Glucokinase Gene Locus Transgenic Mice Are Resistant to the Development of Obesity-Induced Type 2 Diabetes

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Transgenic mice that overexpress the entire glucokinase (GK) gene locus have been previously shown to be mildly hypoglycemic and to have improved tolerance to glucose. To determine whether increased GK might also prevent or diminish diabetes in diet-induced obese animals, we examined the effect of feeding these mice a high-fat high–simple carbohydrate low-fiber diet (HF diet) for 30 weeks. In response to this diet, both normal and transgenic mice became obese and had similar BMIs (5.3 ± 0.1 and 5.0 ± 0.1 kg/m^2 in transgenic and nontransgenic mice, respectively). The blood glucose concentration of the control mice increased linearly with time and reached 17.0 ± 1.3 mmol/l at the 30th week. In contrast, the blood glucose of GK transgenic mice rose to only 9.7 ± 1.2 mmol/l at the 15th week, after which it returned to 7.6 ± 1.0 mmol/l by the 30th week. The plasma insulin concentration was also lower in the GK transgenic animals (232 ± 79 pmol/l) than in the controls (595 ± 77 pmol/l), but there was no difference in plasma glucagon concentrations. Together, these data indicate that increased GK levels dramatically lessen the development of both hyperglycemia and hyperinsulinemia associated with the feeding of an HF diet. *Diabetes* 50:622–629, 2001

Diabetes is characterized by both fasting and excessive postprandial hyperglycemia (1–6). Affected individuals exhibit three major defects in glucose metabolism in the liver: inappropriately high hepatic glucose production (1,2,5,7–15) in the fasting state, a defect in meal- or glucose-induced suppression of endogenous glucose production (1,5,12,16–19), and a defect in hepatic glucose uptake (6,12). The latter results in a reduced rate of synthesis of hepatic glycogen, which is produced from glucose via the so-called direct pathway (i.e., glucose → glucose-6-phosphate → glucose-1-phosphate → UDP-glucose → glycogen) (14,20,21). The same alterations in hepatic glucose metabolism have been found in various animal models of diabetes (22–27).

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ANOVA, analysis of variance; DTT, dithioerythritol; G6Pase, glucose-6-phosphatase; GK, glucokinase; GKRP, GK regulatory protein; HF diet, high-fat high–simple carbohydrate low-fiber diet; NEFA, nonesterified fatty acid.

Glucokinase (GK) plays an important role in determining glucose utilization by the liver. It also opposes glucose-6-phosphatase (G6Pase), which acts to regulate glucose output from the liver. Mice that have diminished GK (heterozygous knockout) exhibit fasting hyperglycemia and impaired glucose tolerance (28–32), a defect in glucose-induced suppression of hepatic glucose production, and decreased glycogen synthesis from glucose (29,30). Moreover, liver-specific GK-knockout mice exhibit mild hyperglycemia and defective glycogen synthesis during a hyperglycemic clamp (32). In contrast, GK-overexpressing transgenic mice (33–37) show a lower fasting plasma glucose level and improved glucose tolerance, even though their plasma insulin levels are similar to (33) or slightly lower than (34–37) those of nontransgenic mice. Furthermore, they exhibit increased hepatic glycogen synthesis despite a smaller increment in plasma insulin during a hyperglycemic clamp (33). Thus, hepatic GK gene expression is a major determinant of plasma glucose levels during fasting and postabsorptive periods.

Diabetes is associated with decreased hepatic GK activity. Streptozotocin-administered rats (19,38–40) and mice (34)—which are used as an experimental model of type 1 diabetes—have extremely low GK activity. Ferre et al. (34) demonstrated that transgenic mice that overexpressed GK in the liver could maintain normal levels of GK activity in the liver and that streptozotocin-induced increases in the serum concentrations of glucose, 3-hydroxybutyrate, triglycerides, and free fatty acids were much smaller than those in nontransgenic animals.

It is widely recognized that mutations in GK are a major cause of maturity-onset diabetes of the young. These individuals, who are heterozygous for a mutation of the GK gene, exhibit mild fasting or postprandial hyperglycemia (41–45) accompanied by impaired hepatic glucose uptake and glycogen synthesis, as well as a defect in glucose-stimulated insulin secretion (41). Defective GK activity has also been reported in a group of type 2 diabetic individuals (46). Mevorach et al. (47) showed in type 2 diabetic subjects that hyperglycemia failed to increase glucose cycling between glucose and glucose-6-phosphate despite unchanged flux through G6Pase, indicating defective GK activity in these patients. It has also been shown in certain type 2 diabetic mouse and rat models that the absolute activity of GK or the relative activities of GK against G6Pase decreased when hyperglycemia developed (48–53). Thus, defective GK activity may be a major contributing factor to the inappropriately high glucose production seen in the fasting state and in the defect in glucose uptake

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and hepatic glycogen synthesis seen in the postprandial state in individuals with type 2 as well as type 1 diabetes.

When fed a high-fat diet, certain strains of mice develop obesity, hyperglycemia, and impaired glucose tolerance and thus display the characteristics of obesity-related type 2 diabetes (54,55). To test the possibility that increased GK can lessen the hyperglycemia associated with obesity-related type 2 diabetes, we studied the effect of high-fat feeding on normal mice and on mice expressing one extra copy of the GK gene locus as a transgene.

RESEARCH DESIGN AND METHODS

Transgenic mice and dietary treatment. GK gene locus transgenic animals (line 37) have been previously described (33,56). These mice were generated by pronuclear DNA microinjection and have an 83-kb transgene containing the entire mouse GK gene (including both the upstream and downstream progenitor region) residing within the X chromosome (56). Hemizygous males and nontransgenic control male mice of mixed genetic background (C57BL/6 and DBA2) were divided into two groups at 6 weeks of age. The first group of both transgenic and nontransgenic control mice was fed a high-fat high–simple carbohydrate low-fiber diet (HF diet) containing 20.5% protein, 35.8% fat, 0.4% fiber, 3.6% ash, 3.1% moisture, and 36.6% carbohydrate (primarily disaccharides) (Diet No. 1850; Bio-Serve, Frenchtown, NJ). The second group was fed a normal Rodent Chow diet containing 23% protein, 4.5% fat, 6.0% fiber, 8.0% ash, 2.5% moisture, and 56% complex carbohydrate (Purina Mills, St. Louis, MO).

Experimental design. Blood glucose level and body weight were measured between 12:00 and 2:00 P.M. every 5 weeks during the 30-week feeding period. Blood samples were obtained from the tail vein, except at the end of the feeding period; at that time, the mice were anesthetized with pentobarbital sodium (70 mg/kg), a laparotomy was performed immediately, and blood was taken from the inferior vena cava. A piece of the liver, as well as a piece of skeletal muscle from the right leg, were also taken at that time and were frozen in situ using Wollenberg tongs precooled in liquid nitrogen.

Northern blot analysis. Total RNA was prepared by acid guanidinium thiocyanate extraction (57). RNA gels, blots, and hybridizations were performed as described previously (58). A 789-bp rat GK cDNA fragment from pGK·Z1 (59) was used as a probe. A 700-bp Hind*III*-EcoR/ fragment of cyclophilin DNA was used to correct for differences in loading (60). Densitometric analysis of autoradiograms was performed using image software (National Institutes of Health, Bethesda, MD) after digital scanning.

Western blot analysis. Western blot analysis was performed as described (60). To visualize GK antibody binding, the washed membranes were incubated with horseradish peroxidase–conjugated donkey anti-sheep IgG at 1:10,000 dilution (Jackson Immunoresearch) for 1 h at room temperature. The same blot was washed and incubated first with a rabbit anti–GK regulatory protein (GKRP) serum (diluted 1:5,000), then with a horseradish peroxidase–conjugated donkey anti-rabbit IgG. A solution consisting of 1.25 mmol/l luminol, 0.2 mmol/l para-coumaric acid, and 0.009% H_2O_2 in 100 mmol/l Tris (pH 8.5) was used to initiate the chemiluminescence reaction. The membranes were then drip-dried, wrapped in polyvinyl chloride film, and exposed to autoradiography film.

Enzymatic assays. For GK and G6Pase activity measurements, freeze-clamped liver was homogenized in 50 mmol/l HEPES, 100 mmol/l KCl, 1 mmol/l EDTA, 5 mmol/l $MgCl_2$, and 2.5 mmol/l dithioerythritol (DTT) (30). Homogenates were then centrifuged at 100,000*g* for 45 min to sediment the microsomal fraction. GK activity was measured in the cytosolic fraction as described by Rossetti et al. (30), but with some minor modifications. Briefly, the supernatants (approximately the equivalent of 1 mg of wet liver) were analyzed for GK in a medium (pH 7.4 at 37°C) containing 50 mmol/l HEPES, 100 mmol/l KCl, 7.5 mmol/l $MgCl_2$, 5 mmol/l ATP, 2.5 mmol/l DTT, 0.5 (for hexokinase) or 18 and 100 mmol/l glucose (total phosphorylating activity), 0.5 mmol/l NAD^+, and four units of glucose-6-phosphate dehydrogenase (leuconostoc mesenterioides). The reaction was initiated by the addition of ATP, and the rate of NAD^+ reduction was recorded at 340 nm. The G6Pase assay was also performed essentially as described by Rossetti et al. (30). The sedimented microsomal fractions were resuspended in a medium containing 50 mmol/l HEPES, 100 mmol/l KCl, 1 mmol/l EDTA, 5 mmol/l $MgCl_2$, and 2.5 mmol/l DTT. The resuspended microsomal fraction was incubated in a medium (pH 7.4 at 37°C) containing 50 mmol/l HEPES, 100 mmol/l KCl, 1 mmol/l EDTA, 5 mmol/l $MgCl_2$, and glucose-6-phosphate at 0.5, 1.0, 2.5, 5.0, and 10 mmol/l. The reaction was carried out at 37°C and stopped after 20 min with a solution containing 6:9 volume of 0.42% ammonium molybdate, 2:9 volume of 10% SDS, and 1:9 volume of 10% ascorbic acid. It was then incubated for 20 min at 45°C, and the absorbance was read at 820 nm. A standard curve was made using different concentrations of Pi.

Metabolites and hormone assays. The blood glucose concentration was determined using a blood glucose meter (Hemocue, Mission Viejo, CA). The values obtained from the Hemocue device were 20% lower than the values obtained from assaying for plasma glucose. The plasma nonesterified fatty acid (NEFA) concentration was determined using the Wako NEFA-C kit (Osaka, Japan). The glycogen content in liver and skeletal muscle and the triglyceride content in liver were assayed as described (61,62). The concentrations of plasma insulin and glucagon were determined by radioimmunoassay using rat insulin and glucagon radioimmunoassay kits (Linco Research, St. Louis, MO), respectively. The binding reactions were modified to allow the assay of insulin and glucagon on 10 μl and 50 μl of plasma, respectively.

Statistical analysis. Data are expressed as means ± SE. A one-way analysis of variance (ANOVA) for repeated measures was used to analyze changes over time in body weight and blood glucose concentrations. A two-way ANOVA for repeated measures was used to analyze time course differences between groups. When significant changes were obtained over time, post hoc comparisons were made using a paired *t* test. Pairwise comparison for BMI; plasma concentration of NEFA and hormones; content of glycogen, lipid, and protein; and enzyme activities were made using a paired *t* test.

RESULTS

We have previously described the production and characterization of GK gene locus transgenic mice (56). These mice express and regulate a single additional copy of the entire GK gene locus; thus, they provide a model for determining the effect of a modest increase in GK gene expression in preventing or limiting glycemic control impaired by high-fat feeding. Because GK expression was twofold higher in the liver but lower in pancreatic β-cells when compared with that in nontransgenic control mice, we concluded that an overexpression of the GK transgene in the liver was primarily responsible for the hypoglycemic phenotype of these animals (56).

At 6 weeks of age, the body weight of the GK transgenic mice (20.9 ± 1.0 g) was not different from the nontransgenic animals (21.6 ± 1.3 g) (Fig. 1). Feeding of a normal Rodent Chow diet caused the body weight of the transgenic and control animals to increase to 33.1 ± 1 and 35 ± 3 g, respectively, by the 30th week. The BMI at the 30th week did not differ between the transgenic and nontransgenic animals (0.33 ± 0.01 and 0.33 ± 0.03, respectively). The feeding of an HF diet increased both the body weight and BMI in both the transgenic and nontransgenic mice. At the 30th week, the body weight and BMI were significantly ($P < 0.05$) but only slightly different in the transgenic and nontransgenic mice (53.2 ± 0.6 vs. 56.3 ± 1.4 g and 0.53 ± 0.01 vs. 0.50 ± 0.01 g, respectively).

Before starting the HF diet at 6 weeks of age, the blood glucose concentration in transgenic mice (8.1 ± 0.2 mmol/l) was modestly lower than in nontransgenic mice (9.1 ± 0.2 mmol/l) (Fig. 2). With control-diet feeding, blood glucose levels in the transgenic and the nontransgenic mice decreased to 6.9 ± 0.1 and 8.0 ± 0.2 mmol/l, respectively, by 5 weeks and then changed minimally, so at 30 weeks of age, the difference between the groups was still evident but slight. Glycogen content in the liver and skeletal muscle (Table 1), as well as plasma insulin and glucagon levels (Fig. 2), were also similar in both groups at the end of the feeding period. In contrast, the nontransgenic mice that were fed the HF diet had a markedly elevated blood glucose concentration by the 30th week (17.0 ± 1.3 mmol/l). These mice had slightly but significantly higher glycogen content in the liver compared with mice fed a normal Rodent Chow diet (Table 1). Plasma insulin rose markedly,

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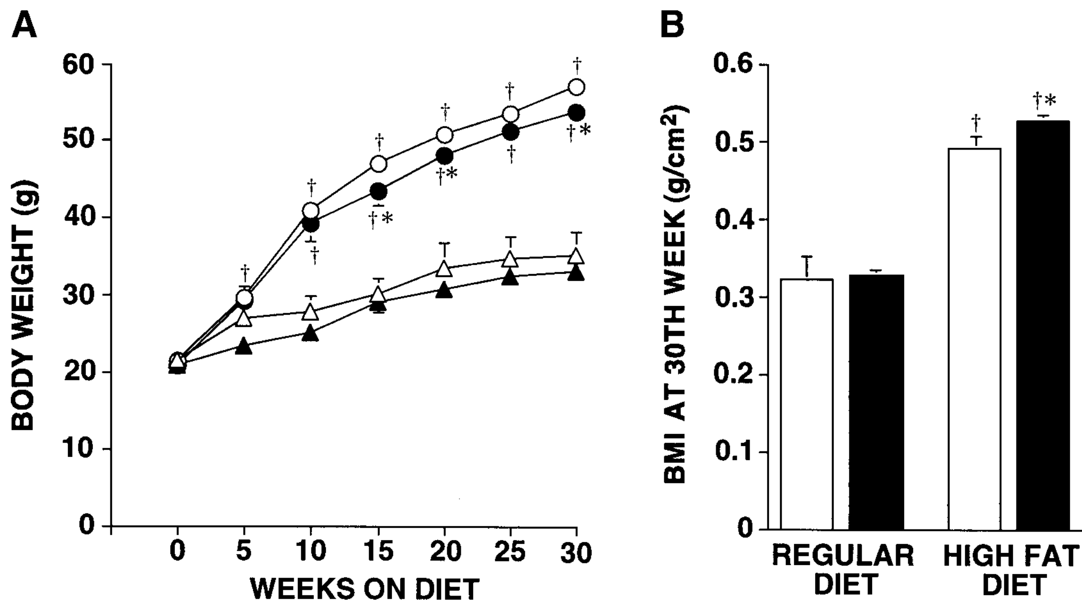

FIG. 1. Body weight (*A*) during 30 weeks of feeding and body mass index (*B*) at the 30th week in transgenic and nontransgenic mice fed with regular diet or HF diet. Each value is the mean ± SE for five animals. ●, HF, nontransgenic; ○, HF, transgenic; ▲, regular transgenic; △, regular, transgenic; ■, transgenic; and □, nontransgenic. †Significantly different from the corresponding value in same genotype animals fed with regular diet ($P < 0.05$); and *significantly different from the corresponding value in nontransgenic mice fed with same type of diet ($P < 0.05$).

by 2,000% (595 ± 77 pmol/l), and plasma glucagon increased by 50% (62 ± 7 ng/l). Thus, the nontransgenic mice fed the HF diet exhibited many of the characteristics of obesity-induced type 2 diabetes, namely, hyperglycemia, hyperinsulinemia, and increased hepatic glycogen stores (3,4). In contrast, the blood glucose concentration in the GK transgenic mice fed the HF diet increased to 9.7 ± 1.2 mmol/l by the 15th week, but returned to 7.6 ± 1.0 mmol/l by the 30th week. Glycogen content in the liver was not significantly different from that in nontransgenic mice fed an HF diet (Table 1). Plasma insulin levels were also dramatically lower in the GK transgenic mice (232 ± 79 pmol/l) than in the nontransgenic animals at the 30th week. The plasma glucagon level increased by 50% (64 ± 8 ng/l) in the transgenic group (Fig. 2).

It has been reported that the increased expression of hepatic GK (resulting from delivery of a recombinant adenovirus containing the GK gene) caused a fatty liver

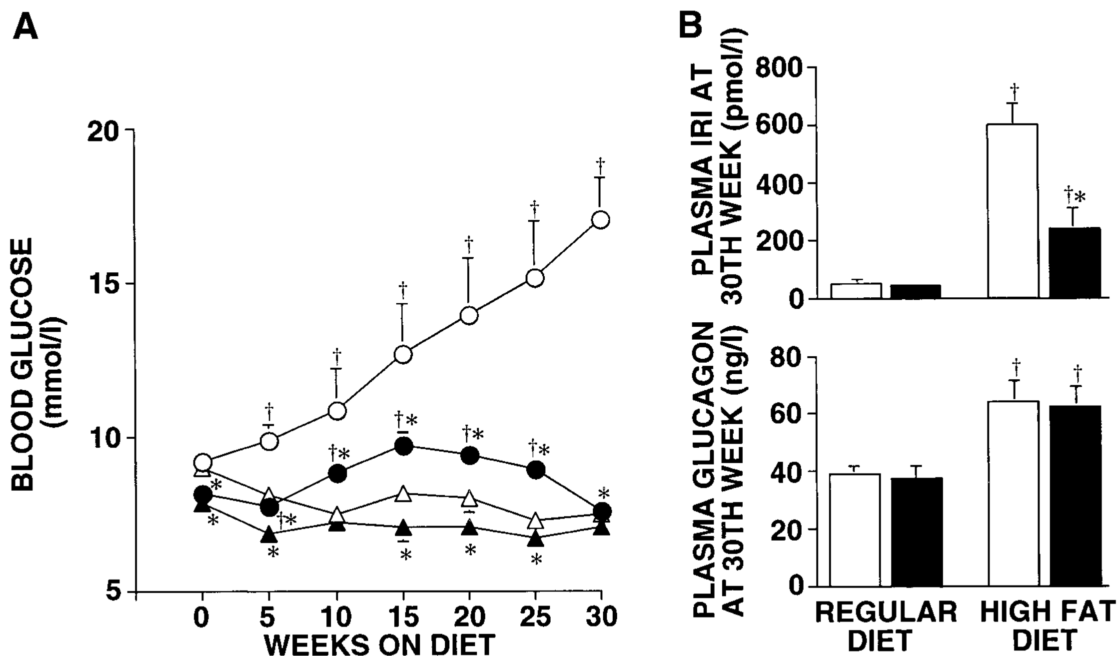

FIG. 2. Blood glucose level (*A*) during the 30-week feeding period and plasma insulin immunoreactivity (IRI) and glucagon levels (*B*) at the 30th week in transgenic and nontransgenic mice fed with regular diet or HF diet. Each value is mean ± SE for five animals. ●, HF, nontransgenic; ○, HF, transgenic; ▲, regular transgenic; △, regular, transgenic; ■, transgenic; and □, nontransgenic. *Significantly different from the corresponding value in nontransgenic mice fed with same type of diet ($P < 0.05$); and †significantly different from the corresponding value in same genotype animals fed with regular diet ($P < 0.05$).

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TABLE 1
Glycogen content in liver and skeletal muscle in transgenic and nontransgenic mice fed regular Purina Rodent Chow or HF diet for 30 weeks

Tissues	Regular diet Nontrans-genic	Regular diet Trans-genic	HF diet Nontrans-genic	HF diet Trans-genic
Liver	162 ± 6	158 ± 5	218 ± 8*	182 ± 23
Skeletal muscle	21 ± 3	24 ± 2	26 ± 2	21 ± 4

Data are means ± SE for five animals. The values are described as μmol/g wet weight of liver and skeletal muscle. * Significantly different from the corresponding genotype of mice fed a regular diet.

(37). In this case, hepatic GK activity was increased sixfold by the treatment. However, in other studies, when hepatic GK expression was increased only 1.2- to 3-fold either by delivering of a recombinant adenovirus containing the GK gene (37) or by using a transgene (33,34,36), there was no increase in either plasma NEFA (33,34,36,37) or triglycerides (34,36,37). In the present study, there was no statistically significant difference in plasma NEFA and hepatic triglyceride content (0.44 ± 0.07 vs. 0.52 ± 0.11 mmol/l and 12 ± 3 vs. 11 ± 3 mg/g, respectively) between the transgenic and nontransgenic mice when they were fed the control diet. The HF diet did not increase plasma NEFA in transgenic (0.55 ± 0.06 mmol/l) or nontransgenic mice (0.47 ± 0.4 mmol/l). On the other hand, the HF diet markedly increased triglyceride content in the liver; however, there was no significant difference in hepatic triglyceride content between the transgenic (148 ± 26 mg/g) and nontransgenic mice (138 ± 19 mg/g). It is unlikely, therefore, that the decrease in plasma glucose levels in GK transgenic mice resulted from an increased incorporation of glucose into lipid.

We have shown previously that the GK transgenic mice have twofold more hepatic GK mRNA, protein, and activity in the liver at 8–10 weeks (29,45) than do nontransgenic animals. To ascertain whether the GK transgene also caused increased GK expression in the present animals, both GK mRNA and protein content in the liver were assessed. At the end of the study (36 weeks), GK transgenic mice fed the normal Rodent Chow had twofold more GK mRNA, but no detectable change in the amount of either GK protein (Fig. 3) or GK activity (Table 2). This is consistent with the finding that the blood glucose levels of the transgenic mice were not different from those of nontransgenic mice at this age (Fig. 2). It is not known why GK expression was not increased in the 36-week-old transgenic mice. When fed the HF diet, the GK transgenic mice had a threefold elevation of GK mRNA and 50% higher GK protein compared with only a twofold increase in GK mRNA and 20% more protein in nontransgenic animals (Fig. 3). Thus, the HF diet seems to have caused a greater increase in the expression of both GK mRNA and protein compared with the nontransgenic mice.

Hepatic GK activity is regulated at both the transcriptional and posttranscriptional levels. The binding of GK to GKRP is a key posttranscriptional regulatory mechanism that inhibits hepatic GK activity by decreasing the apparent affinity of the enzyme for glucose (63). Glucose is known to dissociate GK from GKRP (63–65). Moreover, both GK and GKRP are adaptively regulated genes, and both GK and GKRP mRNA are diminished in response to either starvation or streptozotocin-induced diabetes and are increased in response to refeeding and insulin treatment

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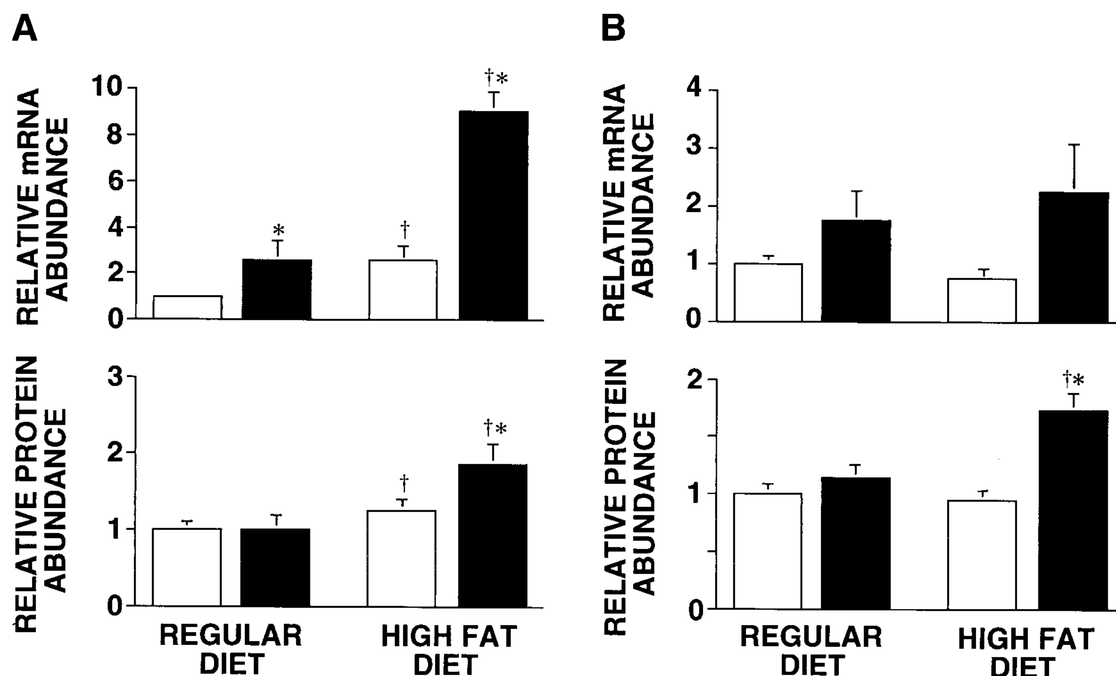

FIG. 3. GK and regulatory protein gene expression in the liver of transgenic and nontransgenic mice fed with regular diet or HF diet for 30 weeks. *A*: Relative amount of GK mRNA and protein. *B*: Relative amount of the regulatory protein mRNA and protein. The values (*n* = 5) were normalized to the mean value in nontransgenic mice fed with a regular diet as 1.0. Each value is mean ± SE for five animals. ■, Transgenic; □, nontransgenic. *Significantly different from the corresponding value in nontransgenic mice fed with same type of diet ($P < 0.05$); and †significantly different from the corresponding value in same genotype animals fed with regular diet ($P < 0.05$).

TABLE 2
Activities of GK and G6Pase in the liver of transgenic and nontransgenic mice fed with regular or HF diet for 30 weeks

	Regular diet		HF diet	
	Nontransgenic	Transgenic	Nontransgenic	Transgenic
Hexokinase (0.5 mmol/l glucose)	3.5 ± 0.2	4.2 ± 0.4	4.9 ± 0.7	5.1 ± 0.9
GK				
(18 mmol/l glucose)	8.1 ± 0.8	8.6 ± 1.8	14.9 ± 1.0†	22.5 ± 0.20*†
(100 mmol/l glucose)	13.7 ± 1.3	14.0 ± 2.8	25.3 ± 2.3†	38.1 ± 4.0*†
G6Pase (V_{max})	181 ± 21	169 ± 13	253 ± 22†	232 ± 21†
GK-to-G6Pase ratio	0.076 ± 0.014	0.082 ± 0.015	0.101 ± 0.008	0.166 ± 0.026*†

Data are means ± SE. Activities of GK and G6Pase are described as nmol/min/mg protein of homogenate. GK activities in the presence of 18 or 100 mmol/l glucose as substrate was obtained by subtracting the phosphorylation rate in the presence of 0.5 mmol/l glucose from phosphorylation rates in the presence of 18 or 100 mmol/l glucose. *Significantly different from nontransgenic mice fed same diet ($P < 0.05$); †significantly different from corresponding genotype of mice fed regular diet ($P < 0.05$).

(63). An excess of GKRP may be necessary to keep GK inactive and thereby maintain the dramatic activation of GK that occurs in response to its substrate and effector despite changes in the V_{max} of the enzyme (63). It has also been reported that both GK activity and protein decrease in GKRP knockout mice (66) and in mice that have a mutation in the GKRP gene (67), suggesting that GKRP serves to preserve and/or stabilize GK in the liver. For this reason, we also assessed the effect of increased GK on GKRP gene expression. As shown in Fig. 3*B*, there was no significant difference in either GKRP mRNA or protein content between the transgenic and nontransgenic mice fed normal Rodent Chow. However, both GKRP mRNA and protein were significantly increased in the GK transgenic mice that were fed an HF diet. A similar change was not observed in nontransgenic mice fed the same diet. Increased GKRP expression may serve to accommodate increased GK, but the mechanism by which GKRP expression changes in parallel with the change in GK expression remains unknown.

Glucose phosphorylation and dephosphorylation, catalyzed by GK and G6Pase, respectively, are competing processes that determine net hepatic glucose flux. Metabolic abnormalities associated with diabetes, such as hyperglycemia and hyperinsulinemia, increase the expression of G6Pase in liver (48–51). Moreover, overexpression of the catalytic subunit of G6Pase in liver causes mild hyperglycemia and impaired glucose tolerance (68). Because a reduction of hepatic GK activity and/or an increase in hepatic G6Pase activity might diminish the rate of hepatic glucose uptake (52), we assessed whether increased expression of GK by the transgene was accompanied by an increase in the ratio of GK activity to G6Pase activity (Table 2). The GK transgenic mice fed the HF diet showed a 50% increase in the GK-to-G6Pase ratio compared with the nontransgenic mice. No difference was seen for mice fed the normal Rodent Chow diet.

DISCUSSION

Feeding of an HF diet to nontransgenic control mice caused a phenotype of obesity, hyperglycemia, hyperinsulinemia, and increased hepatic glycogen storage, all of which resemble obesity-induced type 2 diabetes. However, the development of the hyperglycemia in these mice was not accompanied by a decrease in GK and/or the GK-to-G6Pase ratio, indicating that the changes in GK and/or the GK-to-G6Pase ratio are not the cause of the hyperglycemia in this model. Whatever the cause of hyperglycemia might be, the presence of the GK transgene increased the hepatic GK expression and GK-to-G6Pase activity ratio in the mice fed an HF diet, and it lessened both hyperglycemia and hyperinsulinemia without affecting either the obesity or the glycogen content in the liver and skeletal muscle of these mice.

We have previously shown that GK transgenic mice exhibit a mild hypoglycemia that occurs in the absence of any detectable change in either insulin levels or the glucose turnover rate (33). Net hepatic glucose flux is thought to reflect the balance between the glucose phosphorylation rate due to GK and the glucose-6-phosphate dephosphorylation rate due to G6Pase. The rate of hepatic glucose phosphorylation is largely dependent on the mass effect of glucose and on the activity of GK. The preservation of a high rate of GK activity (and thus a high GK-to-G6Pase ratio) may shift the net hepatic glucose balance favorably toward increased glucose uptake at a given blood glucose level, thereby compensating for a defect both in insulin sensitivity and in glucose effectiveness in suppressing hepatic glucose production. As a result, net hepatic glucose production is maintained at the original rate with a lower blood glucose level.

The GK transgene did not increase hepatic glycogen storage. In our previous study, GK-overexpressing transgenic mice had higher glycogen synthesis rates in the liver despite their lower increment of plasma insulin level during a hyperglycemic clamp (33), indicating that the increased expression of GK augmented the glycogen synthetic capability of the liver. However, when their blood glucose level was not clamped, GK-overexpressing transgenic mice had mild hypoglycemia and similar hepatic glycogen content compared with nontransgenic mice (33). The postprandial glucose level may be lower in the transgenic mice, as shown in the glucose tolerance test (33), and thus the reduced glucose mass influences the effect of increased GK.

It is well known that insulin resistance develops in both the peripheral tissues and the liver in type 2 diabetes. Even if hepatic glucose production was normalized for a given blood glucose level by increasing GK activity, an improvement in peripheral insulin resistance would also be necessary to attain a normalized blood glucose level. Hyperglycemia itself leads to a further aggravation of the

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defect in insulin secretion and insulin resistance in both the peripheral tissues and the liver. Chronic hyperglycemia per se may attenuate the secretory response of pancreatic islet cells to glucose in some animal models of diabetes (69–73). Chronic hyperglycemia also seems to impair both the effects of insulin to stimulate glucose uptake by peripheral tissue and the effects of insulin to suppress glucose production by the liver (74). On the other hand, Rossetti et al. (73) showed that, in diabetic rats, normalization of the plasma glucose profile by a 2-week phlorizin treatment completely normalized insulin sensitivity, and the discontinuation of phlorizin treatment resulted in the reemergence of insulin resistance. The same treatment in diabetic rats also completely corrected the deficient insulin response (both first and second phase) to hyperglycemia (74). Chronic hyperglycemia itself may play a pathogenic role in diabetes by maintaining a self-perpetuating cycle of metabolic deterioration (70,73,74). The supplementation of GK in the liver by the transgene may lessen the hyperglycemia induced by an HF diet, thereby preventing the further development of insulin resistance in both the peripheral tissues and the liver, as well as the attenuation of the secretory response of pancreatic islet cells to glucose. As a result, the transgenic mice may stay in a prediabetic state of obesity, hyperinsulinemia, euglycemia, and normal glucose turnover rates.

Because the expression of the GK transgene was not specific for the liver in the GK transgenic mice used in the present study, it is possible that the HF diet could have altered GK expression in other tissues that express the enzyme. It has been proposed that glucose stimulation of insulin secretion results from enhanced glucose metabolism in the β-cell when the blood glucose rises and that GK is the constituent enabling the β-cell to recognize and measure the fluctuations of blood glucose (75). Recent studies have shown that feeding an HF diet desensitized the insulin secretory response to glucose in C57BL/6J mice (76,77) and that such a desensitization was accompanied by decreased GK activity in the pancreatic islets (77). Because GK activity in the pancreatic islets was not measured in the present study, we cannot rule out the possibility that an alteration in islet GK could have contributed to the protective effect for the HF diet–induced development of hyperglycemia observed in the transgenic mice.

Chronic hyperglycemia has been postulated to be the main factor responsible for the development of diabetes-specific microvascular pathology in the retina and renal glomerular and in neurological and macrovascular complications (78,79). The reduction of hyperglycemia, and thus the maintenance of normoglycemia, is a goal of any therapeutical approach for types 1 and 2 diabetes. Hyperinsulinemia and insulin resistance, both very common in patients with type 2 diabetes, are associated with increased risk of hypertension, coronary artery disease, and stroke, raising the possibility that insulin itself has atherogenic actions. Ferre et al. (34) have shown that the restoration of the normal expression of GK in the liver prevents the hyperglycemia normally seen in streptozotocin-treated mice, a type 1 diabetes model. Here, we show that increased GK normalizes hyperglycemia and reduces hyperinsulinemia induced by high-fat diet feeding in an obese type 2 diabetes model. The present study and a study by Ferre et al. (34) suggest that the maintenance or the supplementation of GK expression in the liver can lessen hyperglycemia in both types of diabetes. We suggest, therefore, that alteration of the expression or activity of GK constitutes a rational target for intervention in the treatment of diabetes.

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