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Critical Review

Liver Glucokinase: An Overview on the Regulatory Mechanisms of its Activity

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Summary

Blood glucose is the primary cellular substrate and in vivo must be tightly maintained. The liver plays a key role in glucose homeostasis increasing or decreasing glucose output and uptake during fasting and feeding. Glucokinase (GCK) is central to this process. Its activity is modulated in a coordinated manner via a complex set of mechanisms: in the postprandial period, the simultaneous rise in glucose and insulin increases GCK activity by enhanced gene expression, changes in cellular location, and interaction with regulatory proteins. Conversely, in the fasting state, the combined decrease in glucose and insulin concentrations and increase in glucagon concentrations, halt GCK activity. Herein we summarize the current knowledge regarding the regulation of hepatic GCK activity.
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Keywords glucokinase; glucose homeostasis; liver; liver glucokinase activity; transcriptional and post-transcriptional glucokinase activity regulation.

Abbreviations GCK, glucokinase; STZ, streptozotocin; PI3K, phosphatiodylinositol-3-kinase; PKB or Akt, protein kinase B; PFK2/FBPase2, 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase; HIF-1α, hypoxia-inducible factor-1a; SREBP1c, sterol regulatory element binding protein; ERRα, estrogen-related receptor-α; PGC-1α, peroxisome proliferator-activated-receptor-gamma co-activator-1α; GCKR, glucokinase regulatory protein; F-6-P, fructose-6-phosphate; F-1-P, fructose-1-phosphate; NLS, nuclear localization-signal; NES, nuclear export sequence; PFK1, 6-phosphofructo-1-kinase; IR, insulin resistance; IGT, impaired glucose tolerance; T2DM, Type 2 diabetes.

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INTRODUCTION

Glucokinase (GCK, hexokinase type IV or D) is a 50 kDa enzyme that phosphorylates glucose. Although initially described in the liver, it is also present in pancreatic β cells, neurons, the pituitary, and the entero-endocrine K and L cells, suggesting that a GCK-expressing cell network acts as a glucose sensor for the maintenance of glucose homeostasis (Fig. 1) (*1*). Such glucose-sensor role is inherent in its kinetic properties: (i) low affinity for glucose ($S_{0.5}$ around 6 mM, mimicking mammalian glucose concentrations), (ii) unlike other hexokinases, GCK is not inhibited by glucose-6-phosphate, and (iii) a cooperative behavior for glucose (Hill coefficient around 1.6), displaying sigmoid kinetics with an inflexion point comparable to normal glycaemia. Accordingly, GCK can tightly maintain extracellular glucose and glucose metabolism within cells with glucose-permeable plasma membranes such as hepatocytes.

GCK monomer with a single glucose-binding site is active, therefore its cooperative behavior cannot be explained by the classical multisubunit allosteric protein mechanism. A mnemonic model mechanism of positive cooperativity has been proposed for GCK in which GCK would alternate between low and high affinity conformations involving a slow interconversion between the conformational states of glucose-free low affinity GCK rapidly evolving to a high affinity state upon binding to glucose. Glucose and an activator modify the space between the large and small globular GCK domains, generating a narrow deep cleft containing the glucose-binding pocket. This "closed" conformation corresponds to the GCK high-affinity-active form while in the absence of ligands, GCK displays a "super-open" low glucose affinity-catalytically-inactive form (*2*). X-ray crystallographic structural analysis of human GCK has provided empirical support to this model.

REGULATION OF GCK GENE EXPRESSION

In mammals, two mutually exclusive tissue specific promoters control GCK gene expression. The upstream, neuroendo-

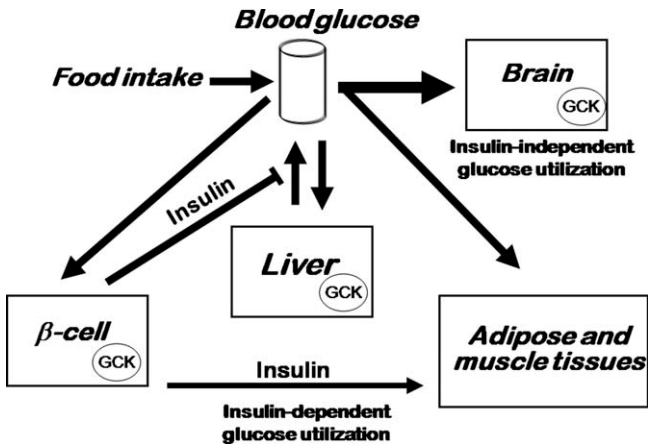

Figure 1. Glucose homeostasis under fasting conditions. Blood glucose concentration results from its provision (food intake or liver production) and consumption by the nervous system, adipose and muscle tissue. The insulin concentration controls glucose release (liver) and tissue uptake, while GCK plays a central role as intracellular signal in these tissues.

crine promoter, and the adjacent leader exon drive GCK mRNA synthesis in nonhepatic tissues, the downstream liver promoter and its associated leader exon are involved in gene transcription in hepatocytes (*3*). Although the fragments of the liver GCK gene promoter have been mapped and characterized in hepatocytes and hepatoma cells, the identity of all transcription factors regulating GCK gene promoter activity has not been fully elucidated (*4*).

Liver GCK gene expression depends on the fasting and refeeding states. Insulin is the primary up-regulator, while glucagon exerts the opposite effect (*3*). In rats with streptozotocin (STZ)-induced diabetes, liver GCK mRNA and protein are reduced and insulin restores their expression, primarily via GCK gene transcription (*5*). In cultured hepatocytes, the insulin enhancing effect is independent of the glucose concentration (*3*). The insulin effect is mediated by phosphatiodylinositol-3-kinase (PI3K)/protein kinase B (PKB or Akt) pathway (Fig. 2) (*4*). *In vivo* transduction of the adenoviruses encoding kinase active versions of 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase (PFK2/FBPase2) into diabetic mice, increased liver GCK mRNA and protein, probably via PKB activation (*6*). Others however, have failed to demonstrate such an active PKB role as an insulin mediator (*7*).

PI3K/Akt would also be involved in the oxygen signaling cascade, regulating the activity of hypoxia-inducible factor-1a (HIF-1α), thereby explaining the association between liver perivenous pO_2 and the zonated hepatic GCK expression. In hepatocytes stimulated with insulin, HIF-1α acts cooperatively with the transcription factor HNF-4 and the coactivator p300 to enhance GCK promoter activity via the PI3K/Akt pathway (*8*), lending molecular support to the synergic effect of hypoxia and insulin upon GCK gene expression in cultured hepatocytes (*9*).

The up-regulating effect of insulin upon GCK transcription is mediated by the transcription factor sterol regulatory element binding protein (SREBP1c). The overexpression of SREBP1c in primary hepatocytes is accompanied by an increase in GCK mRNA, while a dominant negative SREBP1c mutant inhibits the insulin-dependent induction, supporting such assumption (*10*). The following cascade of events involving SREBP-1c and other transcription factors (PPARγ, LXRα and the negative modulator SHP) in the regulation of GCK gene transcription has been proposed: (a) LXRα up-regulates GCK and SREBP-1c gene expression by direct binding to their promoters, (b) insulin activates the SREBP-1c gene and its maturation, and the consequent synergistic increase of LXRα-mediated GCK gene expression, (c) LXRα increases GCK gene expression by up-regulating PPARγ transcriptional activity, and (d) LXRα and PPARγ increase the SHP gene transcription, which in turn represses the transcription of the LXRα- or PPARγ-mediated GCK gene by interaction with RXRα (*11*). However, such a model has been challenged by other observations: (a) the expression of mature SREBP1c in hepatocytes failed to produce a significant GCK mRNA increase (*12*), (b) in SREBP1c knockout mice, induction of GCK gene expression occurs during the fasting refeeding transition (*13*), and (c) in insulin-stimulated hepatocytes, SREBP1c failed to bind to the liver GCK promoter (*14*). So far, no conclusive evidence supports a key role of SREBP1c in the regulation of insulin-induced liver GCK gene expression.

Although insulin's effect upon GCK gene expression is well known, transcriptional regulation by other hormones is less well-characterized. The *in vitro* addition of tri-iodothyronine to primary cultured neonatal rat hepatocytes induces GCK mRNA, while dexamethasone has no direct effect but enhances insulin response (*15*). However, dexamethasone was recently shown to inhibit GCK transcriptional activity in hepatocyte-derived HepG2 and HuH7 cells, suggesting that glucocorticoids and insulin play an antagonistic regulatory role upon liver glycolysis (*16*). These results should be interpreted with caution since tumor cell lines may not necessarily reflect what happens in normal cells.

An estrogen-related receptor-α (ERRα) binding site was demonstrated in the regulatory sequence of GCK gene; also, peroxisome proliferator-activated-receptor-gamma coactivator-1α (PGC-1α) coactivates ERRα, enhancing GCK gene transcription (*17*). The physiological relevance of these findings remains unclear. Hypermethylation of liver GCK promoter occurs in ageing rats suggesting that its epigenetic modification may explain the lower enzyme transcription observed in old rats with a potential diabetogenic effect (*18*).

HEPATIC GCK INTERACTION WITH ITS REGULATORY PROTEIN (GCKR)

GCK-GCKR interaction is a key post-transcriptional short-term control mechanism of liver GCK activity, modulated by

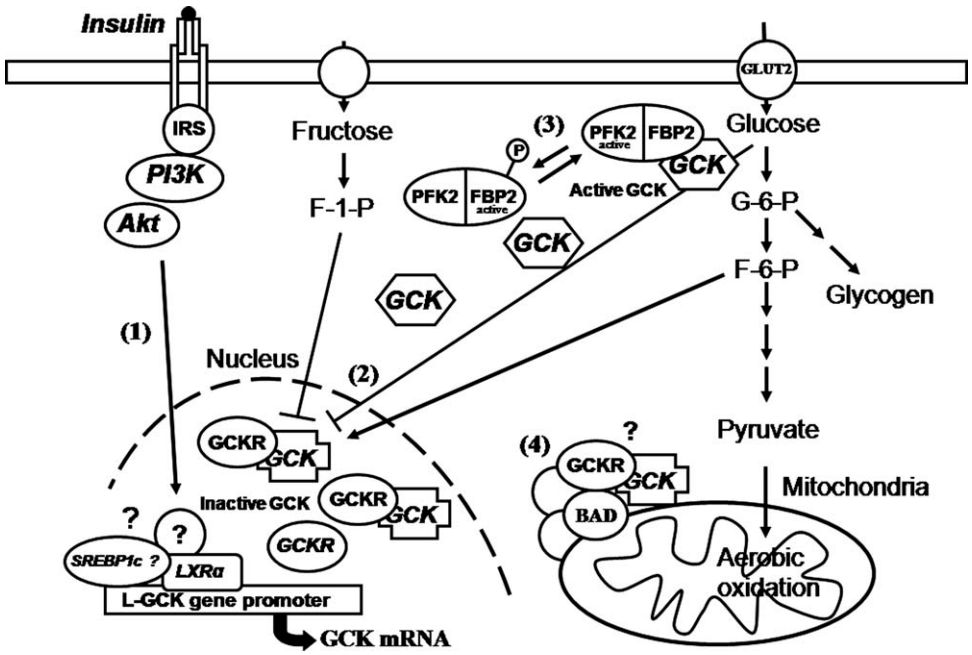

Figure 2. Regulatory mechanisms of GCK activity. (1) Insulin up-regulating effect upon GCK transcription through the PI3K/Akt pathway would be mediated by SREBP-1c and other not well-defined transcription factors. (2) GCK is sequestered in the nucleus in an inactive form by interaction with GCKR favored by binding to F-6-P; F-1-P weakens such interaction releasing active GCK to the cytosol. (3) Positive modulatory role of PFK2/FBP2 on GCK activity. (4) GCK could be colocalized with GCKR, BAD and other proteins in the mitochondria.

the binding of fructose-6-phosphate (F-6-P) to GCKR. Binding of fructose-1-phosphate (F-1-P) weakens such interaction, releasing active GCK (Fig. 2) (*19*). Since fructose is the most important dietary monosaccharide after glucose, the system is modulated by the pre- and postabsorptive state. While in the latter, the intestinal absorption of fructose and its phosphorylation by fructokinase increases liver F-1-P concentration, during starvation, liver GCK is inhibited by its binding to GCKR and F-6-P. Since F-6-P is in equilibrium with glucose-6-phosphate, this mechanism could act as an indirect negative feedback (*19*). Results obtained using cell-free systems suggest that GCKR would be an allosteric GCK-inhibitor that specifically binds to its super-open form (*2*); this interaction can be disrupted by conformational GCK changes (*20*).

Cellular compartmentalization is another regulatory component of GCK activity (Fig. 2). While GCKR is mainly located in the hepatocyte nucleus, the cellular location of GCK depends on the cell's metabolic status. In rat hepatocytes cultured with 5.5 mM glucose, GCK is mainly located in the nucleus, whereas in the presence of >10 mM glucose or 50–1000 μM fructose, the enzyme moves to the cytoplasm suggesting that GCK binding to GCKR is necessary and directly participates in cellular GCK distribution (*21*). Mutational analyses have demonstrated that GCK has different spatial binding motifs located along its large and small domains rather than a unique epitope (*22*). These sites share a common asparagine-leucine motif that confers GCKR binding capacity and is also involved in GCK nuclear shuttling (*23*). GCK mutants with lower GCKR-binding affinity than the wild-type GCK remain in the hepatocyte cytoplasm, while those with higher GCKR affinity display a significantly higher nuclear/cytoplasmic GCK ratio, irrespective of the glucose concentration (*23*). Also hepatic GCK have an extranuclear localization under any condition tested in mice with homozygous inactivation of the GCKR gene (*24*). Since mice with a GCKR gene knockout have a simultaneous loss of GCK protein and activity in their liver, GCKR protein would not only anchor and inhibit GCK in the nucleus, but it would also stabilize and protect the enzyme from degradation. Disruption of this regulation altered glucose metabolism and impaired glucose tolerance (IGT) (*24*).

GCK does not have a nuclear localization-signal (NLS) and GCKR acts as a nuclear chaperone that carries GCK into the nucleus via a piggy-back mechanism. However, at high glucose concentrations, GCK is released from GKRP and translocates to the cytoplasm via an active nuclear export sequence (NES) present in the enzyme (*25*). In the presence of an intracellular increased concentration of glucose or F-1-P and of a sufficient ATP, the GCK-GCKR complex dissociates, exposing the previously masked NES of GCK, thus allowing its rapid translocation. Accordingly, GCKR may simultaneously be a GCK anchoring protein and a chaperone that mediates its nuclear export or import (*25, 26*). This GCK-NES mechanism has been

however challenged in that the GCK nuclear export is not inhibited by leptomycin B, suggesting that the process does not involve interaction with exportin-1 (*26*).

REGULATORY ROLE OF PFK2/FBPase2

PFK2/FBPase2 is a cytosolic bifunctional enzyme involved in the formation and degradation of fructose-2,6-biphosphate, the most potent allosteric activator of the glycolitic enzyme 6-phosphofructo-1-kinase (PFK1). The GCK epitopes that interact with the FBPase2 domain of the PFK2/FBPase2 have also been identified (*27*). Such interaction activates and potentially stabilizes cytoplasmic GCK, thereby preserving its glucose sensor function (Fig. 2) (*28*). In the liver, this complex would promote a coordinated up-regulation of glucose phosphorylation (via GCK activation) and glycolysis (via PFK2 activation), which in turn increase fructose-2,6-biphosphate that activates PKF1, the committing step to glycolysis (*29*). PFK2/FBPase2 overexpression in hepatocytes potentiates GCK expression, and PFK2/FBPase2 phosphorylation–dephosphorylation modulates its binding to GCK, affecting its translocation from the nucleus to the cytoplasm (*30*).

This substrate-induced shuttling of GCK from the nucleus to the cytosol is counteracted by glucagon (*31*); in fact, glucagon inhibits glycolysis by decreasing the PFK1 allosteric modulator fructose-2,6-biphosphate through inhibition of the kinase component of the PFK2/FBPase2 complex and activation of the FBPase2 component by phosphorylation in its Ser-32.

This GCK regulatory mechanism can be strongly affected and even reversed in some abnormal chronic conditions: in insulin-resistant (IR) Zucker *fa/fa* rats with a modest degree of hyperglycaemia, the nucleus-to-cytoplasm GCK translocation is refractory to glucose. However, PFK2 gene overexpression increases the nucleus-to-cytoplasm GCK translocation in a dose-dependent manner and corrects its refractoriness to glucose effect (*32*). We have demonstrated that rats with fructose-induced IR and IGT have an increased GCK cytosolic activity, together with a significantly increased PFK2 mRNA and protein expression. Incubation of liver homogenates from these animals with a specific anti-PFK2 antibody raised against the FBPase2 domain involved in GCK-PFK2 interaction, decreased GCK activity, suggesting that PFK2 plays a positive modulatory role upon GCK activity both under normal and pathological conditions (*33*). Accordingly, changes in liver GCK could represent an adaptive response to the IR state and impaired glucose/lipid metabolism to maintain glucose homeostasis. This assumption is supported by the fact that in STZ-induced diabetic mice, over expression of liver PFK2 isoform reduced hepatic glucose production by enhancing glycolysis and inhibiting gluconeogenesis, thereby decreasing glycaemia (*34*).

The role of PFK2/FBPase2 in the nucleus-to-cytoplasm translocation of GCK has been recently challenged; in primary hepatocytes, glucagon did not affect the localization of GCK and the phosphorylation/dephosphorylation of the liver-type isozyme of PFK2/FBPase2 did not modify the cellular GCK distribution (*35*).

REGULATORY ROLE OF THE MITOCHONDRIA FUNCTIONAL HOLOENZYME COMPLEX

Mitochondria may be a novel regulatory player of liver cell GCK compartmentalization. In fact, a mitochondrial holoenzyme complex, that includes BH3-only protein BAD, GCK, protein kinase A and protein phosphatase 1 catalytic units as well as WAVE-1 (a Wiskott-Aldrich family member), has been identified (Fig. 2). The functional link between BAD and GCK was demonstrated in Bad-deficient hepatocytes which results in a lower mitochondrial-based GCK activity. BAD-deficient mice display IGT, indicating a crosstalk pathway between glucose metabolism and apoptosis (*36*).

GCKR immunoreactivity is also localized in liver mitochondria associated to GCK, undergoing adaptive changes in the ratio of these proteins in response to different substrates, thus suggesting a functional role of GCKR in the cytoplasm (*37*). Further evidence is necessary to support this association since others have failed to detect GCK activity or immunoreactivity in isolated liver mitochondria (*38*).

ALLOSTERIC GCK ACTIVATORS

With diabetes, hepatic glucose metabolism exhibits three major defects: inappropriately increased fasting glucose production, decreased insulin-mediated suppression of glucose output and impaired glucose uptake. Considerable evidence supports the role of GCK in the pathogenesis of these defects: (a) animals with STZ-induced diabetes have decreased hepatic GCK activity (*5*), (b) liver-specific GCK-knockout mice exhibit mild hyperglycemia and defective glycogen synthesis during hyperglycemic clamps (*39*), (c) GCK mutations in humans causes maturity-onset diabetes of the young (MODY 2) (*40*), and d) defective GCK activity has been reported in some people with Type 2 diabetes (T2DM) (*41*).

In their landmark paper Grimsby et al. described the first allosteric small activator that increased recombinant human GCK activity and reversed GCKR inhibition. This compound also reduced glycaemia in normal and T2DM mice. The underlying mechanism for its action involves enhanced release of insulin and reduced hepatic glucose production (*42*). Using other GCK activators, the increased enzyme activity was accompanied by a parallel enhancement of GCK translocation in primary cultured hepatocytes and in rat liver. The increased hepatic glucose disposal is accomplished by increased GCK activity and stabilization of its activated state, thereby interfering with its binding to GCKR (*20*). However, these compounds also increase GCK activity in pancreatic β-cells, with the consequent increase in insulin secretion and the potential induction of hypoglycaemic events. A novel compound that efficiently decreases glucose *in vivo* affecting liver but not pancreatic β-cell GCK activity, represents a potentially useful compound for T2DM treatment (*43*).

Paradoxically, increased GCK activity has been described in obese diabetics (*44*) and in obese hyperinsulinemic Zucker rats (*45*). Moreover, long-term hepatic GCK over expression in

normal mice induces IR, increases triglycerides and results in IGT (*46*). Consequently, inadequate GCK activity rather than simply decreasing/increasing it is a major contributor to the abnormal liver glucose handling in IGT, IR and diabetes.

CONCLUDING REMARKS

Hepatic GCK tightly controls hepatic glucose disposal and is a major contributor to glucose homeostasis. GCK gene expression and activity are regulated by translational and post-translational mechanisms. Insulin is the primary up-regulator of the GCK gene promoter, probably acting through the PI3K/Akt pathway. Further studies will be required to identify both potential regulatory transcription factors of GCK gene expression and their mechanism of action.

The fact that age both increases the methylation of the GCK gene promoter and the prevalence of IR and diabetes, also merits further research to identify possible common underlying mechanisms and to develop preventive strategies.

Hepatocytes quickly shunt GCK from the nucleus to the cytosol parallel to the glucose concentration in the latter, thereby adapting the enzyme activity to metabolic conditions and maintaining peripheral glucose availability. Two major players are involved in such a transition: GCKR protein and PFK2/FBPase2. Even though the evidence for *in vivo* regulation via interaction with PFK2/FBPase2 is not conclusive, it strongly suggests a crosstalk between these two proteins and a critical modulatory role of the complex upon hepatic glucose metabolism. The possible therapeutic implications of this crosstalk with other cytosolic proteins and with other pathways like apoptosis warrant further study. Recently chronic ethanol administration to normal mice was shown to down regulate and inactivate pancreatic β-cell GCK through via tyrosine nitration. Nitrated GCK becomes more susceptible to degradation and triggers pancreatic β-cell dysfunction and apoptosis, processes that result in impaired glucose-induced insulin release and insulin resistance. These animals demonstrate oxidative stress secondary to an increased peroxynitrite production induced by ethanol metabolism, which in turn activates ATF3 that could act as an upstream down regulator of GCK (*47*). Since chronic ethanol consumption sequentially induces fatty liver, steatohepatitis and cirrhosis, it would be worth to evaluate whether oxidative stress could simultaneously impair liver GCK and be a link between enzymatic changes and hepatotoxicity.

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