Metabolic Impact of Glucokinase Overexpression in Liver

Lowering of Blood Glucose in Fed Rats Is Accompanied by Hyperlipidemia

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The balance between hepatic glucose uptake and production is perturbed in both major forms of diabetes. It has been suggested that pharmacologic or genetic methods for enhancing glucokinase (GK) enzymatic activity in liver might be a means of increasing glucose disposal and lowering blood glucose in diabetic patients. To better evaluate this possibility, we used a recombinant adenovirus containing the cDNA encoding GK (AdCMV-GKL) to achieve overexpression of the enzyme at different levels in liver of normal rats. In a first set of experiments, in rats fasted for 18 h, AdCMV-GKL infusion caused a 211% increase in hepatic GK activity relative to animals infused with a control virus (AdCMV-βGAL). AdCMV-GKL–treated fasted rats exhibited no significant changes in circulating glucose, free fatty acids (FFAs), lactate, β-hydroxybutyrate, or insulin levels relative to controls, whereas triglyceride (TG) levels were slightly increased (53%). In a second set of studies, in rats fed ad libitum, GK was overexpressed in liver by 3- and 6.4-fold. Animals with the lower degree of GK overexpression exhibited no significant changes in circulating glucose, FFAs, insulin, TG, or lactate levels relative to controls that received a virus encoding a catalytically inactive mutant GK (AdCMV-GK_{203}), but did show a modest increase in lactate (58%) relative to AdCMV-βGAL–infused controls. In contrast, the higher level of GK overexpression caused a 38% decrease in blood glucose levels and a 67% decrease in circulating insulin levels relative to AdCMV-GK_{203}–infused animals. The decline in glucose levels was accompanied by a 190% increase in circulating TG and a 310% increase in circulating FFAs; total plasma cholesterol was unaffected. Finally, fasted animals treated with AdCMV-GKL had 5.4 times as much liver glycogen as AdCMV-βGAL–treated controls; no significant increases in liver glycogen were observed at either level of GK overexpression in ad libitum–fed rats relative to fed controls. In sum, levels of hepatic GK overexpression associated with a decline in blood glucose are accompanied by equally dramatic increases in FFAs and TG, raising concerns about manipulation of liver GK activity as a viable strategy for treatment of diabetes. ***Diabetes*** **48:2022–2027, 1999**

Glucokinase (GK), or hexokinase IV, catalyzes the conversion of glucose to glucose-6-phosphate and plays a central role in the maintenance of glucose homeostasis. In liver, the enzyme is an important regulator of glucose storage and disposal. In β-cells of the pancreatic islets of Langerhans, GK regulates glycolytic rate and, as a consequence, plays a central role in control of glucose-stimulated insulin secretion (1–4). Much attention has been focused on GK as a result of the discovery that a form of maturity-onset diabetes of the young known as MODY-2 is due to mutations in the GK gene, resulting in reduced activity of the enzyme in both the liver and islets. Decreased hepatic GK activity has been shown to be linked to reduced glucose storage in human MODY-2 patients (5) and decreased glucose disposal in heterozygous GK knockout mice (6). Additionally, it has been suggested that an imbalance in the expression levels of GK and its counteracting enzyme, glucose-6-phosphatase (G-6-Pase), may contribute to loss of control of hepatic glucose output in both classical (late-onset) type 2 diabetes and insulin-deficient type 1 diabetes patients. Based on this suggestion, it has been proposed that strategies for increasing expression or activity of GK in liver of diabetic patients could be a means for ameliorating hyperglycemia.

To fully evaluate the validity of such a strategy, it is critical to understand the effect of acute modulation of GK activity on hepatic function and fuel homeostasis under a variety of physiologic circumstances. GK overexpression has been achieved by several groups in transgenic mice. In one study, the cDNA encoding GK was placed under control of the PEPCK promoter, resulting in liver-specific expression of the enzyme (7). Animals were studied in the ad libitum–fed state, at which time a twofold increase in GK enzymatic activity was observed and shown to be linked to modest decreases in circulating glucose and insulin, a doubling of hepatic glycogen levels and similarly increased glucose-6-phosphate levels, and a small rise in circulating triglycerides (TGs). Other metabolic parameters, such as circulating free fatty acids

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FFA, free fatty acid; G-6-Pase, glucose-6-phosphatase; GK, glucokinase; GRP, glucokinase regulatory protein; TG, triglyceride.

(FFAs), lactate, and ketones, were unchanged. In another study, the full-length GK gene containing both the hepatic and pancreatic β-cell promoters was used to create transgenic animals, resulting in increased enzyme activity in liver and a decrease in pancreatic islet β-cells (8). That study is of interest because it suggests that enhanced glucose disposal by the liver may result in downregulation of β-cell GK expression, but the presence of functional transgenes in both the liver and islets makes it difficult to isolate the hepatic contribution. Finally, another study in which the human apolipoprotein A-I promoter was used to drive expression of GK in liver found only a 20% increase in enzyme activity, resulting in modest decreases in circulating glucose, lactate, and insulin levels (9).

Although the above transgenic studies have provided useful information, a number of issues remain to be addressed. First, all the transgenic models overexpress GK throughout life, making it possible that some of the observed metabolic changes represent compensatory phenomena rather than acute effects of hepatic GK overexpression. Second, metabolic analysis in both fasted and fed animals with significant liver-specific GK overexpression has not been accomplished. Finally, a comparison of the metabolic impact of different levels of liver-specific GK overexpression has not been carried out. We have previously demonstrated that a recombinant adenovirus containing the GK cDNA (AdCMV-GKL) effectively directs overexpression of the enzyme in isolated rat hepatocytes, causing clear increases in glucose storage and utilization (10,11). In the current study, the AdCMV-GKL virus has been used to achieve varying levels of GK overexpression in liver of fasted and fed rats, allowing us to address all of the issues raised above.

RESEARCH DESIGN AND METHODS

Animal maintenance. All procedures were carried out in accordance with the animal care guidelines of the University of Texas and the *Guide for the Care and Use of Laboratory Animals* from the National Research Council. Male Wistar rats (Charles River Breeding Laboratories, Wilmington, MA) weighing 200–225 g were housed at 23°C on a 0900–2100 light cycle and were allowed free access to water and food (65% carbohydrate, 11% fat, 24% protein). The rats were housed under these conditions for ~1 week before the beginning of an experiment.

Recombinant adenoviruses. Adenoviruses containing the cDNA encoding rat liver GK (AdCMV-GKL) or the bacterial β-galactosidase gene (AdCMV-βGAL) were prepared, amplified, and stored as previously described (12–14). As a second control, we also prepared a recombinant adenovirus (AdCMV-GK_{203}) containing a 2.4-kb *Bam*HI cDNA fragment encoding full-length human islet GK with a point mutation (V203A) that strongly impairs the catalytic activity of the enzyme (15), using previously described methods (12). The cDNA encoding the V203A mutant GK was a gift from Drs. Jun Takeda and Graeme Bell, University of Chicago.

Recombinant adenovirus administration. All animals received 15 mg/kg cyclosporine A (Calbiochem, La Jolla, CA) and 1.5 mg/kg prednisone (Upjohn, Kalamazoo, MI) for a period extending from 1 day before to 1 day after the administration of recombinant adenoviruses. Administration of cyclosporine A and prednisone at these doses had no effect on any of the metabolic parameters measured in these studies relative to animals that were not treated with these drugs (data not shown). Recombinant adenoviruses were administered to animals anesthetized with metofane (Mallinckrodt Veterinary, Mundelein, IL) via the tail vein at doses of $0.5–1.0 \times 10^{12}$ particles in a final volume of 500 µl sterile saline using a 24-gauge catheter needle (Becton Dickinson, Santy, UT). After adenoviral administration, rats were individually caged, and food intake and weight gain were monitored until the day of the experiment. Metabolic studies were performed 4–5 days after viral administration.

Experimental design. In fasting experiments, ~96 h after adenovirus administration, animals were deprived of food but allowed access to water. Eighteen hours after food removal (1–2 h after the end of the dark cycle), animals were anesthetized with Nembutal (Abbott Laboratories, Chicago, IL), and a 3-ml blood sample was taken by heart puncture. Plasma from blood samples was rapidly frozen in liquid nitrogen. The liver was removed, washed in ice-cold saline, patted dry, and frozen in liquid nitrogen. This procedure took ~1 min. In ad libitum feeding experiments, a similar procedure was followed except that animals were killed 1–2 h after the end of the dark cycle with no previous fast. In studies with AdCMV-GKL–infused fed animals, GK activities were found to cluster at moderate and high levels of overexpression relative to AdCMV-βGAL– or AdCMV-GK_{203}–infused animals (Fig. 1). The RESULTS section is organized in terms of these discrete groups.

Tissue and plasma analyses. Total glucose phosphorylating activity was assessed in liver extracts by a coupled enzymatic assay that measured the production of NADH from NAD in the presence of glucose-6-phosphate dehydrogenase from Leuconostoc mesenteroides (Sigma, St. Louis, MO) (10). The assay was performed at 100 mmol/l glucose at 25°C, and activity is expressed as milliunits per milligram protein, where 1 unit is defined as the amount of enzyme activity that forms 1 µmol NADPH in 1 min at 25°C and pH 7.2. Total protein concentrations were measured using the Bio-Rad protein assay kit (Hercules, CA). GK protein was measured by immunoblot in protein extracts from rat tissues as described (13). Tissue glycogen was measured as described (16). Plasma measurements of glucose were made using a HemoCue glucose analyzer (Angelholm, Sweden), insulin and glucagon using radioimmunoassay kits (Linco Research, St. Charles, MO), lactate (Sigma, St. Louis, MO), TGs (Sigma), nonesterified fatty acids, and total cholesterol (Boehringer Mannheim, Mannheim, Germany) using kits, and β-hydroxybutyrate using an enzymatic endpoint assay (Sigma).

Statistical analysis. Data are expressed as means ± SE. Data were analyzed with the statistics module of Microsoft Excel 5.0 using an unpaired two-tailed Student's *t* test. Statistical significance was assumed at $P < 0.05$.

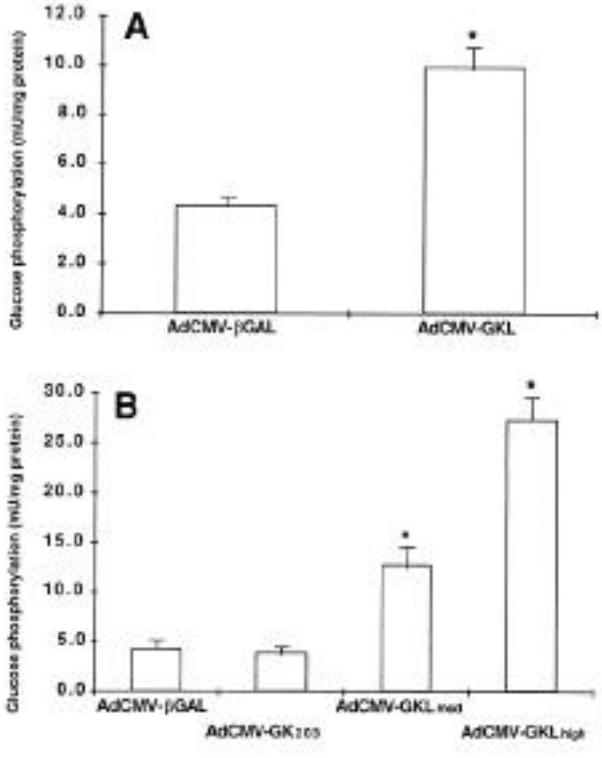

FIG. 1. Glucose phosphorylating capacity in liver of animals infused with recombinant adenoviruses. *A*: Values in animals fasted for 18 h. Animals were infused with AdCMV-βGAL or AdCMV-GKL viruses as described in METHODS. Ninety-six hours after viral infusion, animals were fasted for 18 h and killed for collection of liver samples and measurement of total glucose phosphorylation capacity. Values represent means ± SE for six AdCMV-βGAL–treated and seven AdCMV-GKL–treated animals. *B*: Values in ad libitum–fed animals. Animals were infused with AdCMV-βGAL, AdCMV-GK_{203}, or AdCMV-GKL. Prospective analysis revealed clustering of enzymatic activities at moderate (AdCMV-GKLmod) or high (AdCMV-GKLhigh) degrees of overexpression, as shown. Values represent means ± SE for 14 AdCMV-βGAL–treated, 4 AdCMV-GK_{203}–treated, 7 AdCMV-GKLmod, and 5 AdCMV-GKLhigh animals. Note that the amount of glucose phosphorylating activity in AdCMV-GKL–treated fasted rats is statistically indistinguishable from that in the AdCMV-GKLmod fed rats (12.7 ± 2.8 vs. 9.9 ± 0.7 mU/mg protein, respectively; $P > 0.1$). *Significantly higher hepatic glucose phosphorylating activity than controls ($P < 0.05$).

TABLE 1
Fasted plasma variables of AdCMV-βGal and AdCMV-GKL treated rats

	AdCMV-βGAL	AdCMV-GKL
Lactate (mg/dl)	10.7 ± 2.7	6.0 ± 1.2
TGs (mg/dl)	34.7 ± 5.4	52.3 ± 5.1*
FFAs (mmol/l)	0.29 ± 0.04	0.33 ± 0.10
β-Hydroxybutyrate (mg/dl)	14.1 ± 1.32	16.2 ± 2.14
Glucose (mg/dl)	122 ± 6.2	114 ± 4.8
Insulin (ng/ml)	0.14 ± 0.02	0.15 ± 0.02

Data are means ± SE. Male rats received a recombinant adenovirus expressing either β-galactosidase (AdCMV-βGAL) or rat liver GK (AdCMV-GKL). Approximately 96 h after viral transduction, all animals underwent an 18-h fast. Blood samples were then taken and animals were killed. A number of plasma metabolic parameters were measured. *Significant difference between groups ($P < 0.05$).

RESULTS

Impact of adenovirus-mediated GK expression in liver of fasted rats. We have previously shown that GK overexpression in primary hepatocytes results in dramatic increases in glucose utilization and storage (10,11). To assess the metabolic impact of GK overexpression in liver of fasted rats, we infused the AdCMV-GKL virus, or the AdCMV-βGAL virus as a control, via a tail vein catheter. Animals were allowed to recover for 4 days with free access to food and water and were then fasted for 18 h before collection of blood and liver samples. As shown in Fig. 1*A*, infusion of AdCMV-GKL caused a 2.1-fold increase in glucose phosphorylating capacity in liver compared with animals infused with a similar dose of the control AdCMV-βGAL virus. Circulating hormone and metabolite levels in fasted AdCMV-GKL– and AdCMV-βGAL–infused rats are shown in Table 1. Surprisingly, GK overexpression caused no significant changes in circulating glucose, FFAs, lactate, or insulin levels. TG levels were slightly increased (53%) in AdCMV-GKL–treated animals versus AdCMV-βGAL–treated controls. Thus, in fasted animals, moderate overexpression of GK in liver has only a modest impact on an important set of circulating metabolites and hormones.

Impact of adenovirus-mediated GK expression in liver of fed rats. We next evaluated the effect of hepatic GK overexpression during periods of nutritional repletion. A protocol similar to that described for the studies on fasted rats was used, except that blood and tissue samples were collected from ad libitum–fed animals emerging from the overnight dark period. In addition to studies involving administration of AdCMV-GKL and the AdCMV-βGAL control virus, we added an additional control for untoward effects of high levels of protein overexpression per se, involving infusion of a recombinant adenovirus containing the cDNA encoding a mutant GK with sharply reduced enzyme activity (AdCMV-GK_{203}). Total glucose phosphorylating capacity was the same in liver of ad libitum–fed animals that received AdCMV-βGAL or AdCMV-GK_{203} (Fig. 1*B*). Prospective analysis of fed animals that received 0.5–1.0 × 10^{12} particles of AdCMV-GKL revealed clustering of enzyme overexpression in two groups, with averages of 3- and 6.4-fold increases in glucose phosphorylating activity relative to AdCMV-βGAL– or AdCMV-GK_{203}–infused controls (Fig. 1*B*). These two groups are referred to below as having "moderate" and "high" levels of GK overexpression. Immunoblot analysis revealed that animals with high levels of GK overexpression due to AdCMV-GKL infusion had increases in GK protein in liver similar to those observed in animals infused with AdCMV-GK_{203}, relative to AdCMV-βGAL–treated controls (data not shown).

Table 2 summarizes levels of important circulating metabolites and hormones in all four groups of ad libitum–fed animals. While no changes were observed in levels of FFAs, glucose, or insulin in AdCMV-βGAL– versus AdCMV-GK_{203}–treated animals, overexpression of the mutant GK did cause a modest but significant increase in circulating lactate and a trend toward increased TG that approached statistical significance ($P = 0.06$). In animals with moderately increased GK activity in liver due to AdCMV-GKL infusion, lactate and TG levels were increased to an extent similar to that observed in animals infused with AdCMV-GK_{203}. The changes in metabolite levels observed in response to AdCMV-GK_{203} infusion are somewhat surprising, given that the enzyme is catalytically inactive. These results may be explained by binding of the mutant GK to the glucokinase regulatory protein (GRP), which normally binds GK and inhibits its enzyme activity (17). We suggest that binding of the mutant GK to GRP allows a larger proportion of endogenous, normal GK to exist in an active form. This idea is supported by a recent study showing that binding of GK to GRP is saturable in hepatocytes (18).

TABLE 2
Fed plasma variables of rats treated with AdCMV-βGal, AdCMV-GK_{203}, or AdCMV-GKL

	AdCMV-βGAL	AdCMV-GK_{203}	AdCMV-GKLlow	AdCMV-GKLhigh
Lactate (mg/dl)	30.7 ± 3.0	47.6 ± 4.8*	48.5 ± 6.6*	69.6 ± 14.1*
TGs (mg/dl)	62.3 ± 7.2	90.1 ± 4.4	88.4 ± 14.0	171.1 ± 20.1*†
FFAs (mmol/l)	0.11 ± 0.01	0.15 ± 0.01	0.13 ± 0.02	0.47 ± 0.07*†
Cholesterol (mg/dl)	65.2 ± 8.0	ND	ND	61.6 ± 11.5
β-Hydroxybutyrate (mg/dl)	1.41 ± 0.21	ND	1.49 ± 0.33	3.35 ± 0.66*
Glucose (mg/dl)	228.2 ± 9.5	225.5 ± 10.9	200.0 ± 7.1	139.6 ± 15.9*†
Insulin (ng/ml)	3.56 ± 0.34	2.61 ± 0.17	3.04 ± 0.54	0.86 ± 0.36*†

Data are means ± SE. Male rats received a recombinant adenovirus expressing β-galactosidase (AdCMV-βGAL), a mutant GK (AdCMV-GK_{203}), or liver GK (AdCMV-GKL). Approximately 108 h after viral transduction, blood samples were taken and animals were killed (1–2 h after the end of the dark cycle). A number of plasma metabolic parameters were subsequently measured. *Significant difference compared with AdCMV-βGAL ($P < 0.05$); †significant difference between AdCMV-GKhigh and AdCMV-GK_{203} ($P < 0.05$). ND, not determined.

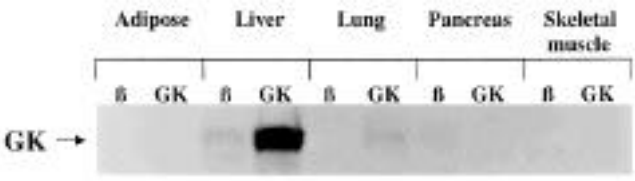

FIG. 2. Immunoblot analysis of GK protein in various tissues of ad libitum–fed animals infused with recombinant adenoviruses. Normal ad libitum–fed rats were infused with AdCMV-βGAL (β) or AdCMV-GKL (GK), and 108 h after viral infusion, the indicated tissue samples were collected for GK immunoblot analysis as described in METHODS. GK was found to be undetectable in brain or kidney of AdCMV-βGAL– or AdCMV-GKL–treated rats (data not shown). The blot is representative of two independent experiments.

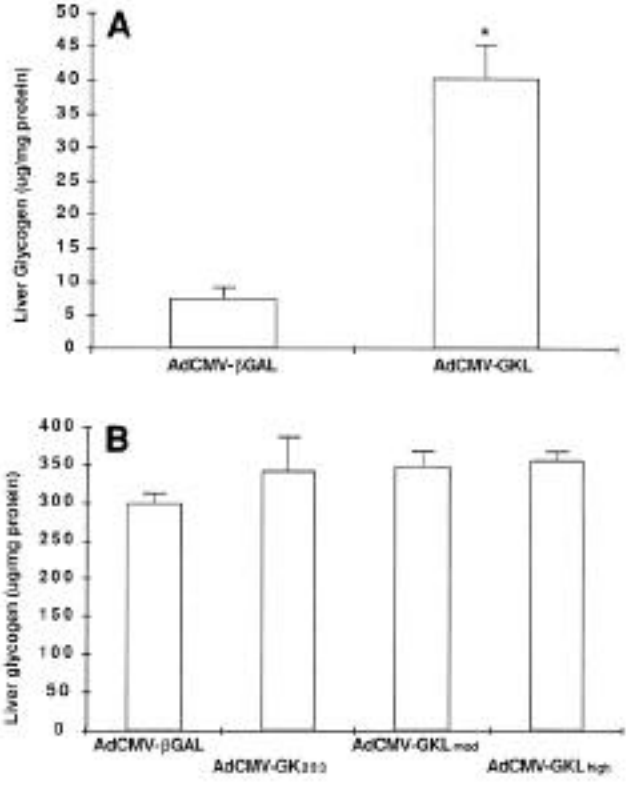

FIG. 3. Liver glycogen levels in rats infused with recombinant adenoviruses. *A*: Values in animals fasted for 18 h. Animals were infused with AdCMV-βGAL or AdCMV-GKL viruses as described in METHODS. Ninety-six hours after viral infusion, animals were fasted for 18 h and killed for collection of liver samples and measurement of glycogen levels. Values represent means ± SE for six AdCMV-βGAL–treated and seven AdCMV-GKL–treated animals. *Significantly higher hepatic glycogen levels than controls ($P < 0.05$). *B*: Values in ad libitum–fed animals. Animals were infused with AdCMV-βGAL, AdCMV-GKL_{203}, or AdCMV-GKL, and glycogen levels were analyzed in liver samples taken from ad libitum–fed animals. Values represent means ± SE for 14 AdCMV-βGAL–treated, 4 AdCMV-GK_{203}–treated, 7 AdCMV-GKLmod, and 5 AdCMV-GKLhigh animals.

However, neither AdCMV-GK_{203} infusion nor the moderate level of GK overexpression induced by AdCMV-GKL infusion had any impact on circulating glucose, insulin, or FFA levels.

In contrast, the higher level of GK overexpression had major effects on nearly all parameters measured (Table 2). Even when compared with the AdCMV-GK_{203}–infused group, these animals exhibited a 38% decrease in blood glucose levels (from 226 to 140 mg/dl), a 67% decrease in circulating insulin levels (from 2.6 to 0.9 ng/ml), and a 44% increase in circulating lactate levels. Importantly, the decline in circulating glucose concentration achieved by the high level of hepatic GK overexpression came at a cost, 190% increase in circulating TG and 310% increase in circulating FFAs. Further, β-hydroxybutyrate levels were increased by 240% in animals with high levels of hepatic GK overexpression relative to AdCMV-βGAL–infused controls. However, total plasma cholesterol was unchanged in animals with high levels of GK overexpression relative to either control group (Table 2).

Site of GK overexpression. Previous studies in mice (14) and rats (19) have indicated that systemic infusion of recombinant adenoviruses results in preferential transgene delivery to liver. However, given that GK expression has recently been shown to enhance glucose disposal in human skeletal muscle myocytes (20), we wished to confirm that GK overexpression was largely confined to liver in our studies. By immunoblot analysis, GK was undetectable in adipose tissue, skeletal muscle, and pancreas of either AdCMV-βGAL– or AdCMV-GKL–treated fed rats (Fig. 2) and was also absent from brain and kidney in both groups (data not shown). As expected, GK was detectable in liver of AdCMV-βGAL–treated animals, and a clear increase in expression was noted in AdCMV-GKL–treated rats (Fig. 2; the blot shown is representative for animals with the high level of GK overexpression). GK was undetectable in lungs of control animals but was clearly expressed in lungs of AdCMV-GKL–treated animals, reaching a level similar to that in control liver. However, glycogen content in lungs of AdCMV-GKL–treated fed rats was less than that in liver of normal fasted rats (data not shown), suggesting that GK expression in lung did not contribute significantly to glucose disposal and altered metabolic status.

Effects of GK overexpression on liver glycogen levels. Previous studies have demonstrated that GK overexpression causes dramatic increases in glycogen synthesis in hepatocytes, hepatic cell lines, and transgenic animals (7–10,21). However, the impact of liver-specific expression of GK at varying levels and under different physiologic conditions has not been evaluated. As shown in Fig. 3*A*, overexpression of GK at moderate levels prevents the complete depletion of hepatic glycogen stores normally seen as a consequence of an overnight fast in rats, such that animals treated with AdCMV-GKL had 5.4 times as much liver glycogen as AdCMV-βGAL–treated controls under these conditions. Interestingly, no significant differences in liver glycogen were noted in the four experimental groups (animals treated with AdCMV-βGAL or AdCMV-GK_{203} or at the two levels of overexpression achieved with AdCMV-GKL) of ad libitum–fed rats (Fig. 3*B*). These findings suggest that some intrinsic limitation exists for glycogen synthesis in fed animals with hepatic GK overexpression, and also that the lowering of blood glucose observed in the high GK-overexpressing group was primarily due to increased hepatic glucose utilization as opposed to glucose storage.

DISCUSSION

The storage and utilization of glucose in liver is impaired in both major forms of diabetes. The impairment occurs in type 1 diabetes because of a deficiency in the anabolic hormone insulin. In type 2 diabetes, insulin resistance is present, and it has been suggested that this could lead to an imbalance in the expression levels of GK, the primary glucose phos-

phorylating enzyme of liver, and the counteracting enzyme, G-6-Pase. Consistent with this idea, insulin is known to induce GK and decrease G-6-Pase gene expression in liver (22–26). Further, adenovirus-mediated overexpression of the catalytic subunit of the G-6-Pase complex in liver of normal animals results in several of the metabolic abnormalities associated with type 2 diabetes, including glucose intolerance, hyperinsulinemia, increased peripheral TG stores, and decreased hepatic glycogen levels (19). Conversely, several groups have suggested that increasing the activity of GK in liver by genetic or pharmacologic methods could increase hepatic glucose disposal and lower blood glucose levels in individuals with either major form of diabetes. Indeed, in one study, near-normalization of blood glucose levels was achieved in streptozotocin-induced diabetic mice transgenic for GK expression under control of the PEPCK promoter (7). Based on these findings, the authors suggested that expression of GK in liver at higher levels could result in complete normalization of blood glucose levels in patients with type 1 diabetes. It should be noted, however, that these animals are completely insulin-deficient, making it difficult to predict the metabolic impact of increased GK expression in the presence of the hormone, as would be the case in type 1 diabetic patients receiving insulin therapy or in patients with type 2 diabetes. Thus, a better assessment of the metabolic alterations that occur as a consequence of different levels of GK overexpression in liver is required. In the current study, this important issue has been addressed by using recombinant adenovirus to manipulate GK expression in liver of normal rats.

In our studies, modest hepatic GK overexpression (two- to threefold) had little impact on circulating metabolite and hormone levels in either fasted or ad libitum–fed states. In fasted animals, only a mild increase in circulating TG was noted, while in fed rats, a similar rise in TG was accompanied by only a modest increase in blood lactate concentrations. The increase in circulating lactate levels in ad libitum–fed animals may reflect increased flux through GK as a function of the increase in circulating glucose levels that occur in fed versus fasted animals. These results differ in some respects from those of Ferre et al. (7) in transgenic mice. Those investigators reported an increase in liver GK activity of ~2.5-fold in ad libitum–fed transgenic animals compared with nontransgenic animals, resulting in decreases in blood glucose and insulin levels of 29 and 45%, respectively, in addition to a 19% increase in TG levels and a 20% decrease in circulating FFAs. In our studies in ad libitum–fed rats, a threefold increase in hepatic glucose phosphorylating activity resulted in 13 and 14% decreases in circulating glucose and insulin levels, respectively, but neither trend was statistically significant. Only with higher levels of GK overexpression (6.4-fold increase in glucose phosphorylation) were substantial decreases in glucose and insulin noted in our studies with fed rats.

The reasons for these differences in metabolic impact of modest levels of GK overexpression in the two models is unclear. One possibility is that GK overexpression in mouse liver may be more effective at stimulating glucose disposal than the same degree of overexpression in a larger animal. Consistent with this, another study in which the apolipoprotein A-I gene enhancer was used to direct liver-specific GK overexpression in transgenic mice resulted in only a 20% increase in hepatic enzyme activity but a substantial lowering of fasting glucose and insulin levels and an improvement in glucose clearance during an oral glucose tolerance test (9). A second possibility is that adenovirus-directed expression of GK may target different subsets of hepatocytes with different efficiency, whereas GK expression in transgenic animals could be more uniform. We and others have demonstrated an 80–90% overall efficiency of gene delivery to hepatocytes of intact rodents (27–29), but periportal hepatocytes appear to stain more intensely after infusion of the AdCMV-βGAL reporter virus than perivenous hepatocytes (29). The relevance of this finding is that periportal hepatocytes have been reported to contain higher levels of glycogenolytic and gluconeogenic enzymes; conversely, perivenous hepatocytes may be enriched in enzymes of glucose utilization (30), suggesting that glucose disposal may be less affected in animals that overexpress GK via adenovirus-mediated gene delivery compared with germ-line manipulation. A formal test of this hypothesis will require repeating the adenovirus studies described herein in mice or the creation of GK transgenic rats. Finally, germ-line manipulated animals overexpress GK throughout life, possibly resulting in compensatory changes in insulin secretion, insulin action, or other metabolic variables that do not occur with acute manipulation of GK via adenovirus technology.

In considering the potential value of manipulation of GK expression or activity as a means of lowering blood glucose levels in diabetic patients, our studies of higher levels of GK overexpression in liver of fed rats introduce an important cautionary note. Dramatic differences in all circulating metabolic parameters assayed are observed when GK overexpression is increased from 3- to 6.4-fold (Table 2). Clearly, overexpression of GK at higher levels is an effective means of lowering blood glucose concentrations, with an even more striking impact on circulating insulin (decrease of >65%). However, these changes occur at a cost: dramatic increases in circulating TG and FFA levels. These effects of GK overexpression on circulating blood lipids may involve both changes in hepatic fuel metabolism and extrahepatic events. In the liver, increased glycolytic flux as a consequence of GK overexpression would be expected to increase concentrations of glycerol-3-phosphate and malonyl CoA. Malonyl CoA serves as both a substrate for de novo lipogenesis and an inhibitor of carnitine-palmitoyl transferase 1, and increases in levels of this intermediate will inhibit fatty acid oxidation and channel fatty acyl-CoA into TG and VLDL synthesis (31). The capacity for TG synthesis would be further enhanced by expansion of the pool of glycerol phosphate. In addition, the increase in circulating FFAs is undoubtedly linked to the substantial decrease in insulin levels, which will result in increased lipolysis in adipose tissue.

An important implication of our findings is that levels of GK overexpression required for therapeutic modulation of hyperglycemia in type 2 diabetes may have the undesired effect of exacerbating the hyperlipidemia associated with the disease. Thus, increases in liver GK activity would be expected to lower blood glucose and insulin levels in such patients, but with attendant increases in hepatic glycolytic flux, malonyl CoA production, and VLDL and TG production. Such elevations in circulating lipids could have deleterious consequences. For example, poor regulation of insulin secretion in type 2 diabetes has been ascribed to a phenomenon of "lipotoxicity," in which chronic exposure of islet β-cells to elevated levels of FFAs results in deterioration of function and increased susceptibility to apoptotic agents (32,33). In addi-

tion, it is unclear if simple alterations in the expression levels of GK or G-6-Pase will be sufficient to achieve therapeutic benefit in type 2 diabetes, since a recent study has demonstrated that such patients lose the capacity to regulate hepatic glucose uptake and glucose production in response to glucose per se (34). This type of regulation may not be occurring at the level of gene expression.

The therapeutic potential of GK overexpression in type 1 diabetes is also unclear. Interestingly, the large increases in TG, FFAs, and ketone levels associated with complete insulin deficiency induced by streptozotocin injection are largely reversed by modest hepatic GK overexpression in transgenic mice (7). The mechanism by which modulation of hepatic GK causes these changes is unknown, but one possible scenario follows. In streptozotocin-treated animals without GK transgene expression, complete insulin deficiency activates peri-pheral lipolysis, accelerates fatty acid oxidation in liver, and results in a rise in TG due to esterification of excess fatty acids. Expression of GK in this environment lowers blood glucose, possibly increasing the dependence of muscle and other peripheral tissues on fatty acids for energy requirements, thereby decreasing circulating FFAs. This decrease in turn reduces the amount of FFAs available for TG synthesis and fatty acid oxidation in liver. If correct, this model suggests that insulin-deficient animals made normoglycemic by hepatic GK expression may oxidize fatty acids at a higher rate in peripheral tissues than normal animals. Further studies are required to carefully evaluate this possibility and its potential ramifications, in both the presence and absence of basal insulin replacement.

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