* synthesis of glucose (gluconeogenesis) from non-carbohydrate precursors such as lactate, amino acids and glycerol [3-5] (Fig 1). The exact contribution of each of these two processes to glucose production remains however controversial. Glycogenolysis occurs within 2-6 hours after a meal in humans, and gluconeogenesis has a greater importance with prolonged fasting. The rate of gluconeogenesis is controlled principally by the activities of unidirectional enzymes such as phosphoenolpyruvate carboxykinase (PEPCK), fructose-1, 6-bisphosphatase (FP₂ase) and glucose-6-phosphatase (G6Pase) (Fig 1). PEPCK catalyses one of the rate limiting steps of gluconeogenesis, the conversion of oxaloacetate to phosphoenolpyruvate (PEP), while G6Pase catalyzes the final step of gluconeogenesis, the production of free glucose from glucose-6-phosphate (G6P). The genes of these

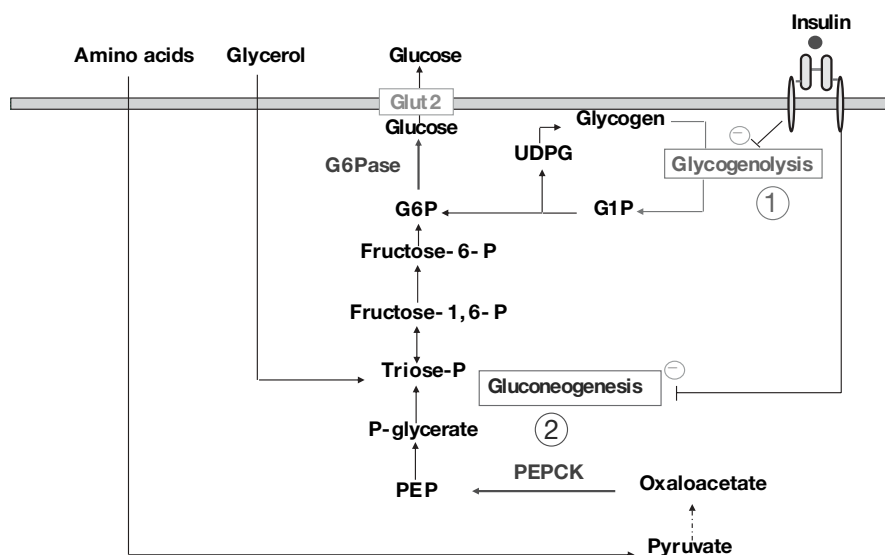


Figure 1

Regulation of hepatic glucose production.

Liver produces glucose by breaking down glycogen (glycogenolysis) and by *de novo* synthesis of glucose (gluconeogenesis) from non-carbohydrate precursors such as lactate, amino acids and glycerol. The rate of gluconeogenesis is controlled principally by the activities of unidirectional enzymes such as phosphoenolpyruvate carboxykinase (PEPCK), fructose-1, 6-bisphosphatase (FP₂ase) and glucose-6-phosphatase (G6Pase). PEPCK catalyses one of the rate limiting steps of gluconeogenesis, the conversion of oxaloacetic acid to phosphoenolpyruvate (PEP), while G6Pase catalyzes the dephosphorylation of G6P, a step shared both by gluconeogenesis and glycogenolysis. The genes of gluconeogenic enzymes are controlled at the transcriptional level by hormones, mainly insulin, glucagon and glucocorticoids. Insulin acting through its receptor inhibits gluconeogenesis acting by suppressing the expression of PEPCK and G6Pase whereas glucagon and glucocorticoids stimulate hepatic glucose production by inducing these genes.

gluconeogenic enzymes are controlled at the transcriptional level by hormones, mainly insulin, glucagon and glucocorticoids. Insulin inhibits gluconeogenesis by suppressing the expression of PEPCK and G6Pase (Fig 1), whereas glucagon and glucocorticoids stimulate hepatic glucose production by inducing these genes [6]. In both type 1 and type 2 diabetes, excessive hepatic glucose production is a major contributor of both fasting and postprandial hyperglycemia [7].

Importance of direct insulin-signaling in liver

As mentioned above, insulin decreases gluconeogenesis through specific transcriptional inhibition of PEPCK, FP₂ase and G6Pase [6]. To address the importance of insulin signaling in the control of gluconeogenesis *in vivo*, liver-specific disruption of the insulin receptor was performed in mice (LIRKO mice). Disruption of insulin action in liver of LIRKO mice leads to severe glucose intolerance, resistance to the blood glucose lowering effect of insulin, and non-inhibited hepatic glucose production due to elevated liver G6Pase and PEPCK expression [8]. Because of the absence of insulin receptor-mediated clearance of insulin by the liver, insulin concentrations are extremely high in LIRKO mice. In addition, LIRKO mice exhibit a 6-fold increase in pancreatic η -cell mass [8]. All together, analysis of LIRKO mice have revealed the critical role for the liver for the maintenance of normal glucose homeostasis and hepatic function [8]. Given that mice with a muscle-specific insulin receptor knock-out (MIRKO) have normal fasting glucose levels and normal glucose tolerance [9], this suggests that the liver plays a more important role in the control of glucose homeostasis than it was previously thought. Furthermore, a considerable portion of the decrease in blood glucose concentration following insulin administration in normal mice may be due to a suppression of hepatic glucose production rather than an increase in skeletal muscle glucose uptake [8].

The LIRKO mouse model has also offered the opportunity to test whether the effect of insulin on hepatic glucose production is direct or indirect [10]. Since the livers of LIRKO mice cannot directly respond to insulin, any effect of insulin administered *in vivo* must be mediated by indirect actions of insulin on extra-hepatic tissues. Several *in vivo* studies had previously suggested that the inhibitory effect of insulin on hepatic glucose production was partly indirect, due to the inhibition of lipolysis in adipose tissue [11], the reduction in plasma free fatty acids inducing an inhibition of gluconeogenesis [12, 13]. In LIRKO mice, in which the direct hepatic effect is fully blocked, even high levels of insulin are inefficient to suppress HPG while fully suppressing HPG in controls, suggesting that both direct and indirect effects of insulin require an intact insulin receptor signaling pathway in liver [10].

Alterations in G6Pase or PEPCK-gene expression

The physiological significance of the dysregulation of hepatic gluconeogenic genes is supported by the fact that overexpression of both G6Pase and PEPCK in the liver induces glucose intolerance [14-16]. Indeed, several groups have studied the metabolic and physiological consequences of altering either G6Pase or PEPCK *in vivo*. The G6Pase enzyme complex catalyzes the hydrolysis of G6P into glucose, a step shared both by gluconeogenesis and glycogenolysis [17] (Fig 1). This complex includes the glucose-6-phosphatase translocase that transports G6P from the cytoplasm to the lumen of the endoplasmic reticulum (ER) as well as the G6Pase phosphohydrolase or catalytic subunit that resides in the lumen. In hepatocytes, G6P concentrations are determined by the balance between G6Pase and glucokinase (GK) activities. In fact, as we will discuss below, hepatic GK expression is decreased, and expression of the catalytic subunit of G6Pase has been shown to increase, in several animal models of diabetes [18-21]. These enzymatic changes are correlated with an increase in hepatic glucose production that seems to contribute to the diabetic phenotype, leading to the suggestion that the ratio GK to G6Pase is crucial for the metabolic fate of glucose in liver [19] (Fig 2). The hypothesis that altered G6Pase expression could be linked to the development of insulin resistance first emerged from a study in which the catalytic subunit of the G6Pase was overexpressed in rat hepatocytes through the use of a recombinant adenovirus [22]. Overexpression of G6Pase in hepatocytes results in a reduction of glycogen synthesis and an increase in glucose production from lactate, suggesting that overexpression of this gene could indeed contribute to the onset of diabetes. Indeed, overexpression of G6Pase *in vivo* results in several of the abnormalities associated with early stage in type 2 diabetes, including glucose intolerance, hyperinsulinemia, decreased hepatic glycogen content, and triglyceride accumulation in skeletal muscle [14]. This suggested that overexpression of G6Pase in rat liver was sufficient to perturb whole animal glucose and lipid homeostasis, possibly contributing to the development of metabolic abnormalities associated with diabetes. Interestingly, overexpression of G6Pase does not increase fasting blood glucose. Hyperglycemia occurred only in the fed state or after an oral glucose tolerance test [14]. These findings are consistent with the fact that the main function of G6Pase is to buffer G6P concentrations in the post-absorptive state when GK is located in the cytoplasmic compartment [23]. In contrast, lack of G6Pase activity in a model of gene knockout recapitulates the model of glycogen storage disease type 1a in humans, characterized by excessive glycogen accumulation in liver, severe hypoglycemia and lactic acidemia [24].

The metabolic impacts of altering the PEPCK expression in mice have also been investigated [15, 16, 25]. Transgenic mice overexpressing PEPCK in liver (a 2-fold

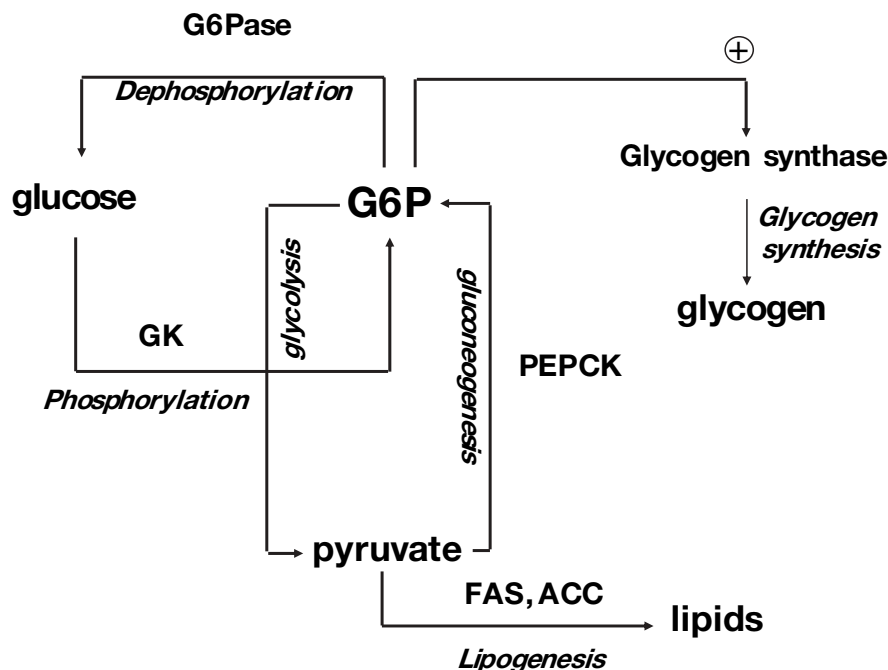


Figure 2

Glucose 6-phosphate is metabolite shared by several pathways in a liver.

Hepatic glucokinase (GK) allows for rapid and efficient phosphorylation of glucose in glucose 6-phosphate (G6P). In hepatocytes, G6P concentrations are determined by the balance between G6Pase and GK activities. G6P is an allosteric activator of glycogen synthase (GS), but is also a key metabolite that can enter glycolysis. In addition, since the major function of glycolysis in liver is to provide carbons from glucose for *de novo* lipid synthesis (lipogenesis), G6P is also considered determinant for this pathway and for the induction of fatty acid synthase (FAS) and acetyl CoA carboxylase (ACC).

increase) under the control of its own promoter show increased basal hepatic glucose production, but normal whole-body glucose disposal during a hyperinsulinemic-euglycemic clamp study compared to wild type controls [16]. In addition, steady state levels of G6Pase are increased in livers of these mice and the expression of both PEPCK and G6Pase is resistant to down-regulation by insulin [16]. This suggests that although transgenic mice overexpressing PEPCK do not develop peripheral insulin resistance their livers were highly insulin-resistant. Consistent with this finding there is also a decrease in the level of IRS-2 (insulin receptor substrate-2) and in the activity of phosphatidylinositol 3-kinase activity in the liver of these mice [16]. This model differs from the one of Valera *et al.* [15] in which a 7-fold increase in PEPCK expression in liver resulted in fasting hyperglycemia and peripheral insulin resistance. The discrepancy between these two studies could be explained by the fact that PEPCK gene expression was only modestly increased in the study of Sun *et al.* [16]. Surprisingly, mice that lack hepatic PEPCK are able to maintain a normal blood glucose concentration in the fasted state, although at the expense of a marked hepatic steatosis [25]. By using both NMR and metabolic tracers, it was shown in a subsequent study that the gluconeogenic flux in mice lacking hepatic PEPCK is still 66% of that of control mice, despite the impairment in whole-body gluconeogenesis from substrate entering the pathway at the level of pyruvate or oxaloacetate [26]. This suggests that either gluconeogenesis from glycerol is capable to compensate for the lack of liver PEPCK or that the kidney and the intestine, two other sites of PEPCK expression, may contribute to systemic glucose production in the total absence of hepatic PEPCK.

Altogether, these models have demonstrated that increased flux through gluconeogenesis, either *via* PEPCK or G6Pase overexpression, is sufficient to recapitulate the metabolic defects observed in type 2 diabetes and that the alteration in a single hepatic gene can have deleterious effects on whole glucose and lipid homeostasis *in vivo*.

Transcriptional control of hepatic glucose production

Both PEPCK and G6Pase genes are regulated at the transcriptional level and their regulation involves a "cross-talk" between a network of transcription factors (Fig 3). The PEPCK promoter has been extensively studied and is known to be induced by various stimuli (glucagon *via* cAMP, glucocorticoids, thyroid hormone) and transcription factors (CREB, C/EBP ζ , HNF-3, HNF-4 ζ , PPAR ζ) [27]. The transcriptional pathway that implies the hepatocyte nuclear factor-4 ζ (HNF-4 ζ) and the peroxisome proliferative activated receptor- ν co-activator-1 ζ (PGC-1 ζ) plays a crucial role in the transcriptional regulation of PEPCK and G6Pase genes. Indeed, PGC-1 ζ affects gluconeogenic gene regulation by directly binding to HNF-4 ζ and to other transcription factors such as Foxo1 (also known as FKHR) (Fig 3). PGC-1 ζ is induced by cAMP and glucocorticoids in isolated hepatocytes and by fasting and insulin deficient states in liver *in vivo* [28]. Adenovirus-mediated overexpression of PGC-1 ζ in rat liver causes an increase in hepatic glucose production, through the activation of all key enzymes of gluconeogenesis. The transcription factor Foxo-1, which directly interacts with PGC-1 ζ [29], also plays an important role in the suppression of hepatic gluco-

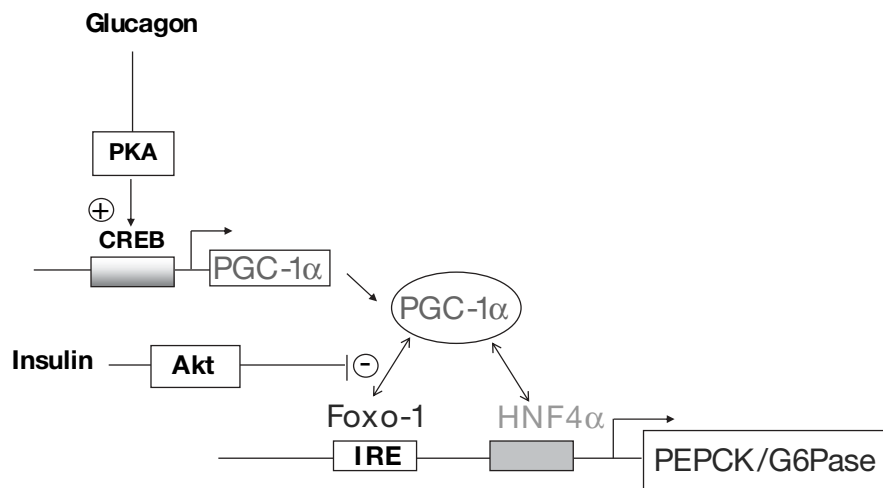


Figure 3
Role of the PGC-1 ζ -Foxo1 complex in the transcriptional control of gluconeogenic genes.

The co-activator-PGC-1 ζ and the transcription factor Foxo-1 both play an important role in the suppression of hepatic gluconeogenesis by insulin. Foxo1 has been shown to be crucial for the regulation of PEPCK and G6Pase through their Insulin Responsive elements (IRE). The nuclear co-activator PGC-1 ζ affects gluconeogenic gene regulation by binding to transcription factors such as Foxo1 or HNF-4 ζ . PGC-1 ζ is induced by cAMP and glucocorticoids and by fasting and insulin deficient states in liver. In addition, the nuclear localization of Foxo1 and its association with PGC-1 ζ are abrogated by Akt-mediated phosphorylation. Therefore, insulin signaling through Akt can attenuate the effects of increased levels of PGC-1 ζ in fasting conditions by promoting the dissociation of PGC-1 ζ from Foxo1. Adapted from Puigserver *et al.* [29].

neogenesis by insulin (Fig 3). *In vitro* studies have shown that Foxo1 regulates both PEPCK and G6Pase genes through an interaction with their consensus insulin response elements (IRE) [30] present in their promoters [31]. Insulin repression of these genes occurs through a phosphatidylinositol 3-kinase (PI3K)/Akt-mediated phosphorylation of the Foxo1 protein, resulting in its translocation out of the nucleus [32, 33]. After being activated by insulin, Akt phosphorylates Foxo1 at 3 conserved sites (Thr-24, Ser-253 and Ser-316). Phosphorylation of these sites leads to inhibition of Foxo-stimulated transcription and a redistribution of the transcription factor from the nucleus to the cytoplasm, therefore preventing Foxo1 from activating its target genes. Interestingly, the association of Foxo1 with PGC-1 ζ is also abrogated by Akt-mediated phosphorylation [29]. Therefore, insulin signaling through Akt can attenuate the effects of increased levels of PGC-1 ζ in fasting and other conditions by promoting the dissociation of PGC-1 ζ from Foxo1 (Fig 3). Altogether, these results indicate that the complex formed by PGC-1 ζ and Foxo1 is of crucial importance for the regulation of gluconeogenesis and may be considered, through its inactivation by phosphorylation for example, as a potential target for the anti-gluconeogenic treatment of type 2 diabetes.

Insulin is the most potent inhibitor of PEPCK gene expression [6]. SREBP-1c, which has emerged as a major mediator of insulin action on lipogenic gene expression in liver [34, 35], was also shown to inhibit PEPCK gene expression [36]. The mechanism of action of SREBP-1c inhibition on PEPCK gene transcription was further elucidated in the recent study by Yamamoto *et al.* [37]. This study reveals that SREBP-1 exerts a novel role as negative regulator of gluconeogenic genes through a “cross-talk” with HNF-4 ζ interference with PGC-1 ζ recruitment.

Also consistent with these results, both hepatic PEPCK and G6Pase mRNA levels are suppressed by overexpression of SREBP-1a and -1c in transgenic mice [37].

Although gluconeogenic genes are known to be mostly regulated by insulin and glucagon, the Interleukin-6 (IL-6) family of cytokines is also implicated in the regulation of these genes. The transcription factor STAT-3, which is an important signal transducer used by the IL-6 family contributes to various physiological processes, including the transcriptional regulation of hepatic gluconeogenic genes [38]. Hepatocytes treated with IL-6 show reduced PEPCK gene expression [39] and transplantation of an IL-6 producing tumor into mice results in an inhibition of G6Pase gene expression [40]. The recent mouse model of liver-specific inactivation of STAT-3 (L-ST3KO), has revealed that STAT-3 signaling is essential for glucose homeostasis [38]. L-ST3KO mice show insulin resistance, associated with a 1.5 fold increased in co-activator PGC-1 ζ expression, associated with a parallel increased in gluconeogenic gene expression (both PEPCK and G6Pase). Rescued expression of STAT-3 in liver through the use of an adenovirus restores the insulin resistance phenotype and down-regulates the expression of PEPCK and G6Pase. In addition, the demonstration that overexpression of the constitutively active form of STAT-3 in liver of diabetic db/db mice ameliorates their glucose tolerance, suggests that STAT-3 may be a potential target for anti-diabetic therapies [38].

Glucose storage during the fed state

The liver maintains glucose homeostasis in the normal state by net glucose uptake of post-prandial glucose when blood glucose exceeds the euglycemic threshold. Enzymes

that have a high control strength on hepatic glucose metabolism [41] are potential targets for controlling hepatic glucose balance and thereby glucose levels in type 2 diabetes. One strong candidate in that respect is hepatic glucokinase (GK), which catalyzes the first step of glucose metabolism (Fig 4). Hepatic GK, by virtue of kinetic characteristics that distinguish it from other hexokinases (HK) [42] allows, for rapid and efficient phosphorylation of glucose in glucose 6-phosphate (G6P), a key step of glycolysis, glycogen synthesis and pentose phosphate pathway [43]. In fact, as revealed by a series of *in vivo* analysis, hepatic GK is crucial for glucose uptake and glycogen synthesis in liver, as well, as we will discuss in further details for the expression of an entire network of hepatic glucose-responsive genes.

Role of hepatic GK in glucose homeostasis and glucose sensing

In hepatocytes, extracellular and intracellular glucose concentrations are in equilibrium due to the high capacity of hepatic glucose transporter GLUT2 [44]. Glucokinase (GK), which is the predominant glucose-phosphorylating enzyme in hepatocytes, is a monomeric enzyme of 50 kDa protein that exhibits kinetic properties quite distinct from the those of the other members of the mammalian HK family [42, 45]. GK is not inhibited by its end-product reaction, G6P, and has a relatively low affinity for glucose ($S_{0.5}$ 8 mM) [45]. A variety of genetically mouse models have been generated and characterized to determine the role of hepatic GK in regulating the glycolytic flux *in vivo* [46, 47]. Both overexpression and conditional gene knock-out strategies proved to be valuable for determining the effects of GK gene dosage on blood glucose concentrations [47]. In transgenic mice overexpressing hepatic GK, glycolysis and glycogen synthesis are increased [48-50]. These transgenic mice have lower glycemia than controls after a glucose tol-

erance test indicating that GK overexpression in liver increases glucose uptake by the liver [51]. Similar results have been obtained with mice we generated that express one or more copies of the entire GK gene locus [50, 52]. Furthermore, these mice overexpressing one extra copy of the GK gene locus are protected against high-fat diet induced diabetes [53]. In these mice, increased GK activity reduces hyperglycemia and hyperinsulinemia induced by high-fat diet. Ferre *et al.* [48] had also reported that the streptozotocin-induced increase in glucose concentrations is normalized in GK overexpressing mice. All these studies confirm that GK has a high control strength on hepatic glucose metabolism as well as on glycogen synthesis [41]. In addition, the recent finding that allosteric activators of hepatic GK improve glucose tolerance and increase hepatic glucose uptake [54, 55] makes of hepatic GK a feasible gene therapy-based approach to reduce hyperglycemia in diabetes.

Regulation of glycogen synthesis

After a meal, the secretion of insulin from η cells results in about 20-30% of the carbohydrate intake being stored in the form of glycogen in the liver and in skeletal muscle [56, 57]. Glycogen is considered the principal storage form of glucose and with up to 10% of its weight as glycogen, the liver has the highest specific content of any tissue. Therefore, defects in this process can be a major contributor to postprandial hyperglycemia, and glycogen contents of both liver and skeletal muscle are reduced in individuals with type 2 diabetes [58, 59].

Hepatic glycogen metabolism is controlled by the coordinated action of two enzymes, glycogen synthase (GS) and glycogen phosphorylase (GP), both of which are regulated by phosphorylation and allosteric modulators [60]. Although glucose and its metabolites can modulate their

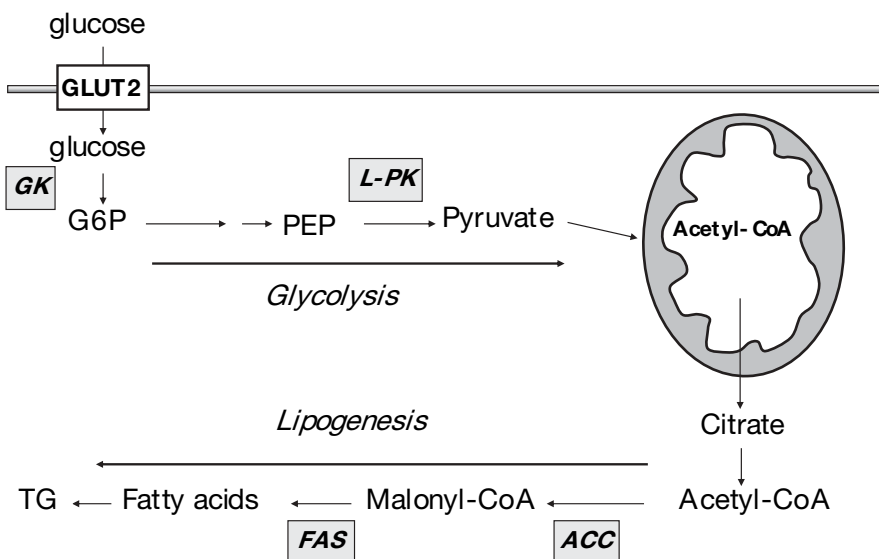


Figure 4
Regulation of the glycolytic and lipogenic pathway.

Metabolic flux in the liver reflects the net activities of several major pathways, including glycolysis and lipogenesis. The major function of glycolysis in liver is to provide carbons from glucose for *de novo* lipid synthesis. Glycolytic genes: glucokinase (GK) and L-pyruvate kinase (L-PK); lipogenic genes: fatty acid synthase (FAS) and acetyl coA carboxylase (ACC).

enzymatic activity and localization, as we will discuss below, GS and GP are principally regulated by phosphorylation. GS, which is the rate limiting enzyme of glycogen synthesis, is inactivated by phosphorylation on up to 9 regulatory residues by several kinases, including protein kinase A (PKA) and glycogen synthase kinase-3 (GSK-3) [61]. Insulin regulates glycogen metabolism by promoting the dephosphorylation and the activation of GS through activation of the protein phosphatase-1 (PP1) and the inactivation of the upstream kinases. The identification of “targeting” subunits that would selectively allow the activation of PP1 only at specific sites has shed some light on the regulation of glycogen metabolism. Among them, protein targeting to glycogen (PTG) [62-64] is a scaffolding protein that links PP1 ζ to glycogen, and links it to enzymes involved in glycogen degradation or synthesis [64]. The recent inactivation of PTG in mice has revealed that this protein is crucial for glycogen synthesis [65]. While the homozygous mutation is lethal, heterozygous PTG knockout mice (PTG^{+/-}) are viable but display a 40-50% decrease in steady-state glycogen concentrations and in glycogen synthase activity in fat, liver and muscle. In addition, PTG^{+/-} mice develop a progressive glucose intolerance that is probably due to decreased glycogen content in liver but also in multiple sites [65]. Similarly, overexpression of PTG in rat liver by adenoviral infection produces a dramatic increase in steady-state glycogen accumulation and the glycogen synthase activity [66]. Moreover, cells overexpressing PTG do not respond to glycolytic stimuli and are thus store high levels of glycogen. All together these results demonstrate that PTG plays a crucial role in regulating glycogen synthesis *in vivo*.

As mentioned above, GS and GP are also regulated by allosteric modulators. It is now clear that glucose must be phosphorylated to allow the activation of GS [60] (Fig 2). Indeed, G6P, which is the main metabolite involved in the regulation of the enzyme, binds to GS, causing the allosteric activation of the enzyme through a change in conformation that converts it to a better substrate for glycogen synthase, leading to the covalent activation of GS. The potency of the activation of GS seems to be determined by the origin of G6P since only G6P produced by hepatic GK is able to efficiently promote glycogen synthesis. Indeed, *in vitro* studies have demonstrated that adenovirus-mediated overexpression of GK, but not of HKI, has a potent effect on glycogen synthesis in primary cultured hepatocytes [22] and that only G6P produced by overexpressed GK is glycogenic because it effectively promotes the activation of glycogen synthase. We have also recently confirmed that the ability of hepatocytes to efficiently synthesize G6P and glycogen is directly dependent on GK activity and not on HK's. Using a model of liver-specific inactivation of GK (hGK-KO mice) [67], we have shown that the efficiency of hGK-KO hepatocytes to synthesize G6P and glycogen was severely reduced in absence of GK, despite a marked increase in low K_m HK activity [68].

Regulation of lipid synthesis in liver

Metabolic flux in the liver reflects the net activities of several major pathways, including glycolysis and lipogenesis. The major function of glycolysis in liver is to provide carbons from glucose for *de novo* lipid synthesis (Fig 4). Since dysregulations in hepatic lipid synthesis have been associated with obesity and type 2 diabetes, a perfect understanding of the regulation of lipogenesis seems crucial in both physiology and physiopathology.

The synthesis of fatty acids from glucose is nutritionally regulated and it has been proposed that two signaling pathways elicited in response to dietary carbohydrates play a synergic role in regulating lipogenic gene expression (acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS)) [69, 70]. The transcriptional induction of ACC and FAS requires both glucose metabolism and insulin and we and others have shown that the role of insulin is to induce GK gene expression which is then essential for subsequent glucose phosphorylation [43, 68, 71] (Fig 5). Although important progress has been made in the identification of the partners involved in the events following insulin binding to its receptor, factors involved in the transcriptional effects of insulin are still largely unknown. SREBP-1c has however emerged as a major mediator of insulin action on hepatic GK [72] and lipogenic gene expression [34, 35]. SREBP-1c, which is induced by insulin in primary cultures of hepatocytes [73], is able to induce lipogenic genes by its capacity to bind to the Sterol Regulatory Element (SRE) present in their promoters [74-76] (Fig 5). Not only SREBP-1c controls the transcription and expression of lipogenic genes, but it is remarkable that this transcription factor is also able to regulate the rate of triglyceride synthesis as well as the amount of their storage in liver [77, 78]. Several studies have suggested that SREBP-1c may be involved in the pathogenesis of hepatic insulin resistance. Indeed, elevated levels of SREBP-1c are observed in liver of insulin-resistant animals [79, 80] and a recent study demonstrates that high levels of SREBP-1c exacerbates insulin resistance through suppression of IRS-2 signaling in liver [81]. In fact, SREBP-1c directly represses IRS-2 gene transcription and inhibits hepatic insulin signaling [81]. In contrast, and from a therapeutic point of view, it is interesting to note that the disruption of SREBP-1c gene expression in ob/ob mice improves their hepatic steatosis. More importantly, overexpression of the mature isoform of SREBP1c in livers of streptozotocin-induced diabetic mice leads to increased hepatic glycogen and triglyceride content as well as a marked decrease in hyperglycemia in these mice [82]. All together these results indicate that SREBP-1c plays a major role in the long-term control of glucose homeostasis by insulin demonstrating that this transcription factor must be considered as a major therapeutic target.

With the exception of hepatic GK which is exclusively induced by insulin [83, 84], most of the genes of the glycolytic and lipogenic pathways are also regulated by glucose

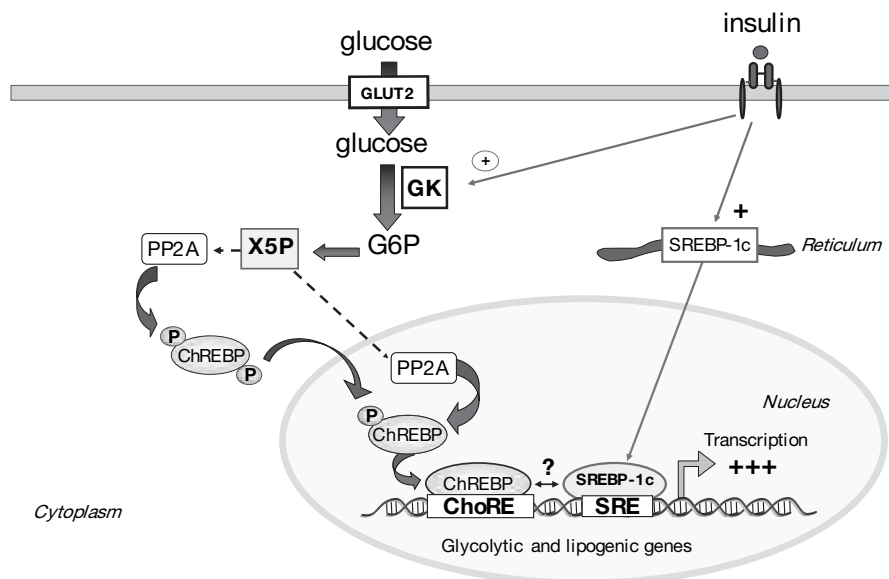


Figure 5
SREBP-1c and ChREBP act in synergy to regulate glycolytic and lipogenic gene expression.

Hepatic glucokinase (GK) catalyzes the phosphorylation of glucose in glucose 6-phosphate (G6P), a step which is essential for glucose metabolism as well as for the induction of glycolytic and lipogenic genes. Except for GK, which is only induced by insulin, genes of the glycolytic and lipogenic pathways are also regulated by glucose. The recent identification of ChREBP, a glucose-responsive transcription factor, has recently shed light on the possible mechanism whereby glucose affects gene transcription and in fact, glucose-response elements (ChoRE) have been identified in most of these genes through promoter-mapping analysis. The activity of ChREBP requires a mechanism of phosphorylation/dephosphorylation which is determined by the relative activity of protein phosphatase 2A (PP2A), regulated by xylulose 5-phosphate (X5P) concentrations. SREBP-1c, which is induced by insulin, is able to induce lipogenic genes by its capacity to bind to the Sterol Regulatory Element (SRE) present in the promoters.

[43, 69]. In fact, glucose- or carbohydrate-response elements (ChoRE) have been identified in the promoters of these genes through promoter-mapping analysis [85-87]. Until recently, the nature of the glucose-signalling compound was not known, although it had been established that a metabolite of glucose, and not glucose *per se*, was responsible for the glucose signal [43]. The recent identification of a glucose-responsive basic/helix-loop-helix/leucine zipper (bHLH/LZ) transcription factor named ChREBP (Carbohydrate Responsive Element Binding Protein) [88] has recently shed light on the possible mechanism whereby glucose affects gene transcription. ChREBP is regulated in a reciprocal manner by glucose and cAMP [89]. Under basal conditions ChREBP is localized in the cytosol, and its nuclear translocation is rapidly induced under high glucose concentrations. Nuclear translocation of ChREBP is controlled by dephosphorylation of several serine residues. Serine residue 196 (Ser-196) is the target of protein kinase A (PKA) phosphorylation, and its dephosphorylation allows ChREBP translocation in the nucleus. Two other residues, Ser-568 and Thr-666 are dephosphorylated in the nucleus, thus alleviating DNA binding inhibition. Protein phosphatase 2A (PP2A) was recently shown to be activated by xylulose-5-phosphate (Xu5P), a metabolite generated by the hexose monophosphate pathway in presence of high glucose, and was likely to be responsible for both cytosolic and nuclear dephosphorylation of ChREBP [90 (Fig. 5)]. Mlx, (Max like protein) which is also a member of the bHLH/LZ family was recently identified as an heteromeric partner of ChREBP [Stoekman, 2004 #612]]. The fact that ChREBP interacts with Mlx, suggests that a network of transcription

factors or co-factors may be required to fully regulate glucose-responsive gene expression in liver.

The discovery of ChREBP and its potential role in glucose action prompts us to perform a series of experiments in primary hepatocytes of both control and hGK-KO mice [67]. We have shown that increased glucose metabolism via GK is necessary for both expression and function of ChREBP in primary cultures of hepatocytes [68]. To address the role of ChREBP in mediating glucose signaling in liver, we have used the siRNA approach to silence ChREBP gene expression in control hepatocytes. Our studies have revealed, for the first time in a physiological context, that ChREBP mediates the glucose effect on both glycolytic and lipogenic gene expression and that this transcription factor is a key determinant of lipid synthesis in liver [68]. Our results have been confirmed by the recent global inactivation of ChREBP gene expression in mice (ChREBP^{-/-} mice) [91]. The fact ChREBP^{-/-} mice are intolerant to glucose and insulin resistant suggests that this transcription factor may also play a role in the pathogenesis of type 2 diabetes.

Concluding remarks

Although genetically-altered mice may not always reproduce human metabolism and physiology, the development of transgenic and knockout mice have helped, in the past ten years, to a better understanding of liver physiology. Key enzymes of hepatic glucose production, such as PEPCK and G6Pase, have shown, when overexpressed, to induce insulin resistance *in vivo*, and disturbances of both glucose and lipid homeostasis. Dissection of insulin signal-

ing in liver, through the analysis of LIRKO mice, has revealed that the liver may play a more important role in the control of glucose homeostasis than it was previously thought and that a considerable portion of the decrease in blood glucose following insulin administration may be due, at least in mice, to a suppression of hepatic glucose production rather than an increase in skeletal muscle glucose uptake [8]. More importantly, these mice models have helped identify potential therapeutic targets for the treatment of diabetes. Indeed, the possibility of controlling hepatic glucose utilization as a treatment of type 2 diabetes has been explored through the modulation of expression of GK, one key glycolytic enzyme. Both overexpression and conditional gene knockout strategies proved to be valuable for determining the effects of GK gene dosage on blood glucose concentrations. In addition, the fact that allosteric activators of hepatic GK have recently been proven to be promising in lowering blood glucose and in reverting the diabetic phenotype in mice, suggests that clinical trials in diabetic patients may occur in the near future. Finally, functional *in vivo* studies of key transcription factors, such as the Foxo1-PGC-1 ζ complex or ChREBP and SREBP-1c for their control of hepatic gluconeogenesis and lipid synthesis respectively, has undoubtedly helped unravel the transcriptional regulation of metabolic pathways in liver.

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