

# Targeting Hepatic Glucokinase in Type 2 Diabetes

## Weighing the Benefits and Risks

Loranne Agius

**G**lucokinase (hexokinase IV) has a major role in the control of blood glucose homeostasis because it is the predominant hexokinase expressed in the liver, has a very high control strength on hepatic glucose disposal (1), and is the glucose sensor for insulin secretion in pancreatic  $\beta$ -cells (2). Glucokinase is currently considered a strong candidate target for antihyperglycemic drugs for type 2 diabetes (2–4). This is supported by the impact of mutations in the glucokinase gene on blood glucose concentration in humans. Inactivating mutations that lower the enzyme affinity for glucose or compromise glucokinase expression cause diabetes (maturity onset diabetes of the young type 2), whereas activating mutations lower blood glucose (2). Pharmacological activators of glucokinase (GKAs) that mimic the effect of activating mutations represent a potential novel strategy for antihyperglycemic therapy (2–4).

Type 2 diabetes is associated with defective regulation of hepatic glucose metabolism, involving elevated glucose production in euglycemic conditions and subnormal clearance of glucose by the liver after a meal because of delayed suppression of hepatic glucose production and impaired conversion of glucose to glycogen (5,6). This inefficient clearance of glucose by the liver is due at least in part to impaired regulation of glucose production and usage by hyperglycemia, a process sometimes described as decreased “autoregulation” or “glucose effectiveness” (7). Whether the defect in diabetes in humans involves decreased glucokinase activity is not established. Hepatic glucokinase activity was shown to be either elevated in newly diagnosed type 2 diabetic patients (8) or decreased in obese subjects with diabetes (9). Hepatic glucokinase is regulated by an inhibitory protein (glucokinase regulatory protein [GKRP]) that binds glucokinase with high affinity at basal glucose concentrations (5 mmol/l) and sequesters glucokinase in the nucleus in an inactive state (1,10). In the postprandial state, hyperglycemia causes dissociation of glucokinase from GKRP and translocation to the cytoplasm (Fig. 1). It could be speculated that decreased “glucose effectiveness” in type 2 diabetes in humans may involve decreased glucokinase expression or impaired regulation by GKRP, as occurs in animal models of insulin resistance (11,12), in addition to other metabolic defects.

For hepatic glucokinase to be an effective target for antihyperglycemic drugs (GKAs) in type 2 diabetes in humans, three essential criteria must be met. First, there must be sufficient expression of endogenous glucokinase such that the GKAs can elicit a substantial increment in glucose phosphorylating capacity. Second, glucokinase must have a high control strength on hepatic glucose disposal in the dysregulated diabetic state, such that increments in glucokinase activity caused by the GKAs relay corresponding changes in glucose flux. A third, important criterion is that glucokinase activation in diabetes must not have untoward metabolic effects, such as excessive enhancement of hypertriglyceridemia (13) or accumulation of fat in the liver that would further aggravate hepatic insulin resistance. Although several studies have demonstrated the efficacy of liver-specific up regulation or down regulation of glucokinase activity in nondiabetic models (rev. in 3,14), there have been few detailed, thorough studies based on either glucokinase overexpression or pharmacological activation that have specifically addressed these issues in models of diabetes with insulin resistance.

Torres et al. (15) report a detailed study of the effect of overexpression of glucokinase in liver on glucose turnover in the Zucker diabetic fatty (ZDF) rat (20 weeks old) in basal conditions and during a hyperglycemic clamp. The ZDF rat is characterized by increased body weight and progressive insulin resistance, beginning at ~7 weeks and leading to development of overt diabetes after 10 weeks. Hepatic glucokinase activity was elevated before the development of diabetes (at 7–10 weeks) but declined subsequently in parallel with the fall in circulating insulin to an activity level about one third that of nondiabetic controls at 20 weeks. GKRP expression remained constant and, accordingly, the glucokinase-to-GKRP ratio (and thereby the hepatocellular affinity for glucose [1]) paralleled the changes in glucokinase expression. The development of diabetes was associated with increased endogenous glucose production, glucose cycling between phosphorylation of glucose and dephosphorylation of glucose-6-phosphate, and increased flux through glucose-6-phosphatase, determined from the sum of glucose cycling and endogenous glucose production.

Torres et al. (15) overexpressed glucokinase in the liver of ZDF rats by treatment with an adenoviral vector at 19–20 weeks. The viral titers used enabled selection of two groups of treated diabetic rats: one with glucokinase activity that was restored to the level of nondiabetic controls (threefold increase) and another with activity twice that of nondiabetic controls (sixfold increase). The hepatic glucose-6-phosphate content and glycogen synthesis from glucose (Fig. 1) closely paralleled glucokinase expression and were normalized in the group with restored glucokinase activity and further increased in the

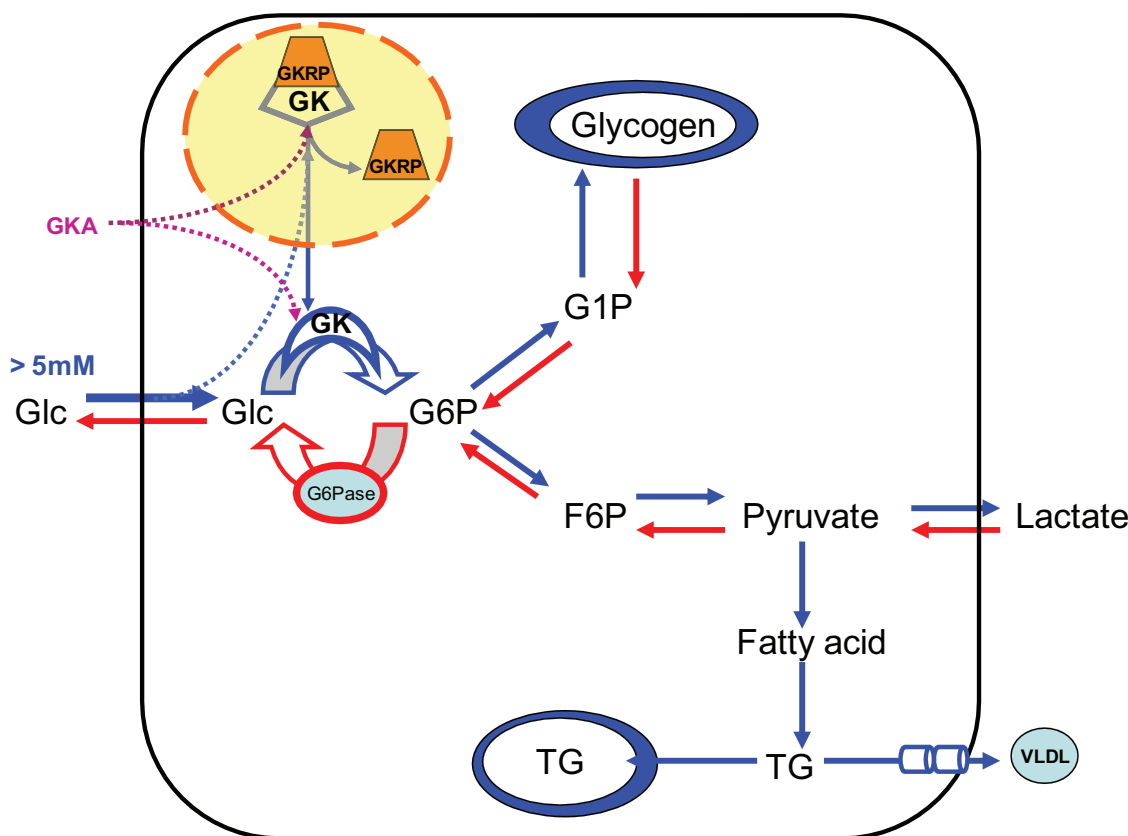
From the Institute of Cellular Medicine, The Medical School, Newcastle University, Newcastle upon Tyne, U.K.

Corresponding author: Loranne Agius, loranne.agius@ncl.ac.uk.

DOI: 10.2337/db08-1470

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**FIG. 1. Hepatic glucose metabolism.** In the postabsorptive state, glucokinase (GK) is sequestered in the nucleus bound to GKRP and the liver maintains blood glucose homeostasis by glucose production (red arrows) by glycogenolysis from glycogen and by gluconeogenesis from lactate and other gluconeogenic substrates. In the postprandial state (blue arrows), a rise in blood glucose concentration in the portal vein  $>5$  mmol/l causes dissociation of glucokinase from GKRP and translocation of glucokinase to the cytoplasm, resulting in increased glucose phosphorylation to glucose-6-phosphate (G6P) and conversion to glycogen, lactate, and triglyceride (TG). If the production of triglyceride via glycolysis to pyruvate and synthesis of fatty acids exceeds the VLDL secretion capacity, triglyceride accumulates in the hepatocyte. Pharmacological GKAs mimic the effect of glucose in causing translocation of glucokinase to the cytoplasm, and they also directly increase glucokinase activity. F6P, fructose-6-phosphate; Glc, glucose; G6P, glucose-6-phosphate; G6Pase, glucose-6-phosphatase.

group with twofold elevation of glucokinase. The latter indicates that the high control strength of glucokinase on both the glucose-6-phosphate content and glycogen synthesis is preserved in the diabetic model. In the experimental group with restored glucokinase activity, hyperglycemia was partially corrected (60%), and elevated endogenous glucose production and flux through glucose 6-phosphatase, both before and during the hyperglycemic clamp, were partially rectified. In the group with overcompensated glucokinase activity (twice normal), the correction of hyperglycemia was further improved (87%), though still not completely normalized, and endogenous glucose production was still elevated in basal conditions (before the clamp) but was suppressed during the hyperglycemic clamp (similar to nondiabetic controls). While these results demonstrate the efficacy of glucokinase overexpression in reversing hyperglycemia in diabetes, they also show that overcompensation of glucokinase activity to twofold above that for nondiabetic controls is necessary to achieve glycemic control and near-normal endogenous glucose production. This indicates that the defect in the diabetic model is clearly not confined to glucokinase regulation and, accordingly, overcompensation of glucokinase activity is necessary to achieve glycemic control.

An important issue is whether treatments that raise hepatic glucokinase activity to a sufficient level to achieve adequate glycemic control in diabetes cause excessive elevation in plasma and hepatic triglycerides (Fig. 1) by

stimulation of glycolysis and lipogenesis. A previous study demonstrated that overexpression of glucokinase by sixfold in nondiabetic rats raised plasma triglycerides and cautioned against possible hypertriglyceridemia resulting from glucokinase activation (13). However, other studies involving a more modest increase in hepatic glucokinase activity (less than twofold) did not show abnormalities in plasma triglycerides on either a normal diet or a high-fat diet (rev. in [3]). At 20 weeks the ZDF rat has very marked hypertriglyceridemia (15). In this model, glucokinase overexpression was associated with markedly raised lactate concentrations and a trend toward elevated plasma triglycerides (15). It is noteworthy, however, that in 20-week ZDF rats, impaired clearance of triglycerides is an important contributing factor to excessive triglyceridemia (16). Further studies are needed on the effects of glucokinase overexpression or activation with GKAs on both plasma triglycerides and hepatic triglycerides in experimental animal models where plasma triglycerides correlate with hepatic triglyceride secretion and in clinical studies.

At this point, some lessons can be learned from the recent genome-wide analysis studies that identified associations among two common GKRP gene (*GCKR*) variants (an intron and a coding single nucleotide polymorphism) and elevated serum triglycerides and lower fasting plasma glucose (17–20). In type 2 diabetes, hypertriglyceridemia generally correlates with hyperglycemia because conversion of glucose to triglyceride is a major hepatic route of

glucose disposal (Fig. 1). However, the inverse correlation between plasma triglycerides (elevated) and glucose (lowered), in association with two GKR variants (rs780094 and rs1260326), suggests that the increased glucose phosphorylation capacity (inferred from the lowering of plasma glucose because no molecular characterization of the human GKR variants is available as yet) correlates with elevated triglycerides, as was indicated by glucokinase overexpression (13). Nonetheless, at least in some circumstances (20), the benefits of blood glucose lowering may outweigh the risks posed by higher triglycerides. Likewise, although targeting hepatic glucokinase in type 2 diabetes may enhance plasma triglycerides through an inevitable physiological process, the benefits of improved glycemic control might still outweigh the risks of elevated triglycerides. Alternatively, the latter can be treated by combination therapy. Thorough scrutiny of the impact of novel treatments on hepatic and plasma triglycerides remains of paramount importance.

#### ACKNOWLEDGMENTS

No potential conflicts of interest relevant to this article were reported.

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