Abnormal regulation of HGP by hyperglycemia in mice with a disrupted glucokinase allele

Luciano Rossetti, Wei Chen, Meizhu Hu, Meredith Hawkins, Nir Barzilai, and Shimon Efrat

Diabetes Research and Training Center, Departments of Medicine and Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, New York 10461

Abnormal regulation of HGP by hyperglycemia in mice with a disrupted glucokinase allele. Am. J. Physiol. 273 (Endocrinol. Metab. 36): E743–E750, 1997.—Glucokinase (GK) catalyzes the phosphorylation of glucose in β-cells and hepatocytes, and mutations in the GK gene have been implicated in a form of human diabetes. To investigate the relative role of partial deficiencies in the hepatic glucose phosphorylation capacity occur in humans with diabetes mellitus and may contribute to its pathophysiology. (6). Mutations in the GK gene are responsible for a form of maturity-onset diabetes of the young (MODY 2) (13). Although in the case of MODY 2 this enzymatic defect is inherited (13), it may also be acquired secondarily to associated hormonal and metabolic alterations in some patients with insulin-dependent diabetes mellitus (8, 19) and in others with non-insulin-dependent diabetes mellitus (6). The rate of hepatic glucose phosphorylation is largely dependent on the mass effect of glucose, i.e., its portal concentration, and on the in vivo activity of GK (26, 27, 32). Acutely, hyperglycemia per se promotes hepatic glucose uptake, decreases net liver glycogenolysis, and inhibits hepatic glucose production (HGP) (26–28). We have suggested that the increase in the rates of hepatic glucose phosphorylation, which is induced by hyperglycemia, is pivotal to its ability to inhibit HGP (26).

In a recent communication (1) we reported that the disruption of one allele of the GK gene (GK+/−) in mice resulted in a modest decrease (~30%) in the level of gene expression and/or activity of hepatic GK and in a decreased response of HGP and direct pathway of glycogen synthesis to acute changes in the plasma glucose concentrations (1). However, the presence of a concomitant partial deficit in the β-cell GK and the lower plasma insulin concentrations during the hyperglycemic clamp studies could have accounted for some or all of the hepatic metabolic alterations in this mouse model (1, 10). In fact, chronic changes in the circulating insulin levels may have been sufficient to alter the hepatic gene expression and activity of GK and other key hepatic enzymes. Furthermore, the differences in the circulating plasma insulin concentrations observed during the in vivo studies complicate the interpretation of the glucose flux data. In the present study we aimed to investigate the specific regulatory components of whole body glucose homeostasis that are affected by long-term partial deficits in the activities of GK in the β-cells of the pancreas and in the parenchymal cells of the liver. In particular, we wished to discern whether the metabolic changes in hepatic glucose fluxes and in their responses to changes in extracellular glucose levels, which are present in mice and humans with whole body GK deficiency, are due to a specific hepatic defect or are secondary to chronic and/or short-term alterations in circulating insulin levels. To this end, the responses of insulin secretion and of hepatic and peripheral glucose fluxes to standardized hyperglycemic challenges were compared in two transgenic models with a moderate deficit in glucose phosphorylation, selectively in the pancreatic β-cells or in both liver and pancreas. Thus mice with a disrupted GK allele (GK+/−) (1) were compared with a mouse model with a selective disruption of GK gene expression and activity in β-cells that was generated with a GK ribozyme (10). Additionally,
we made use of a pancreatic clamp technique that allowed us to investigate the role of a sustained decrease in liver GK in the regulation of hepatic glucose fluxes in the presence of similar and fixed pancreatic hormone levels (26).

**METHODS**

**Animals**

Three groups of male mice were studied with two experimental protocols. Group 1 included 11 control (C57BL/6j) mice (Jackson Breeding Laboratories, Bar Harbor, ME); group 2 included 16 GK +/− mice; and group 3 included 14 RIP-GKRZ mice (10). Figure 1 provides a schematic representation of the two experimental protocols. Protocol 1 consisted of a hyperglycemic clamp study (n = 6 or more for each group). Protocol 2 combined pancreatic and hyperglycemic clamp studies (n = 5 or more for each group).

At 4–6 mo of age, all mice (28–35 g) were anesthetized with chloral hydrate (400 mg/kg of body wt ip), and an indwelling catheter was inserted into the right internal jugular vein, as previously described (23, 25). The venous catheter was used for the multiple infusions; blood samples were obtained from the tail vein. Mice were studied 4–6 days postsurgery.

**Euglycemic and Hyperglycemic Clamp Studies**

Studies were performed in awake, unrestrained, chronically catheterized mice by use of the pancreatic and hyperglycemic clamp techniques (1, 23, 25, 26) in combination with high-performance liquid chromatography (HPLC)-purified [3-3H]glucose and [U-14C]lactate infusions, as previously described (15, 26). Food was removed for 8 h before the in vivo study infusions. All studies lasted 170 min and included an 80-min euglycemic period for assessment of basal turnover rates and a 90-min hyperglycemic clamp period. Eighty minutes before the start of glucose, insulin, and/or somatostatin infusions, a prime-continuous infusion of HPLC-purified [3-3H]glucose (New England Nuclear, Boston, MA; 10 µCi bolus and 0.1 µCi/min) was initiated and maintained throughout the remainder of the study. [U-14C]lactate (5 µCi bolus and 0.25 µCi/min) was infused during the last 10 min of the study.

Protocol 1. Briefly, a variable infusion of a 25% glucose solution was started at time 0 and periodically adjusted to sequentially clamp the plasma glucose concentration at ~5 mM for 80 min (euglycemic period) and at ~17 mM for 90 min (hyperglycemic period).

Protocol 2. Briefly, a primed-continuous infusion of somatostatin (3 µg·kg⁻¹·min⁻¹), insulin (~0.3 mU·kg⁻¹·min⁻¹), and glucagon (5 ng·kg⁻¹·min⁻¹) was administered, and a variable infusion of a 25% glucose solution was started at time 0 and periodically adjusted to sequentially clamp the plasma glucose concentration at ~5 mM for 80 min (euglycemic period) and at ~17 mM for 90 min (hyperglycemic period).

Plasma samples for determination of [3H]glucose specific activity (~45 µl blood/each) were obtained at 40, 60, 70, and 80 min during the basal period and at 40, 60, 70, 80, and 90 min during the clamp period. Steady-state conditions for the plasma glucose concentration and specific activity were achieved within 40 min in both the basal and clamp periods of the studies. Plasma samples for determination of plasma insulin concentrations (~40 µl blood/each) were obtained at ~30, 0, 20, 40, 60, 75, and 90 min during the study. Additional plasma samples for the determination of plasma glucose concentration (~20 µl) were obtained at ~40 and ~20 min and at 10-min intervals thereafter. The total volume of blood withdrawn was ~0.9 ml/study; to prevent volume depletion and anemia, a solution (1:1, vol/vol) of ~1.2 ml of fresh blood (~0.6 ml obtained by heart puncture from littermates of the test animals) and heparinized saline (10 U/ml) was infused at a rate of 7 µl/min. Furthermore, after larger samples at time 0 and 40, 60, and 90 min, red blood cells were resuspended in saline and immediately returned through the infusion catheter. All determinations were also performed on portal vein blood obtained at the end of the experiment. To minimize stress during the sampling procedures, all mice were accustomed to handling and tail sampling, were freely moving within a large cage, and were allowed sufficient time for postsurgical recovery (4 days or more). Blood samples for assessment of glucose concentration were obtained every 10 min from a cut at the tip of the tail (1, 23, 25).

At the end of the in vivo studies, mice were anesthetized (pentobarbital sodium, 60 mg/kg body wt iv), the abdomen was quickly opened, portal vein blood was obtained, and the liver was freeze-clamped in situ with aluminum tongs precooled in liquid nitrogen. The time from the injection of the...
anesthetic until freeze-clamping of the liver was <45 s. All tissue samples were stored at −80°C for subsequent analysis. The study protocol was reviewed and approved by the Institutional Animal Care and Use Committee of the Albert Einstein College of Medicine.

Analytic Procedures

Plasma glucose was measured by the glucose oxidase method (Glucose Analyzer II, Beckman Instruments, Palo Alto, CA). Plasma insulin was measured by radioimmunoasay with use of rat and porcine insulin standards. Plasma [3H]glucose radioactivity was measured in duplicates in the supernatants of Ba(OH)2 and ZnSO4 precipitates (Somogyi procedure) of plasma samples (25 μl) after evaporation to dryness to eliminate tritiated water. Uridine 5'-diphosphate-glucose (UDP-Glc), uridine 5'-diphosphate-galactose (UDP-Gal), and phosphoenolpyruvate (PEP) concentrations and specific activities in the liver were obtained through two sequential chromatographic separations, as previously reported (14, 15, 23, 26).

GK Activity

Hepatic GK activity was measured by a continuous spectrophotometric method (3, 9, 26). Liver homogenates (−200 mg) were prepared in (mM) 50 N-2-hydroxymethylpiperazine-N'-2-ethanesulfonic acid (HEPES), 100 KCl, 1 EDTA, 5 MgCl2, and 2.5 dihydroethrythritol. Homogenates were centrifuged at 100,000 g for 45 min to sediment the microsomal fraction. The postmicrosomal fraction was assayed at 37°C in a medium containing 50 mM HEPES (pH 7.4), 100 mM KCl, 7.5 mM MgCl2·6 mM ATP, 2.5 mM dihydroethrythritol, 10 mg/ml albumin, and glucose at 0.5 mM (for hexokinase activity), 18 mM (for GK activity at in vivo glucose levels), or 100 mM (maximal glucose phosphorylation capacity), 0.5 mM NAD+, 4 U glucose-6-phosphate 1-dehydrogenase (L. mesenteroides), and the equivalent of −1 mg of wet liver. The reaction was initiated by the addition of ATP, and the rate of NAD+ reduction was measured at 340 nm. Glucose phosphorylation was determined as the absorbency change in the complete medium minus the absorbency change in the absence of ATP, under conditions in which the absorbency is increasing linearly with time (usually from 20 to 40 min). Kinetic analysis of GK was also performed at glucose concentrations of 0.5, 8, 10, 15, 18, 25, and 100 mM from livers obtained at the end of the in vivo studies.

Calculations

Under steady-state conditions for plasma glucose concentrations, the rate of steady-state glucose appearance (Ra) equals the rate of glucose disappearance (Rd). The latter was calculated as the ratio of the rate of infusion of [3H]glucose [disintegration · min−1 · dpm−1] and the steady-state plasma [3H]glucose specific activity (dpm/mg). When exogenous glucose was given, the rate of endogenous glucose production was calculated as the difference between Rd and the infusion rate of glucose (GIR). The percentage of the hepatic glucose 6-phosphate pool directly derived from plasma glucose was calculated as the ratio of [3H]UDP-Glc and plasma [3H]glucose specific activities. The percentage of the hepatic glucose 6-phosphate pool derived from PEP gluconeogenesis was calculated as the ratio of the specific activities of [3H]UDP-Glc and 2 × [14C]PEP after in vivo labeling with [U-14C]lactate (15, 26).

Table 1. General characteristics of WT, GK+/-, and RIP-GKRZ mice receiving either hyperglycemic (protocol 1) or hyperglycemic/pancreatic (protocol 2) clamp studies

<table>
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<th>WT</th>
<th>GK+/-</th>
<th>RIP-GKRZ</th>
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<tr>
<td>n</td>
<td>11</td>
<td>16</td>
<td>14</td>
</tr>
<tr>
<td>Body wt, g</td>
<td>31.6±2.6</td>
<td>32.2±1.9</td>
<td>30.3±1.4</td>
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<tr>
<td>Plasma glucose, mM</td>
<td>5.5±0.9</td>
<td>5.8±0.7</td>
<td>5.7±0.6</td>
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<tr>
<td>Plasma insulin, ng/ml</td>
<td>2.2±0.4</td>
<td>1.9±0.3</td>
<td>1.9±0.3</td>
</tr>
<tr>
<td>HGP, mg·kg−1·min−1</td>
<td>22.5±1.5</td>
<td>21.2±1.2</td>
<td>22.4±1.2</td>
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</tbody>
</table>

Values are means ± SE; n, no. of mice/group. WT, wild type (control) mice; GK+/-, mice with 1 disrupted glucokinase (GK) allele; RIP-GKRZ, transgenic mice with GK ribozyme-generated disruption of β-cell GK gene expression and activity; HGP, (basal) hepatic glucose production.

RESULTS

General Characteristics of the Experimental Animals

Three groups of conscious male mice were studied: 11 wild type (WT) controls, 16 mice with one GK allele disrupted by homologous recombination in mouse embryonic stem cells (GK+/-), and 14 transgenic mice whose GK activity was attenuated specifically in the β-cells of the pancreas by a GK ribozyme approach (RIP-GKRZ). Some of the data obtained in 5 of the 16 GK+/- mice were included in a previous publication (1) and are reported here solely to facilitate comparison with the RIP-GKRZ mice. There were no differences in the mean body weights among the three groups of mice (Table 1). After a −6-h fast (postabsorptive state), the plasma glucose and insulin concentrations and the basal rate of HGP were also similar in the three experimental groups (Table 1).

Impact of Genotype on Hepatic GK Activity

We examined the kinetic parameters of hepatic GK in extracts prepared from liver samples obtained at the completion of the in vivo studies. Because the insulin promoter targets the ribozyme to the pancreatic β-cells, GK gene expression and activity should not be affected in the liver of RIP-GKRZ mice, whereas the disruption of one allele of the GK gene is expected to result in a reduction in the hepatic enzymatic activity. Indeed, as shown in Table 2, hepatic GK maximum velocity (Vmax) was reduced by 10.2±0.3 on April 13, 2017 http://ajpendo.physiology.org/ Downloaded from

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<th>WT</th>
<th>GK+/-</th>
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<tr>
<td>Kmic, mM</td>
<td>Protocol 1</td>
<td>12.7±0.8</td>
<td>11.6±0.9</td>
</tr>
<tr>
<td>Vmax, µmol·g−1·min−1</td>
<td>Protocol 2</td>
<td>12.9±0.9</td>
<td>8.3±1.1*</td>
</tr>
<tr>
<td>Vmax, µmol·g−1·min−1</td>
<td>Protocol 2</td>
<td>13.6±0.9</td>
<td>12.3±0.9</td>
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Values are means ± SE. Kmic, Michaelis-Menten constant; Vmax, maximum velocity. *P < 0.01 vs. WT.
was significantly decreased in the GK+/- mice in liver samples obtained at the completion of both protocol 1 (by 35% vs. WT) and protocol 2 (by 50% vs. WT). Conversely, GK activity was unchanged in the RIP-GKRZ group compared with WT. GK K_m was similar (~12 mM) in all groups (Table 2). Thus these two transgenic models differ considerably in their glucose phosphorylation capacity in the liver, and their comparison should allow one to gain insight into the specific impact of a decrease in hepatic GK per se on whole body and hepatic glucose fluxes and their regulation by hyperglycemia.

Effect of Hyperglycemia on Insulin Secretion and Glucose Fluxes

Protocol 1 was designed to examine the effect of a similar increase in the circulating glucose concentrations on insulin secretion and on peripheral and hepatic glucose fluxes. The steady-state plasma glucose concentration averaged ~5.5 mM during the euglycemic period, whereas it was raised by ~12 mM during the hyperglycemic period. Steady-state conditions for plasma glucose concentration and specific activity were achieved within 40 min during the basal and clamp periods of the studies. However, the steady-state plasma insulin concentration was significantly decreased in the two groups of mice with decreased GK activity in the pancreatic β-cells (Table 3). Thus, in keeping with previous observations in conscious mice (1) and the perfused pancreas (10), the ability of an increase in glucose concentration to elicit insulin secretion was partially impaired in both GK+/- and RIP-GKRZ mice. Interestingly, the decline in the plasma insulin concentration compared with WT was more evident and reproducible in the RIP-GKRZ mice than in the GK+/- mice. The latter may reflect our previous finding of a ~70% decrease in islet GK activity in the RIP-GKRZ model (10) compared with a ~40% decrease in islet GK activity in the GK+/- model (1). Our experimental approach does not allow one to identify the glycemic threshold at which the defect in GK activity results in decreased insulin secretion.

The whole body Rd and the GIR during the hyperglycemic clamp study are depicted in Fig. 2. GIR and Rd were decreased by ~30% in both GK+/- and RIP-GKRZ mice compared with WT (P < 0.01 for all). Figure 3 depicts the rate of glucose production during the basal period and its suppression by hyperglycemia and endogenous hyperinsulinemia. Basal HGP was similar and averaged ~22 mg·kg^{-1}·min^{-1} in the three experimental groups. In contrast, HGP during the hyperglycemic

<table>
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<th>Table 3. Steady-state plasma insulin and glucose concentration in WT, GK+/-, and RIP-GKRZ mice during hyperglycemic (protocol 1) and hyperglycemic/pancreatic (protocol 2) clamp studies</th>
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<tr>
<td>Protocol 1</td>
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<td>Protocol 2</td>
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Values are means ± SE; n, no. of mice/group. *P < 0.05 vs. WT; **P < 0.01 vs. WT.
clamp studies was significantly (63%) higher in the GK +/- mice compared with the WT. Similarly, when the inhibition of HGP during the hyperglycemic clamp was expressed as a percent decrease from basal levels, it was significantly impaired in GK +/- mice (48% inhibition) compared with WT (70% inhibition; P < 0.01). Conversely, the absolute rate of HGP and the percent inhibition from basal were unchanged in the RIP-GKRZ mice (Fig. 3).

Effect of Hyperglycemia Per Se on Glucose Fluxes

The steady-state plasma glucose concentration was kept at the basal level (~5.5 mM) during the euglycemic period, whereas it was raised by ~12 mM during the hyperglycemic period (protocol 2 in Table 3). Steady-state conditions for plasma glucose concentration and specific activity were achieved within 40 min during the basal and clamp periods of the studies. In this protocol, however, the plasma insulin concentration was kept at similar levels (~9 ng/ml) in all groups during the hyperglycemic clamp studies, allowing us to discern the metabolic responses to hyperglycemia per se. Under these experimental conditions, the body Rd was similar in the three experimental groups (Fig. 4A). However, the average GIR required to maintain the target hyperglycemic level during the last 50 min of the clamp study was significantly less in the GK +/- mice than in either WT or RIP-GKRZ mice (Fig. 4B). HGP was significantly and similarly inhibited by ~45% in response to hyperglycemia in WT and RIP-GKRZ (Fig. 5). In contrast, hyperglycemia caused only a 12% decline in HGP in GK +/- mice, and the rate of HGP was 45% higher in the latter group compared with WT (Fig. 5; P < 0.01). These data support the hypothesis that hepatic glucose phosphorylation fails to properly adapt to increased circulating glucose levels, leading to the blunting of the suppression of HGP by hyperglycemia in the GK +/- mice. Estimates of the relative contributions of the direct phosphorylation of glucose and of gluconeogenesis to the hepatic glucose 6-phosphate pool can be derived by assessment of the specific activities of hepatic substrates after the infusion of labeled lactate and glucose.

Effect of Reduced GK Activity on Stimulation of the "Direct Pathway" of Liver UDP-Glc Formation by Hyperglycemia

An impairment in hepatic glucose phosphorylation may affect the relative contribution of plasma glucose to the hepatic glucose 6-phosphate pool. Table 4 displays the [3H]UDP-Glc, [3H]UDP-Gal, and [3H]Glc specific activities that are used to calculate the contribution of plasma glucose ("Direct Contribution" in Table 4) to the hepatic glucose 6-phosphate pool. The UDP-Gal specific activities confirmed the values obtained with UDP-Glc, suggesting rapid and complete isotopic equilibration between the two intracellular pools. The ratio of the specific activities of [3H]-labeled hepatic UDP-Glc and UDP-Gal and portal vein plasma glucose provided an estimate of the contribution of the direct pathway. As shown in Table 4, the contributions of the direct pathway to the hepatic UDP-hexose pool measured at the end of both protocols 1 and 2 were significantly diminished in the GK +/- mice compared with both WT and RIP-GKRZ mice. These changes in the composition of the intrahepatic UDP-hexose pools provide evidence for decreased flux through GK in vivo in GK +/- mice.
Table 4. Substrate specific activities used to calculate “direct pathway” at end of [3-3H]glucose-[U-14C]lactate infusion in WT, GK +/-, and RIP-GKRZ mice during hyperglycemic (protocol 1) and hyperglycemic/pancreatic (protocol 2) clamp studies

<table>
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<th>Protocol 1</th>
<th>Dpm/nmol</th>
<th>Direct Contribution, %</th>
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<tr>
<td>WT</td>
<td>11.4 ± 0.8</td>
<td>5.4 ± 0.8 5.1 ± 0.6 52.8 ± 2.8 52.1 ± 2.7</td>
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<tr>
<td>GK +/-</td>
<td>17.4 ± 2.3</td>
<td>6.4 ± 1.1 6.8 ± 1.2 37.9 ± 2.9 39.0 ± 2.7*</td>
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<tr>
<td>RIP-GKRZ</td>
<td>17.3 ± 1.2</td>
<td>8.4 ± 0.5 8.1 ± 0.7 51.4 ± 1.4 50.8 ± 1.7</td>
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<th>Protocol 2</th>
<th>Dpm/nmol</th>
<th>Direct Contribution, %</th>
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<tbody>
<tr>
<td>WT</td>
<td>40.9 ± 4.8</td>
<td>21.3 ± 4.6 20.9 ± 3.7 52.4 ± 3.2 52.2 ± 4.2</td>
</tr>
<tr>
<td>GK +/-</td>
<td>39.8 ± 5.4</td>
<td>16.2 ± 2.8 15.8 ± 2.7 40.7 ± 3.3 40.2 ± 2.9*</td>
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<tr>
<td>RIP-GKRZ</td>
<td>38.9 ± 7.6</td>
<td>19.7 ± 2.8 19.5 ± 2.6 50.9 ± 3.4 50.6 ± 2.8</td>
</tr>
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Values are means ± SE. UDP-Glc, uridine 5-diphosphate glucose; UDP-Gal, uridine 5-diphosphate galactose; Glc, plasma glucose; Direct Contribution, % of hepatic glucose 6-phosphate (Glc-6-P) pool derived from plasma Glc, calculated as ratio of specific activities of [3H]UDP-Glc (Glc) or [3H]UDP-Gal (Gal) to [3H]Glc. *P < 0.01 vs. WT and RIP-GKRZ.

which likely reflects the decrease in GK activity measured in vitro.

Effect of Reduced GK Activity on the “Indirect Pathway” of Liver UDP-Glc Formation

Table 5 displays the [14C]UDP-Glc, [14C]UDP-Gal, and [14C]PEP specific activities that are used to calculate the contribution of gluconeogenesis (“Indirect Contribution” in Table 5) to the hepatic glucose 6-phosphate pool. The indirect pathway accounted for 30–35% of the hepatic UDP-Glc pool in WT and RIP-GKRZ mice. This contribution was increased to 45–55% in the GK +/- mice. These data indicate that the reduced liver GK activity in the GK +/- mice leads to a significantly higher proportion of hepatic glucose 6-phosphate being derived from gluconeogenesis vs. plasma glucose compared with WT and RIP-GKRZ mice.

DISCUSSION

A fundamental premise of our experimental design was that the genetic manipulations implemented in our animal models resulted in discordant effects on the hepatic activity of GK. The extent of deficit in the β-cell and liver glucose phosphorylating capacity induced by the disruption of one allele of the GK gene has been debated regarding both human MODY and animal models (1, 4, 17, 30). In humans, in whom the enzymatic activity of the GK protein encoded by the mutant allele is generally negligible (16), GK activity should be ~50% of the normal levels. However, a recent study has suggested that compensatory mechanisms may be activated in β-cells of individuals with MODY 2 and may account for an insulin secretory function that is higher than expected (5, 29). In mouse models in which one allele of the GK gene had been disrupted, the decrease in GK activity in β-cells varied between 37 and 50% (1, 17, 30). Similarly, the decrease in the hepatic GK activity in the same mouse models varied between 28 and 44% (1, 17). The hepatic isofrom of GK is generated by a different promoter than the pancreatic isoform (20–22). Because hepatic GK is regulated by insulin, it has been postulated that changes in circulating levels of the hormone are likely to upregulate the transcription of the normal GK allele in the liver and perhaps diminish the impact of this genetic manipulation at this site (1, 3, 22). Our findings show that the activity of GK in vitro was decreased in the GK +/- mice by 35 and 50% at the end of protocols 1 and 2, respectively. Interestingly, the decrease in hepatic GK activity was more severe and reproducible at the end of the hyperglycemic clamp studies, in which the plasma insulin concentration was kept at near basal levels, than at the completion of the hyperglycemic clamp studies performed at high circulating insulin levels. It can be postulated that the intact GK allele is quite sensitive to insulin regulation and that the degree of compensation may vary on the basis of the nutritional and hormonal status of the animal at the time of sampling. Overall, the present finding of a partial decrease in the hepatic GK activity in GK +/-, but not in RIP-GKRZ, mice is consistent with previous reports (1, 10) and allowed us to use these two experimental models to further dissect the metabolic impact of a partial and chronic inhibition of hepatic GK.

During the hyperglycemic clamp studies, the ability of hyperglycemia to promote insulin secretion was significantly diminished, particularly in the RIP-GKRZ mice. This observation reproduces in the intact animal the decreased glucose-induced insulin secretion we had previously observed in this model with use of the perfused pancreas technique (10). This is also consistent with elegant secretory regulatory processes observed in humans carrying a mutant GK allele (5). Peripheral glucose uptake did not appear to be specifically affected by the alterations in GK activity. In fact, although moderate decreases in the rates of whole body Rg were...
observed in both GK-deficient models during the hyperglycemic clamp studies, this observation appeared to be due to the lower plasma insulin concentrations. However, our findings in protocol 1 may also indicate lower insulin sensitivity in the GK+/− than in the RIP-GKRZ mouse. In fact, the R_{b} was similar in these two groups in the presence of higher insulin levels in the GK+/− mice. It should be pointed out that the differences in circulating plasma insulin concentrations between these two groups did not achieve statistical significance because of the large variability in the levels measured in the GK+/− mice. Finally, the decreased R_{b} was not reproduced when the circulating insulin levels were kept at the same levels in all groups (protocol 2). Overall, our data do not allow one to suggest or to exclude a modest effect of either mild glucose intolerance or of decreased GK activity in other tissues (brain) on peripheral insulin action in the GK+/− mice. Conversely, reproducible and specific features of liver glucose metabolism were demonstrated in the GK+/− mice. In fact, our findings indicate that the diminished glucose phosphorylation capacity in the liver of GK+/− mice causes an impairment in the ability of hyperglycemia to inhibit HGP. This defect is not observed in mice in which GK activity is reduced solely in β-cells. Furthermore, these observations cannot be ascribed to differences in pancreatic hormone levels among the groups, because they were reproduced during hyperglycemic-pancreatic clamp studies. The ability of hyperglycemia to inhibit HGP and the contribution of the direct pathway to the hepatic glucose 6-phosphate pool were markedly decreased in the GK+/− group compared with both WT and RIP-GKRZ groups. Several recent findings support the independent role of a decrease (even modest) in hepatic GK activity in the pathophysiology of carbohydrate intolerance in MODY and in animal models. Hepatic insulin resistance appears to represent an early finding in patients with MODY 2 (7), and impaired hepatic glycogen synthesis and decreased contribution of the direct pathway have recently been reported in an elegant study using 13C nuclear magnetic resonance spectroscopy in a group of MODY 2 patients (31). We have also shown that transient and short-term inhibition of hepatic GK activity with use of glucosamine can reproduce some of the defects in hepatic glucose fluxes (2). Finally, two recent studies have demonstrated improved glucose tolerance in transgenic mice with liver-specific overexpression of GK (11, 18).

An important finding in the present study is that the decreased flux through GK was paralleled by a marked increase in the contribution of the gluconeogenic pathway to the hepatic glucose 6-phosphate pool. The increased HGP in the GK+/− mice was associated with a marked increase in the relative contribution of gluconeogenesis to HGP. Interestingly, a transient and acute inhibition of hepatic GK activity in rats resulted in similar effects on HGP and the direct pathway of hepatic glycogen repletion, but not on gluconeogenesis (2). In fact, the increased HGP in the latter rat study was due to an increased rate of glycogenolysis. Conversely, a recent study by Velho et al. (31) in MODY 2 patients demonstrated that the decreased contribution of direct hepatic phosphorylation of glucose to postmeal glycogen synthesis was compensated in part by a parallel increase in the contribution of the indirect or gluconeogenic pathway. This apparent discrepancy regarding the metabolic consequences of short-term vs. chronic decreases in the hepatic GK activity may be due to the noteworthy role of hepatic glucose phosphorylation in regulating gene expression of key hepatic enzymes in the gluconeogenic and glycolytic pathways, such as phosphoenolpyruvate carboxykinase and L-type pyruvate kinase. Indeed, Ferre and co-workers (11, 12) reported that the hepatic overexpression of GK leads to a marked increase in pyruvate kinase mRNA and activity. Thus a prolonged moderate decrease in the rate of hepatic glucose phosphorylation is likely to alter the intrahepatic distribution of glucose fluxes, probably through regulation of gene expression of key enzymes. In conclusion, our data indicate that decreased activity of GK in the liver can cause increased HGP in the face of hyperglycemia.

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REFERENCES


GLUCOKINASE AND HEPATIC GLUCOSE PRODUCTION


