Correction of diabetic alterations by glucokinase

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Communicated by Charles R. Park, Vanderbilt University Medical Center, Nashville, TN, February 26, 1996 (received for review September 15, 1995)

ABSTRACT Hyperglycemia is a common feature of diabetes mellitus. It results from a decrease in glucose utilization by the liver and peripheral tissues and an increase in hepatic glucose production. Glucose phosphorylation by glucokinase is an initial event in glucose metabolism by the liver. However, glucokinase gene expression is very low in diabetic animals. Transgenic mice expressing the P-enolpyruvate carboxykinase/glucokinase chimeric gene were generated to study whether the return of the expression of glucokinase in the liver of diabetic mice might prevent metabolic alterations. In contrast to nontransgenic mice treated with streptozotocin, mice with the transgene previously treated with streptozotocin showed high levels of both glucokinase mRNA and its enzyme activity in the liver, which were associated with an increase in intracellular levels of glucose 6-phosphate and glycogen. The liver of these mice also showed an increase in pyruvate kinase activity and lactate production. Furthermore, normalization of both the expression of genes involved in gluconeogenesis and ketogenesis in the liver and the production of glucose and ketone body by hepatocytes in primary culture were observed in streptozotocin-treated transgenic mice. Thus, glycolysis was induced while gluconeogenesis and ketogenesis were blocked in the liver of diabetic mice expressing glucokinase. This was associated with normalization of blood glucose, ketone bodies, triglycerides, and free fatty acids even in the absence of insulin. These results suggest that the expression of glucokinase during diabetes might be a new approach to the normalization of hyperglycemia.

The liver has a central role in maintaining glucose homeostasis. When the plasma glucose is high, the liver takes up glucose, replenishes depleted glycogen stores, and then synthesizes fatty acids (1). Glucose transport and phosphorylation are the first steps in glucose utilization. In the liver, glucose is transported by two members of the facilitative glucose transporter (GLUT) family, GLUT1 and GLUT2 (2). In nonobese diabetic animals, GLUT2 provides glucose to hepatocytes at a rate that is 600 times greater than the rate of phosphorylation by hexokinase and 100 times higher than the rate of phosphorylation by glucokinase (3). Thus, glucose phosphorylation by GK seems to be key in the regulation of glucose utilization by hepatocytes. This enzyme, in contrast to other hexokinases, has a high K_m for glucose (5–8 mM), and its activity is not inhibited by physiological concentrations of glucose 6-phosphate (4, 5). Regulation of GK activity is mainly due to changes in the transcription of its gene. Insulin increases while glucagon inhibits liver GK gene transcription, and these effects are not glucose-dependent. However, the transcriptional activation of glycolytic and lipogenic genes in hepatocytes requires the presence of both glucose and insulin, neither of which is active alone (6, 7). The main role of insulin in the activation of glycolytic genes appears to be the stimulation of GK synthesis, which leads to increased glucose phosphorylation. The expression of GK in rat hepatoma cell lines, which lack endogenous GK gene expression, results in an induction of glucose uptake and metabolism (8). Glucose/carbohydrate regulatory elements have already been located in the promoter of genes coding for some of the enzymes of glycolysis and lipogenesis (6). The insulin effect on glucose-dependent activation of the L-type pyruvate kinase gene promoter in hepatocytes can be mimicked by a GK expression vector (9). The lack of insulin in insulin-dependent diabetes mellitus (IDDM) and the sensitivity to insulin and relative insulin deficiency in non-insulin-dependent diabetes mellitus (NIDDM) lead to a decrease in glucose utilization by the liver, muscle, and adipose tissue and to an increase in the hepatic glucose production (10, 11). During diabetes, GK gene expression and the enzyme activity are very low, and thus the liver is unable to metabolize blood glucose (4, 5).

The cytosolic form of P-enolpyruvate carboxykinase (PEPCK) is a regulatory enzyme of gluconeogenesis. PEPCK activity is regulated by the transcription rate of its gene. Glucagon activates while insulin inhibits PEPCK gene transcription (12, 13). Thus, the expression of the PEPCK gene is greatly induced during diabetes. PEPCK gene transcription is suppressed during fetal development and enhanced at birth (14). Fragments of the PEPCK promoter of ∼500 bp can regulate the expression of chimeric genes, in a manner like the endogenous PEPCK gene, in the liver of transgenic mice (14, 15).

Chronic hyperglycemia has been postulated to be the main factor responsible for the development of diabetes-specific microvascular pathology in the retina and renal glomerulus and in neurological and macrovascular complications (16). The reduction of hyperglycemia, and thus the maintenance of normoglycemia, is a goal of any therapeutical approach for IDDM and NIDDM. In this study, we examine the capacity of the PEPCK promoter to direct the expression of the GK gene in the liver of diabetic transgenic mice. We aimed to determine whether the return of GK expression in diabetes was able to induce glucose utilization and to block glucose production by the liver and, if so, to assess the contribution of the liver to the reduction of hyperglycemia and the other diabetic alterations.

MATERIALS AND METHODS

Construction of the PEPCK/GK Chimeric Gene and Generation of Transgenic Mice. To obtain the PEPCK/GK chimeric gene, we used a 2.3-kb EcoRI–EcoRI fragment containing the entire coding sequence and the polyadenylation signal of the rat GK cDNA (gift of P. Iynedjian, University of Geneva, Geneva, Switzerland). It was introduced at an EcoRI site newly created in the PEPCK/insulin chimeric gene (17). This chimeric gene was used as a liver expression vector to obtain regulated expression and mRNA stabilization from

Abbreviations: GK, glucokinase; PEPCK, P-enolpyruvate carboxykinase; GLUT, glucose transporter; IDDM, insulin-dependent diabetes mellitus; NIDDM, non-insulin-dependent diabetes mellitus; Siz, streptozotocin; HMG-CoA synthase, 3-hydroxy-3-methylglutaryl-CoA synthase; CPT, carnitine palmitoyltransferase.

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cDNAs in transgenic mice. The construction of the PEPCK/GK chimeric gene was initiated by subcloning the \textit{BglII–SphI} fragment of the PEPCK/insulin chimeric gene, which includes the first exon and the translation initiation site of the human insulin gene at the pSP72 polylinker. To destroy the ATG translation start site, which was contained in a unique \textit{NcoI} restriction site, this fragment of the insulin gene was digested with \textit{NcoI} and treated with mung bean nuclease to remove the single-stranded overhangs produced by the restriction enzyme. Afterwards, an \textit{EcoRI} linker (Boehringer Mannheim) was introduced to generate a new \textit{EcoRI} subcloning site. Then, the \textit{EcoRI–EcoRI} fragment of \textit{GK} cDNA was introduced at this new \textit{EcoRI} site. Finally, the \textit{BglII–SphI} fragment of the human insulin gene containing the full-length \textit{GK} cDNA was reinserted into the \textit{EcoP151}/insulin chimeric gene. The final plasmid was designated pPEPCK/GK. A 4.5-kb \textit{XbaI–SphI} fragment, containing the entire PEPCK/GK chimeric gene, was microinjected into fertilized eggs (see Fig. 1A). The expression of this chimeric gene leads to a 2.5-kb mRNA transcript when polyadenylated at the end of the \textit{GK} cDNA.

The general procedures for microinjection of the chimeric gene were as described (18). At 3 weeks of age, the animals were tested for the presence of the transgene by Southern blot of 10 \( \mu \)g of tail DNA digested with \textit{EcoRI}. Blots were hybridized with a 2.3-kb \textit{EcoRI–EcoRI} fragment containing the entire \textit{GK} cDNA radiolabeled with \([\alpha-32P]\)dCTP [3000 Ci/mmol (1 Ci = 37 GBq); Amersham] by random oligopriming (Boehringer Mannheim). Mice were fed ad libitum with a standard diet (Panlab, Barcelona, Spain) and maintained under a light/dark cycle of 12 h (lights on at 8:00 a.m.). Diabetes was induced by injection through the jugular vein of 2 mg of streptozotocin (Stz) per 10 g of body weight on 2 consecutive days. Stz (Sigma) was dissolved in 10 mM sodium citrate with 0.9% NaCl (pH 4.5) immediately before administration. Mice were used 7 days after Stz treatment. Diabetes was assessed by blood glucose and urine glucose and ketone levels (Accutrend and Gluketur Test; Boehringer Mannheim).

Animals were killed and samples were taken between 9:00 and 10:00 a.m. In the experiments described below, male mice aged 4 to 8 weeks were used.

**RNA Analysis.** Total RNA was obtained from liver by the guanidine isothiocyanate method (19), and RNA samples (30 \( \mu \)g) were electrophoresed on a 1% agarose gel containing 2.2 M formaldehyde. Northern blots were hybridized to internally labeled \textit{GK}, \textit{L-type pyruvate kinase}, and \textit{GLUT2} and \textit{GLUT1} cDNA probes as indicated in ref. 20 and also to the following probes: PEPCK, a 1.1-kb \textit{PstI–PstI} fragment from the 3′-end of the rat PEPCK cDNA (gift of R. W. Hanson, Case Western Reserve University, Cleveland, OH); tyrosine aminotransferase, a 0.6-kb \textit{PstI–PstI} fragment that includes the 3′-end of the rat cDNA (gift of G. Schütz, Institut für Zell und Tumorniologie, Heidelberg, Germany); mitochondrial 3-hydroxy-3-methylglutaroyl-CoA synthase (HMGS-CoA synthase), a 1.43-kb \textit{EcoRI–EcoRI} fragment of the rat cDNA (gift of F. G. Hegardt, University of Barcelona, Barcelona, Spain); carnitine palmitoyltransferase (CPT)-I, a 2.6-kb \textit{EcoRI–EcoRI} fragment of rat CPT-I cDNA (gift of J. D. McGarry, University of Texas, Dallas, TX); and CPT-II, a 1.8-kb \textit{EcoRI–BamHI} fragment of rat CPT-II cDNA (gift of J. D. McGarry). The \( \beta \)-actin probe corresponded to a 1.3-kb \textit{EcoRI–EcoRI} fragment of the rabbit cDNA. These probes were labeled using \([\alpha-32P]\)dCTP, following the method of random oligopriming as described by the manufacturer. Specific activity of the DNA probe was 1-10\(^{10}\) cpm/\( \mu \)g DNA. Membranes were placed in contact with Kodak XAR-5 films. The \( \beta \)-actin signal was used to correct for loading inequalities.

**Preparation and Incubation of Hepatocytes.** Hepatocytes were isolated between 10:00 and 11:00 a.m. from fed normal and diabetic mice as described (21). After removing nonparenchymal cells and debris, hepatocytes were resuspended in DMEM (GIBCO) containing 0.2% albumin and 10% fetal calf serum (Boehringer Mannheim). Cells (5.5 \( \times \) 10\(^6\)) were plated in 10 ml of this medium on collagen-coated dishes and maintained at 37°C under a 5% CO\(_2\) atmosphere. After 4 h, the medium was removed and cells were washed three times in DMEM in the absence of either serum or glucose. Subsequently, 10 ml of DMEM without serum but with 25 mM glucose were added to the cells, which were maintained in this medium for up to 24 h. Aliquots of 100 \( \mu \)l of medium were taken at different times, and lactate production was determined. To measure glucose and ketone body production, other hepatocytes were incubated in 10 ml of DMEM without glucose and supplemented with 16 mM lactate plus 4 mM pyruvate for up to 24 h. Aliquots of 100 ml of medium were taken at different times and glucose and \( \beta \)-hydroxybutyrate concentrations were determined.

**Enzyme, Metabolite, and Hormone Assays.** To measure \textit{GK} activity, liver samples were homogenized in an ice-cold buffer (pH 7.4) containing 50 mM Tris-HCl, 300 mM sucrose, 100 mM KCl, 1 mM EDTA, and 0.7 \( \mu \)l of 2-mercaptoethanol per ml. To determine pyruvate kinase activity, the liver was homogenized in an ice-cold buffer containing 100 mM KCl, 1 mM EDTA, and 0.7 \( \mu \)l of 2-mercaptoethanol per ml. These activities were analyzed in 12,000 \( \times \) g supernatants (22, 23). \textit{GK} activity was calculated as the difference between the glucose phosphorylation capacity at 100 and 0.5 mM glucose, and hexokinase activity as the glucose phosphorylating capacity at 0.5 mM glucose. Pyruvate kinase activity was determined at 5 mM P-enolpyruvate (total activity). To measure PEPCK activity, liver samples were homogenized in an ice-cold buffer (pH 7.4) containing 50 mM glycylglycine, 120 mM KCl, 1 mM DTT, 20 units of Trasylol per ml, and 100 \( \mu \)M phenylmethylsulfonyl fluoride. Afterwards, PEPCK activity was analyzed in 12,000 \( \times \) g supernatants as indicated by Chang and Lane (24). The concentrations of insulin and glucose in serum, of glycojen, glucose 6-phosphate, and lactate in liver extracts, and of glucose and lactate in the incubation media of hepatocytes were measured as described in ref. 20. Glucose was also determined in 20 \( \mu \)l of blood by using an Accutrend analyzer (Boehringer Mannheim). The \( \beta \)-hydroxybutyrate levels in serum and in the incubation medium of hepatocytes were measured by the \( \beta \)-hydroxybutyrate dehydrogenase technique (Boehringer Mannheim). Serum triglycerides were determined enzymatically (GPO-PAP, Boehringer Mannheim). Serum-free fatty acids were measured by the acyl-CoA synthase and acyl-CoA oxidase method (Wako Chemicals, Germany). Enzyme activities and metabolite concentrations are expressed as the means \pm SEMs. Analysis of statistical differences between data were performed using the Student-Newmann-Keuls test. Differences were considered statistically significant at \( P < 0.05 \).

**RESULTS AND DISCUSSION**

The PEPCK/GK chimeric gene (Fig. 1A) was microinjected into fertilized eggs and nine transmitter–transgenic founder mice were obtained. In this study, we used F1 and F2 mice from the transgenic lines PEPCK/GK-6 (TgA) and PEPCK/GK-47 (TgB). TgA and TgB carried about five and 20 intact copies, respectively, of the PEPCK/GK chimeric gene when analyzed by Southern blot (data not shown). We used littermates as controls for the transgenic animals. A 2.5-kb transcript was detected in the livers of control and transgenic mice, resulting from the expression of both the endogenous \textit{GK} gene (4, 5) and also the transgene, because of the chimeric gene used (Fig. 1B). No \textit{GK} mRNA transcripts were detected in the liver of nontransgenic diabetic mice, while Stz-treated transgenic mice expressed significant levels of \textit{GK} mRNA (Fig. 1B). Non-Stz-treated transgenic mice also showed an increase (\( \approx 2\)-fold) in \textit{GK} mRNA compared to control mice (Fig. 1B). The increase
that noted in healthy control mice, whereas diabetic TgB animals even showed higher GK activity than healthy control mice (Fig. 1C). These findings suggest that GK mRNA was translated and that the protein was stable in a diabetic environment. These results also indicated that the establishment of a certain level of GK mRNA in the liver of Stz-treated transgenic mice was enough to produce high enzyme activity. The results described below were obtained from the TgB line, which showed higher GK activity. However, an identical phenotype was observed with the TgA line.

In Stz-treated control mice, the reduction of GK activity was associated with a 75% decrease in the intracellular concentration of glucose 6-phosphate compared with healthy controls. In contrast, Stz-treated transgenic mice presented high levels of this metabolite, and they were similar to those of healthy control mice (Table 1). Glucose 6-phosphate is a substrate for the synthesis of glycogen as well as an allosteric activator of glycogen synthase (25). Transgenic mice expressing the PEPCCK/GK chimeric gene accumulate more glycogen (~2-fold) than control mice (Table 1). During diabetes, because of the lack of insulin and the increase in glucagon, glycogen synthase is phosphorylated and inactive (26, 27). As expected, no glycogen was stored in the liver of diabetic control mice. However, Stz-treated transgenic mice showed similar levels of glycogen to control healthy mice (Table 1), probably resulting, at least in part, from the increase of glucose 6-phosphate. Thus, the expression of the PEPCCK/GK chimeric gene led to the rescue of glucose storage during diabetes and indicated that GK activity is probably rate-limiting for glycogen synthesis in hepatic cells. Similarly, we have already shown that FTO-2B and H411E hepatoma cells, which are unable to store glycogen (8), accumulate high levels of glycogen when infected with a retroviral vector carrying a PEPCCK/GK chimeric gene expressing GK (8).

An induction of glucose utilization was also observed in transgenic mice. A main regulatory enzyme of glycolysis is the L-type pyruvate kinase. The L-PK gene is transcriptionally regulated—positively by glucose plus insulin and negatively by glucagon (6). A reduction in L-PK gene expression and enzyme activity was noted in the liver of diabetic control mice compared with healthy controls. However, both L-PK mRNA concentration and enzyme activity were higher (~4-fold) in diabetic transgenic mice than in diabetic control mice and were similar to those of healthy transgenic mice (Fig. 2 and Table 1). Similarly, in hepatocytes in culture, transfection with a GK expression vector confer an insulin-independent glucose activation of L-PK gene promoter (9). The activation of L-PK during diabetes was associated with an increase in the intrahepatic content of lactate in the transgenic mice (Table 1). In addition, when hepatocytes from diabetic transgenic mice were cultured for up to 24 h in DMEM without serum, an increase in lactate production was detected compared to Stz-treated nontransgenic animals (Fig. 3A). The concentration of this metabolite was similar to that produced by non-Stz-treated control hepatocytes.

**Table 1. Glucose storage and utilization**

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<th>Non-treated</th>
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<th>STZ-treated</th>
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<tr>
<td></td>
<td>Con</td>
<td>Tg</td>
<td>Con</td>
<td>Tg</td>
</tr>
<tr>
<td>Glucose 6-P, nmol/g of liver</td>
<td>215 ± 15</td>
<td>337 ± 21</td>
<td>56 ± 12</td>
<td>230 ± 26</td>
</tr>
<tr>
<td>Glycogen, mg/g of liver</td>
<td>44 ± 5</td>
<td>98 ± 5</td>
<td>4 ± 2</td>
<td>49 ± 6</td>
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<tr>
<td>Pyruvate kinase, units/mg of prot</td>
<td>0.24 ± 0.02</td>
<td>0.53 ± 0.06</td>
<td>0.15 ± 0.03</td>
<td>0.59 ± 0.08</td>
</tr>
<tr>
<td>Lactate, μmol/g of liver</td>
<td>0.23 ± 0.03</td>
<td>0.44 ± 0.04</td>
<td>0.06 ± 0.02</td>
<td>0.25 ± 0.04</td>
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Glucose storage and utilization in the liver of diabetic mice expressing GK. The concentrations of glycogen, glucose 6-phosphate, and lactate, and also the activity of pyruvate kinase, were measured in liver extracts obtained from non-treated and Stz-treated control (Con) and transgenic (Tg) mice, as indicated. Results are means ± SEMs of 12 different animals in each group.

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**Fig. 1.** Expression of GK in liver of diabetic mice. (A) Schematic representation of the PEPCCK/GK chimeric gene. A 4.5-kb XbaI-SphI fragment, containing the entire PEPCCK/GK chimeric gene, was microinjected into fertilized eggs. The construction of this chimeric gene is described. (B) Expression of the transgene in liver. Total cellular RNA was obtained from non-treated and Stz-treated control (Con), and transgenic mice from the lines PEPCCK/GK-6 (TgA) and PEPCCK/GK-47 (TgB), as indicated. After transfer, membrane was tested for GK. (C) GK activity was determined in liver extracts obtained from non-treated and Stz-treated control (Con) and transgenic (Tg) mice from the lines PEPCCK/GK-6 (TgA) and PEPCCK/GK-47 (TgB), as indicated. Results are means ± SEMs of 15 different animals in each group.
The expression of key genes in the regulation of glucose and ketone body metabolism in the liver of diabetic mice expressing GK was determined by Northern analysis of total hepatic RNA obtained from non-treated and Stz-treated control (Con) and transgenic (Tg) mice, as indicated. Representative Northern blots hybridized with L-type pyruvate kinase (Pyr K), PEPCK, tyrosine aminotransferase (TAT), GLUT2, CPT-I and CPT-II, and mitochondrial HMG-CoA synthase (HMG-CoAS) specific probes are presented.

In contrast to control mice treated with Stz, the Stz-treated transgenic mice showed a reduction in the expression of the gene for PEPCK, which was induced by the diabetic process (Fig. 2). The changes in PEPCK mRNA levels were parallel to changes in the enzyme activity. Although no differences were noted in PEPCK activity between healthy control and transgenic mice [control, 4.9 ± 0.9 milliunits of protein per mg of body weight (n = 10) versus transgenic, 4.3 ± 0.3 milliunits of protein per mg of body weight (n = 10)], a strong reduction in PEPCK activity was observed in diabetic transgenic mice compared to diabetic animals [Stz-control, 19.6 ± 1.0 milliunits of protein per mg of body weight (n = 8) versus Stz-transgenic, 5.9 ± 0.8 milliunits of protein per mg of body weight (n = 8)]. It has been reported that glucose reduces the rate of PEPCK gene transcription in hepatocytes in culture and in FAO rat hepatoma cells (28, 29). In these cells, when the glucose concentration was increased, the rate of glucose uptake and utilization increased. Administration of glucose to diabetic rats also decreases PEPCK mRNA levels in liver (29). The expression of the gene for the tyrosine aminotransferase, another enzyme involved in gluconeogenesis (30), was also normalized in the liver of diabetic transgenic mice compared to controls (Fig. 2). In agreement with these findings, glucose production from gluconeogenic precursors by hepatocytes in primary culture from diabetic transgenic mice was lower than that of diabetic mice and similar to that of healthy control mice (Fig. 3B). The decrease in hepatic glucose production by the liver of diabetic transgenic animals was also related with a decrease in the glucose transporter GLUT2 mRNA levels, which were increased in the liver of diabetic mice (Fig. 2), as reported (31). No changes were observed in the expression of GLUT1 between control and transgenic mice under the same conditions (data not shown). These results are similar to those reported in the liver of diabetic rats treated with vanadate (32). However, in the Stz-treated transgenic mice expressing the PEPCK/GK chimeric gene, a signal was generated that blocked gluconeogenesis in the absence of insulin and thus overcame the effects of glucagon. These findings suggest that the increase in glucose 6-phosphate in the liver might induce
Table 2. Serum parameters

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<th>Non-treated</th>
<th>Stz-treated</th>
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<tr>
<td></td>
<td>Con</td>
<td>Tg</td>
<td>Con</td>
<td>Tg</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>207 ± 11</td>
<td>148 ± 9</td>
<td>&gt;800</td>
<td>265 ± 14</td>
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<tr>
<td>Insulin, ng/ml</td>
<td>2.7 ± 0.3</td>
<td>1.5 ± 0.1</td>
<td>&lt;0.2</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td>β-OH-butyrate, mmol/l</td>
<td>0.37 ± 0.06</td>
<td>0.30 ± 0.07</td>
<td>2.94 ± 0.3</td>
<td>0.49 ± 0.12</td>
</tr>
<tr>
<td>Triglycerides, mg/dl</td>
<td>129 ± 15</td>
<td>154 ± 12</td>
<td>365 ± 28</td>
<td>181 ± 15</td>
</tr>
<tr>
<td>FFAs, mmol/l</td>
<td>0.98 ± 0.1</td>
<td>0.78 ± 0.1</td>
<td>1.95 ± 0.2</td>
<td>0.83 ± 0.1</td>
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Serum parameters in transgenic mice expressing the PEPCK/GK chimeric gene. Serum parameters from non-treated and Stz-treated control (Con) and transgenic (Tg) mice were determined, as described. Results are means ± SEMs of at least 15 different animals in each group.

a common mechanism that both increased the expression of key genes in glucose utilization and decreased the expression of those involved in glucose production. However, other mechanisms cannot be ruled out. The regulation by glucose 6-phosphate of specific transcription factor or factors, which could mediate these effects, has not been reported to date.

Induction of ketogenesis is a common feature of untreated IDDM (10, 11). The mitochondrial β-oxidation of fatty acids is initiated by the sequential action of two membrane-bound enzyme activities, CPT I and CPT II (33–35). These enzymes, responsible for the entrance of acyl-CoA to mitochondria and the mitochondrial HMG-CoA synthase, a regulatory enzyme of ketone body pathway (36), have been considered to control liver ketogenesis. Their activities increase during diabetes. It has also been described that CAMP, FFA, and diabetes induce, and insulin inhibits, the expression of HMG-CoA synthase gene (32, 37). No changes in the expression of these genes between healthy control and transgenic mice were noted. An increase in the mRNA concentration of CPT I, CPT II, and HMG-CoA synthase was observed in the liver of diabetic mice. However, Szt-treated transgenic mice showed similar mRNA levels to non-treated controls (Fig. 2). The reduction of CPT I, CPT II, and HMG-CoA synthase was related with a decrease in ketone body production by hepatocytes from diabetic transgenic mice cultured in the presence of gluconeogenic precursors, as compared to diabetic mice (Fig. 3C). The concentration of ketone body in the incubation medium of Szt-treated transgenic mice was similar to that of healthy control mice. These results indicated that ketogenesis was blocked in diabetic mice expressing the PEPCK/GK chimeric gene. The results obtained in the Szt-treated transgenic mice suggest that the activation of GK during diabetes induces a mechanism or mechanisms that coordinately block the expression of key genes in ketogenesis in the absence of insulin. This mechanism might be shared with that controlling the genes involved in gluconeogenesis, because both pathways are activated in the diabetic liver.

The changes in liver metabolism were associated with normalization of serum parameters altered during diabetes. Whereas diabetic control mice had high levels of blood glucose, Stz-treated transgenic mice showed a strong reduction of hyperglycemia (Table 2). All these mice had very low levels of circulating insulin. Healthy, transgenic mice showed a 20% decrease in the serum concentration of glucose and a 40% reduction in insulin levels compared with their non-transgenic siblings (Table 2), indicating that liver glucose metabolism determined the blood glucose and insulin set points in the mice. These results were consistent with the key role of the liver in whole body glucose homeostasis. Furthermore, in agreement with the blockage of CPT I, CPT II, and HMG-CoA synthase gene expression and ketone body production, serum β-hydroxybutyrate was normalized in diabetic transgenic mice compared with diabetic animals. The expression of the PEPCK/GK chimeric gene also led to normalization of circulating triglycerides and free fatty acids, which are markedly increased during diabetes (Table 2). These results indicated that the return of glucose uptake by the liver was sufficient to normalize glucose, lipid, and ketone body metabolism. Finally, as expected, diabetic control mice lost body weight [a 25% reduction 15 days after Stz, from 21.4 ± 0.5 g to 15.7 ± 0.8 g (n = 25)]. However, the normalization of liver metabolism and serum parameters in the Stz-treated transgenic mice was also accompanied by the maintenance of body weight [from 20.3 ± 0.6 g to 21.1 ± 0.9 g (n = 25)].

The Diabetes Control and Complications Trial (38), designed to test the hypothesis that the complications of diabetes mellitus are related to a rise in the plasma glucose concentration, demonstrated that intensive insulin therapy can delay the onset and slow the progression of retinopathy, nephropathy, and neuropathy in patients with IDDM. Patients with NIDDM were not studied in the Diabetes Control and Complications Trial. Improvements in glycemic control could potentially reduce microvascular complications in patients with NIDDM. One concern about using intensive insulin therapy in NIDDM is that it might exacerbate macrovascular complications, the leading cause of morbidity and mortality in this disease (16). Hyperinsulinenia and insulin resistance, both very common in patients with NIDDM, are associated with increased risk of hypertension, coronary artery disease, and stroke, raising the possibility that insulin itself has atherogenic actions. However, both IDDM and NIDDM hyperglycemia might be reduced by increasing glucose uptake by liver and peripheral tissues. Our results suggest that the expression of GK during diabetes might be a useful approach to the reduction not only of diabetic hyperglycemia but also of ketoacidosis.

We thank R. W. Hanson for PEPCK promoter and cDNA; P. Iynedjian, A. Kahn, G. Schütz, G. Bell, M. Birnbaum, F. G. Hegardt, and J. D. McGarry for cDNAs; J. E. Feliu for helpful suggestions; R. Casamitjana for insulin measurements; R. Rycroft for critical reading of the manuscript; and C. H. Ros for technical assistance. A.P. and E.R. were recipients of predoctoral fellowships from Dirección General d'Universitats, Generalitat de Catalunya, Spain, and T.F. was a recipient of a fellowship from Fondo Investigación Sanitaria, Spain. This work was supported by grants from Fondo Investigación Sanitaria (FIS 94/0795) and the Juvenile Diabetes Foundation International.