Characterization of the gene expression profile of heterozygous liver-specific glucokinase knockout mice at a young age

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Abstract

In the liver, glucokinase (GCK) facilitates hepatic glucose uptake during hyperglycemia and is essential for the regulation of a network of glucose-responsive genes involved in glycolysis, glycogen synthesis, and lipogenesis. To better understand the consequences of changes in response to a liver-specific deficiency of GCK function we examined the expression profiles of genes involved in glucose metabolism in the liver, pancreas, muscle and adipose tissue in heterozygous liver-specific *Gck* knockout (*Gck*^{w/-}) mice. Our results showed that with the development of a liver GCK deficiency, significant decreases in the mRNA levels for insulin receptor and *Glut2* were observed in the liver, and *HkII* in muscle, while glucagon mRNA increased markedly in the pancreas. The levels of circulating glucagon hormone levels increased with increased mRNA levels. Despite a decrease in muscle *HkII* levels, the hexokinase activity level did not change. Our findings suggest that in liver-specific *Gck*^{w/-} mice, peripheral tissues use different strategies to tackle with hyperglycemia even at a young age. By identifying the specific changes that occur in different tissues at an early stage of glucokinase deficiency, potentially we can develop interventions to prevent further progression to diabetes.

Keywords

liver glucokinase; gene expression; diabetes

Introduction

Glucokinase (GCK) is a 50 KD enzyme that catalyzes the ATP-dependent phosphorylation of glucose to glucose-6-phosphate as the first, and rate-limiting step, in glucose utilization

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[1][2]. In humans, gene mutations in *GCK* cause a monogenic form of diabetes mellitus known as maturity onset diabetes of the young, type 2 (MODY2) [3][4], a disease that is characterized by early onset and persistent hyperglycemia. The *GCK* gene has two promoters that are expressed in a tissue-specific pattern[5][6][7]. The two promoters each generate unique leader exons that specify a 5' untranslated region for the mRNA as well as the first 15 codons of the GCK protein sequence, thus there is difference in the N-terminal ends of the protein synthesized in the liver compared to other tissues, but there is no evidence for a difference in enzymatic function [8]. The pathophysiological basis of GCK-deficient MODY2 is generally viewed as involving both pancreatic β cells and hepatocytes [2][9][10], however, the relative contributions of each cell type to the disease have not yet been established.

Heterozygous pancreatic beta-cell-specific *Gck* knockout mice show mild early-onset diabetes due to an impaired insulin-secretory response to glucose [11]. In contrast, overexpression of *Gck* in isolated rat islets shows an enhanced insulin secretion in response to stimulatory levels of glucose [12], clearly illustrating that the pancreatic levels of GCK impact insulin secretion. Efforts have also been made to understand the role of GCK in the liver. Hepatic GCK activity is decreased in obese subjects with diabetes [13]. Loss of beta cells in rats [14] and mice [15] due to streptozotocin treatment leads to extremely low levels of liver GCK activity. These results are in accord with the observation that expression of *Gck* in the liver is dependent upon insulin signaling [16], and thus changes in pancreatic GCK function can influence liver-specific GCK function. Liver-specific function of GCK has been examined by overexpression of *Gck* in rat primary hepatocytes, which was shown to have potent effects on glucose storage and utilization and that similar effects were not observed if hexokinase I was overexpressed in these cells [17]. Overexpression of *Gck* in the liver of rats resulted in decreased blood glucose in liver, but also hyperlipidemia, in the fed state [18]. To examine GCK deficiency our lab, and others, have generated hepatocyte-specific *Gck* knockout mice using the Cre-loxP gene targeting strategy [19][20]. The liver-specific *Gck* knockout mice display hyperglycemia, impaired glucose tolerance, and impaired insulin secretion[2][19][20]. The importance of these changes in GCK function in the liver, and its consequences for diabetes, are far from being completely understood.

In the liver, GCK facilitates glucose uptake during hyperglycemia and is essential for the regulation of a network of glucose-responsive genes involved in glycolysis, glycogen synthesis, and lipogenesis [21]. Phosphorylated glucose generated by GCK not only functions as a substrate for carbohydrate and lipid metabolism, but also regulates the expression of genes that are involved in glycolytic and lipogenic metabolism, and potentially other functions [22]. A modest hepatic overexpression of *Gck* is sufficient to promote fed expression levels of glycolytic and lipogenic enzyme genes in the fasted state [23]. In contrast, little is known about the metabolic and gene expression changes due to a liver-specific deficiency of GCK, especially at an early age. Functional alterations in pancreatic beta-cell, liver, skeletal muscle and adipose tissue may disrupt glucose homeostasis and lead to the development of hyperglycemia. By knowing the specific changes that occur in these tissues, especially those that occur soon after the development of a GCK deficiency, should allow the development of interventions that may prevent further progression to diabetes. The aim of the present work is to characterize changes in the expression and function of genes

involved in metabolism that occur in mice at an early age in diverse tissues of liver-specific *Gck* deficient mice. Our goal is to better understand the mechanisms that regulate the metabolic interaction between the various organs and tissues in the whole animal as diabetes begins to develop. Here we focused on the early steps involved in glucose metabolism, including insulin action and glucose utilization. We believe that a more complete understanding of the regulation and the functions of GCK in the liver will help us understand the role of the deficiency hepatic GCK in the pathogenesis of MODY2 and hyperglycemia.

Materials and methods

Animals

Liver-specific *Gck*^{W/-} mice were previously generated by our lab [19]. Mice were housed in a pathogen-free animal facility maintained at 25°C and illuminated with a 12:12-h light-dark cycles. Mice were provided with standard rodent chow and water ad libitum. The Animal Care and Use Committee of Peking University approved all animal protocols. Sacrificed mice were deprived of food from 8:00AM to 14:00PM on the day of sacrifice to generate a fasted state and sacrificed at 14:00PM. To obtain mice with a fed status, mice were first fasted for 8-h and then injected with a 20% glucose solution at a dose of 2g/kg [24]. Fed mice were then sacrificed, 40 minutes after the glucose injection, with tissues quickly removed from the sacrificed mice and placed into liquid nitrogen.

Biochemical analysis

Blood glucose concentration was measured from blood obtained from the tails of mice with a Roche blood glucose monitor (Glucotrend 2). Serum insulin levels were determined by radioimmunoassay (RIA) using a human insulin RIA kit [19] (Beijing North Institute of Biological Technology, China, F01PJA). Serum glucagon levels were determined by radioimmunoassay (RIA) using a human glucagon kit [25] (Beijing North Institute of Biological Technology, China, F03PJA). Cholesterol, triglyceride, HDL-cholesterol and LDL-cholesterol (enzyme methods) were determined using a commercial kit (BIOSINO, China) and an automatic analyzer (HITACHI7080).

HK activity

Muscle HK activity was measured by a HK activity kit (Nanjing Jiancheng, China, A077). Protein concentration was determined using the BCA method (APPLYGEN, China, p1511).

Isolation of total RNA and quantitative RT-PCR

Total RNA was isolated from ~50mg of frozen liver, muscle and pancreatic tissue and ~150mg of frozen adipose tissue using Trizol Reagent (Invitrogen) according to the manufacture's instructions. cDNA was synthesized from 2ug of total RNA using a high capacity DNA reverse transcription kit (TAKARA, D6110A). Amplifications were performed in the BIO-RAD MiniOpticon™ Real-Time PCR Detection system CFB-3120 using iQ™ SYBR Green Supermix 170-8880 (Bio-Rad) with primers listed in Table 1. All annealing temperatures were listed in the table 1. Transcription levels were normalized to those of beta actin.

Western blot

About 50mg of liver or muscle tissues was homogenized in 500ul of lysis buffer containing 50mmol/L Tri-HCl (pH8.0), 150mmol/L NaCl, 0.02%NaN₃, 0.1%SDS, 1mmol/L EDTA, 100mg/L PMSF, 1mg/L leupeptin and 1% NP-40. The mixture incubated at 4°C for 30min, and the supernatant was removed after centrifuging at 10,000×g for 10 min. Protein concentration was determined using the Bradford method. Quantified proteins were mixed with an equal volume of 2×SDS loading buffer containing 100 mmol/L Tris-HCl (pH6.8), 4% SDS, 0.2% bromophenol blue, 20% glycerol and 200 mmol/L DTT mixed and boiled for 10 min. The supernatant was collected after centrifugation at 10,000×g for 10 min, and proteins were separated by 10% SDS-PAGE. Separated proteins were electrotransferred to PVDF membranes (Millipore). After blocking (2h at room temperature in 5% nonfat dry milk in lysis buffer), membranes were incubated overnight at 4°C with an antibody against beta-actin (4967, CST), insulin receptor (ab69508, Abcam), HKII (2867, CST), GCK (ab70857, Abcam). Antibody binding was detected after incubation with HRP-linked secondary antibodies, with the membrane-bound antibodies visualized by luminal chemiluminescence ChemiDoc XRS (BIO-RAD).

Statistical analysis

Data are presented as means ± SE.M. with statistical differences between or among groups evaluated by the Student's t test or ANOVA.

Results

1. Biochemical parameters

To examine the initial effects of decreased glucokinase levels we measured the fasting blood glucose levels in heterozygous liver-specific *Gck* knockout (*Gck^{w/-}*) and wild-type mice (*Gck^{w/w}*) at 2, 4, 6 and 8 weeks of age (Fig. 1A). Except at 2-weeks of age, the fasting blood glucose levels in the *Gck^{w/-}* mice are significantly higher than those in age-matched *Gck^{w/w}* mice. The change in blood glucose levels did not affect body weight as the weights of *Gck^{w/-}* mice and age-matched *Gck^{w/w}* mice were not significantly different (Fig. 1B).

In figure 2, we show the insulin, glucagon, cholesterol and lipid levels in knockout and wild-type mice. No difference in the serum insulin concentrations was observed in age-matched *Gck^{w/-}* and *Gck^{w/w}* mice at any ages (Fig. 2A), but serum glucagon levels of the *Gck^{w/-}* mice was higher than seen in the *Gck^{w/w}* mice at 8 weeks (Fig. 2B). Cholesterol levels of the *Gck^{w/-}* mice were higher than those of *Gck^{w/w}* mice at 8 weeks (Fig. 2C), which is due to the LDL-C rather than the HDL-C component (Figs. 2D and 2E). The triglyceride levels of the *Gck^{w/-}* mice were similar to those of the *Gck^{w/w}* mice at all ages (Fig. 2F).

2. Changes in the mRNA levels of genes expression in the liver of liver-specific *Gck^{w/-}*-knockout mice at a young age

Since blood glucose levels in the liver-specific *Gck^{w/-}* mice were elevated we examined changes in the levels of expression of genes for glucokinase (*Gck*), hexokinase I and II (*HkI* and *HkII*), glucose transporter 2 and 4 (*Glut2* and *Glut4*), insulin receptor (*IR*), apolipoproteins B and E (*ApoB* and *ApoE*), and key lipid metabolism regulators *HMG-CoA*

reductase and *SREBP-1c* (Fig. 3A). As expected, the mRNA levels of *Gck*, *IR* and *Glut2* were much higher than those for *Glut4*, *HkI*, and *HkII* (Fig. 3A), consistent with glucokinase being the primary glucose phosphorylating enzyme, *Glut2* being the principal glucose transporter, and insulin being an important hormonal regulator of carbohydrate and lipid metabolism. Heterozygous liver-specific *Gck^{w/-}* mice expressed levels of *Gck* that were lower than age-matched *Gck^{w/w}* mice, with this difference being statistically significant at 4, 6, and 8 weeks of age (Fig. 3B). Changes in the glucokinase protein levels were confirmed by Western blot (Fig. 4). At 2 weeks of age, a decreased level of *Gck* mRNA was detected, but the decrease was not statistically significant (Fig. 3B). The decrease in *Gck* levels was not compensated by an increase in hexokinase levels, as *HkI* and *HkII* transcripts did not show an increase at any age (Figs. 3C and 3D). *Glut2* levels were lower in the *Gck^{w/-}* mice, but only at 8 weeks of age did this decrease reach statistical significance (Fig. 3E) and were not associated with a compensating increase in *Glut4* levels (Fig. 3F). Insulin receptor, however, was like glucokinase and showed decreased levels of expression as early as 4 weeks in *Gck^{w/-}* mice (Fig. 3G), and was also reflected in Western blots (Fig. 4).

Total cholesterol and LDL-C of *Gck^{w/-}* mice increased significantly at 8 weeks of age, indicating that with the development of a liver GCK deficiency that lipid metabolism dysregulation is initiated by 8 weeks of age. As shown in figure 3A, *ApoE* is expressed abundantly in the liver, with *ApoB* levels being about one percent of those for *ApoE*. The increase in LDL-C levels may be due to an upregulation of *ApoE*, as *ApoE* in *Gck^{w/-}* mice is significantly decreased at 8 weeks while *ApoB*, *HMG-CoA* reductase and *SREBP-1C* mRNA levels were found not to change at any age (Fig. 3).

3. Increased glucagon secretion by the pancreas of *Gck^{w/-}* mice: An early change in the pancreas in the fasted state

Glucokinase-expressing cells construct a network to maintain glucose homeostasis [26]. Besides the liver, the pancreas is a second important site for glucokinase function. To determine if liver *Gck* deficiency would affect glucose metabolism in the pancreas we measured the expression of genes involved in glucose metabolism in pancreas tissue. As shown in figure 5, the levels of expression of *Gck* and *Glut2* mRNA are similar, while those for the insulin receptor are about twice that of *Gck* or *Glut2*. The hormone encoding genes--insulin and glucagon have the most abundant mRNA in pancreas compared to the other genes examined (Fig. 5A).

Glucokinase acts as a glucose sensor in pancreatic beta cells and plays a crucial role in glucose-induced insulin secretion [27][28]. Liver *Gck* deficiency did not affect pancreatic *Gck* expression at young ages (Fig. 5B) consequently both insulin mRNA levels and serum insulin content of *Gck^{w/-}* mice are similar to the wild-type mice (Fig. 5E and 2A). However, *Gck^{w/-}* with a specific liver GCK deficiency has a significantly increased level of serum glucagon at 8 weeks of age (Fig. 2B), which is reflected at the mRNA level (Fig. 5F). In contrast, the *Glut2* and insulin receptor mRNA levels in *Gck^{w/-}* mice did not change with age, now were they different from those in wild-type mice (Figs. 5C and 5D).

4. Early change in the muscle of liver-specific *Gck^{w/-}* mice in the fasted state: decreased *HKII* mRNA levels but normal hexokinase activity

Skeletal muscle is the primary tissue responsible for the disposal of glucose load [29] and diabetes is characterized by the decreased insulin-stimulated glucose uptake by muscle [30]. To explore whether liver-specific GCK deficiency leads to a dysregulation of glucose metabolism in muscle we examined the levels of *HkI*, *HkII*, *Glut4* and insulin receptor expression in skeletal muscle tissue. HkI and HkII are the major expressed isoforms of hexokinase in skeletal muscle [31][32] and our results showed that *HkII* is expressed at a level of about thirty times greater than *HkI* (Fig. 6A). Insulin and muscle contraction have been reported to increase expression of *HkII*, but not *HkI*, in rodents [33] and humans [34]. The activity of HKI is not affected by insulin while HKII activity is regulated by insulin. These findings indicate that the HKII is the most important isoform in mouse muscle that participates in glucose metabolism.

No change in gene expression was detected for any gene until at age of 8 weeks age (Fig. 6). At 8 weeks the levels of *HkII* mRNA in *Gck^{w/-}* mice was decreased significantly compared to wild-type mice (Fig. 6C). Change in the HKII protein levels was confirmed by Western blot (Fig. 7A). Despite the change in HKII levels, the HK activity level of *Gck^{w/-}* mice was similar to that of wild-type mice (Fig. 7B). This change in activity level was not due to an increase in *HkI* mRNA levels as this did not change at any age (Fig. 6B). The levels of the other genes examined, including insulin receptor and *Glut4* did not change at any age (Figs. 6D and 6E).

5. Liver glucokinase deficiency did not affect genes involving in glucose metabolism in adipose tissue

In addition to muscle, adipose is another important site in of glucose uptake and utilization. Although the liver-specific *Gck^{w/-}* mice were not obese, we explored whether the liver-specific defects had effects on gene expression in adipose tissue. As in other tissues we examined the expression of *HkI*, *HkII*, *Glut4* and *IR*, but also added two key hormones that are secreted by adipose cells, leptin and resistin, in *Gck^{w/-}* and wild-type mice at 6 and 8 weeks of ages. Figure 8A shows that leptin and resistin are the most abundantly expressed genes examined, and that the mRNA levels for *Glut4* were about three times greater than that those for the insulin receptor. Both *HkI* and *HkII* are expressed in adipose tissue, with *HkII* expressed at a level about three times greater than *HkI*.

No change in expression of any of the examined genes was detected in the *Gck^{w/-}* mice compared with wild-type mice (Fig. 8B–8G), suggesting that the liver-specific GCK deficiency did not affect glucose metabolism in adipose tissue at young age.

6. No change in gene expression profiles was seen in the liver and pancreas of *Gck^{w/-}* mice compared to wild-type mice, in the fed state

Liver glucokinase is the rate-limiting enzyme in glucose usage, especially glucose flux. To elucidate the consequence of liver GCK deficiency on glucose flux we examined the expression profile of genes in glucose-stimulated state. The protocol to generate a glucose-stimulated state is described in materials and methods. The genes examined are those studies

above in mice in fasted state. As shown in figure 9B and 10B, insulin and glucagon mRNAs are expressed at much higher levels after feeding, compared to the fasted state, indicating that the administration of glucose mimics the fed state.

However, in glucose-stimulated state, the mRNA levels of liver *Gck* of *Gck^{w/-}* mice was the same as that of wild-type mice, and the amount was as much as wild-type mice at fasted state. The mRNA levels for insulin receptor and *Glut2* in *Gck^{w/-}* mice were similar with that of wild-type mice at the fed state, although both were significantly decreased in the fasted state (Figs 9A and 10A).

Figures 8B and 9B showed that in the glucose-stimulated state, pancreatic *Gck* expression was normal as pancreatic *Gck* and insulin expression of liver-specific *Gck^{w/-}* mice was similar to that of wild-type mice at young ages. Although the mRNA levels for glucagon in the *Gck^{w/-}* mice were increased in the fasted state, no difference was seen between *Gck^{w/-}* and wild-type mice in the fed state (Figs. 9B and 10B).

Discussion and Conclusion

Expression of *Gck* in the liver is strictly dependent on the presence of insulin [8]. In a large study of French patients using HOMA analysis Velho showed an increase in insulin resistance in glucokinase patients with diabetes compared to those with fasting hyperglycemia and to normal controls [35]. A deficiency of GCK in the liver could lead to insulin resistance in the liver, but the mechanism underlying this is unknown. Our findings suggest that in the liver-specific *Gck^{w/-}* mice, insulin levels stay at normal level in both the fasted and fed states (Figs. 2A, 5E and 9B, 10B), while the number of insulin receptors on liver cells start decreasing by 4 weeks in the fasted state (Fig. 3G). Glucokinase has been reported to be a measure of the biochemical activity of insulin [36]. We speculate that the decrease in glucose usage lead to a down regulation of the expression of the insulin receptor. The down-regulation of the insulin receptor then restricts insulin action in the liver, which contributes to the impairment of the liver and its ability to sense and respond to insulin. This mechanism may underlie the early appearance of insulin resistance in liver. In liver-specific insulin receptor knockout mice, *Gck* expression is also decreased, showing a decrease of between 60%–95% [37]. Overexpression of hepatic *Gck* can compensate, in part, for the metabolic disorders developed by the insulin receptor-deficient mice [38], however, the liver insulin receptor levels in *Gck^{w/-}* mice do not change in the fed state (Figs. 9A and 10A). This lack of change, may be due to the observation that in the fed state, the liver *Gck* levels of *Gck^{w/-}* mice do not show a deficiency. These phenomena indicated that there is a link between GCK and insulin receptor in liver, but molecular mechanisms underlying this are unclear.

GLUT2 is the facilitative glucose transporter predominantly expressed in the liver and pancreatic beta cells, and our results suggest that *Glut2* is expressed at higher levels than other genes involved in glucose metabolism in the liver (Fig. 3A). Previous studies have shown that glucose increases *Glut2* mRNA expression in vivo and in vitro [39] [40]. In addition, increase in *Glut2* mRNA abundance in the presence of glucose is not due to the stabilization of the transcript, but rather was a direct effect on gene transcription [40].

However, in those studies, liver GCK expression was normal, thus after glucose is transported into the liver cell, there was enough GCK to phosphorylate the glucose. In our liver-specific *Gck*^{w/-} mice, *Glut2* mRNA levels are decreased at 8 weeks of age (Fig. 3E). This decrease may be due to the liver cells having less GCK and thus phosphorylating less glucose yielding a lower intracellular glucose concentration. The lower intracellular glucose concentration then may down-regulate *Glut2*, reducing surface GLUT2 transporters, which then cause hyperglycemia as liver cells take up less glucose. This model is consistent with observation from GLUT2-null cells. In GLUT2-null liver cells, no up-regulation of *Gck* mRNA or GCK activity is induced by feeding [41]. These findings suggest coordination between *Gck* and *Glut2* in liver at the transcription level.

Decreased expression GLUT2 in of pancreatic beta cells has been reported in *db/db* mice [42] and Goto-Kakizaki rat [43]. In liver-specific *Gck*^{w/-} mouse model, *Glut2* expression in pancreatic beta cells was not change at an early age (Fig. 5D), suggesting that the glucose transport system was normal in the pancreas of the liver GCK deficiency mice.

In the liver, GCK is essential for the appropriate regulation of a network of glucose-responsive genes involved in glycolysis, glycogen synthesis, and lipogenesis [21]. It has been reported that the overexpression of apoE reduces plasma cholesterol and triglyceride levels due to the disappearance of LDL and VLDL [44]. Data from our mice show that the level of LDL-C and cholesterol in serum of liver *Gck*^{w/-} mice increased by an age of 8 weeks (Fig. 2). This result may in part be due to the dysregulation of ApoE, which we found was significantly decreased at 8 weeks (Fig. 3I).

In the pancreatic beta cells, GCK functions as the glucose sensor, determining the threshold for insulin secretion, and the phosphorylation of glucose is tightly coupled to insulin secretion [45]. Our results suggest that a liver-specific GCK deficiency does not affect pancreatic GCK action at early ages (Fig. 5B). Our results also show changes in glucagon secretion occur before changes in other genes involved in glucose metabolism (Fig. 5F). Glucagon is a major counteracting hormone to insulin in regulating glucose homeostasis, with a major role to promote hepatic gluconeogenesis and glycogenolysis to produce a raise in blood glucose levels during hypoglycemic conditions [46]. Both serum glucagon levels and pancreatic glucagon mRNA levels were increased, compared with wild-type mice, in *Gck*^{w/-} mice that were 8 weeks old (Figs. 2B and 5F). Increased glucagon levels, and its secondary effects, should result in the progression of hyperglycemia. Glucagon receptor knockout mice, which show lower blood glucose levels throughout the day and improved glucose tolerance [47], are resistant to diet-induced obesity and STZ-mediated beta cell loss and hyperglycemia [48]. Thus increases in glucagon secretion by the pancreas may be a good approach to prevent or slow the progression of MODY2 and liver-specific GCK deficient associated hyperglycemia at early stages of the disease.

Skeletal muscle and adipose tissue are also important sites participating in glucose metabolism. The loss of hepatic GCK activity may cause additional glucose disposal into these tissues, but also attenuate insulin action. Our data show that liver-specific GCK deficiency did not affect the expression of genes involved in glucose metabolism in adipose tissue, but that changes were observed in muscle at an early age (Fig. 6 and 8). In muscle,

although *Glut4* is more abundantly expressed than members of the hexokinase gene family or insulin receptor (Fig. 6A), it was only the levels of *HkII* that were decreased in response to the liver-specific GCK deficiency (Fig. 6C). Despite the decrease in *HkII* mRNA, the activity of HK was normal (Fig. 7B). As previously described, HKII is the most important glucose phosphorylate enzyme in muscle [49][50]. HKII deficiency in muscle led to insufficient glucose utilization, just as seen in the liver of *Gck^{w/-}* mice, which makes hyperglycemia more severe. It has been reported that SREBP-1C plays a major role in the response of the *HkII* gene to nutritional in rodents [51], however we did not observe a change in SREBP-1C levels in the muscle of liver-specific GCK deficient mice (S1).

In summary, our findings suggest that in liver-specific *Gck^{w/-}* mice, different peripheral tissues tackle hyperglycemia by different response at the early stage. In the liver, it responds by changing how it sense insulin; in the pancreas, it secretes large amounts of glucagon; and in muscle, the activity of *HkII* changes. Liver-specific GCK deficiency did not lead to any observed change in adipose tissue at early ages. Understanding the early consequence of a liver-specific GCK deficiency should help us prevent further progression of the disease.

In conclusion, this study reveals the early development of gene expression profiles secondary to liver *Gck* deficiency. We believe that a more complete understanding of the regulation and the functions of GCK in the liver will help us understand the role of the deficiency hepatic GCK in the pathogenesis of MODY2 and hyperglycemia.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Changes in blood glucose levels and body weight in heterozygous liver-specific *Gck* knockout and wild-type mice at 2, 4, 6, 8 weeks of age. Fasted blood glucose concentrations (A) and body weight (B) of liver *Gck*^{w/-} mice and *Gck*^{w/w} mice at 2, 4, 6, and 8 weeks of age. n=5–8. Means±S.E. Asterisk indicates significant differences in liver *Gck*^{w/-} mice vs age-matched *Gck*^{w/w} mice. *P<0.05; **p<0.01.

Figure 2.

Changes in serum parameters in fasted heterozygous liver-specific *Gck* knockout and wild-type mice at 2, 4, 6, 8 weeks of age. Fasted serum insulin content (A), serum glucagon content (B), serum cholesterol (C), triglyceride (D), LDL-C (E) and HDL-C content (F) of liver *Gck*^{w/-} mice and *Gck*^{w/w} mice at different age. n=5-8. Means $\pm$ S.E. Asterisk indicates significant differences in liver *Gck*^{w/-} mice vs age-matched *Gck*^{w/w} mice. * $P < 0.05$.

Figure 3.

Changes in the mRNA levels of genes in the liver of liver-specific *Gck* knockout mice at young age. (A) Relative amounts of mRNA of examined genes in the liver of *Gck*^{w/w} mice at 8 weeks in the fasted state was determined by real-time PCR and corrected with beta actin as an internal standard. Real-time PCR results of *Gck* (B), *Hkl* (C), *Hkll* (D), *Glut2* (E), *Glut4* (F), *Ir* (G), *ApoB* (H), *ApoE* (I), *HMG-CoA* (J) and *SREBP-1C* (K) in the liver of liver *Gck*^{w/-} mice and *Gck*^{w/w} mice in fasted state at different ages. n=5–8. Means±S.E. Asterisk indicates significant differences in liver *Gck*^{w/-} mice vs age-matched *Gck*^{w/w} mice. *P<0.05.

Figure 4. Western blot analysis of GCK and insulin receptor levels in the liver of liver $Gck^{w/-}$ mice and $Gck^{w/w}$ mice in fasted state at different ages.

Figure 5.

Changes in the mRNA levels of genes in the pancreas of liver-specific *Gck* knockout mice at a young age. (A) Relative amounts of mRNA in the pancreas of *Gck*^{w/w} mice at 8 weeks in fasted state determined by real-time PCR and corrected with beta actin as the internal standard. Real-time PCR results of *Gck* (B), *IR* (C), *Glut2* (D), insulin (E) and glucagon (F) in the pancreas of liver *Gck*^{w/-} mice and *Gck*^{w/w} mice in fasted state at different ages. n=5–8. Means±S.E. Asterisk indicates significant differences in liver *Gck*^{w/-} mice vs age-matched *Gck*^{w/w} mice. *P<0.05.

Figure 6.

Changes in the mRNA levels of genes in the muscle of liver-specific *Gck* knockout mice at young age. (A) Relative amounts of mRNA in the muscle of *Gck^{w/w}* mice at 8 weeks in fasted state determined by real-time PCR and corrected with beta actin as the internal standard. Real-time PCR results of *HkI* (B), *HkII* (C), *Glut4* (D) and *IR* (E) in the muscle of liver *Gck^{w/-}* mice and *Gck^{w/w}* mice in fasted state at different ages. n=5–8. Means±S.E. Asterisk indicates significant differences in liver *Gck^{w/-}* mice vs age-matched *Gck^{w/w}* mice. *P<0.05.

Figure 7.

Changes in the protein levels of HKII and the activity of HK in the muscle of liver-specific *Gck* knockout mice at a young age. (A) Western blot results of HKII in the muscle of liver *Gck*^{w/-} mice and *Gck*^{w/w} mice in fasted state at different ages. (B) HK activity in the muscle of liver *Gck*^{w/-} mice and *Gck*^{w/w} mice in fasted state at different ages. n=5–8. Means±S.E. Asterisk indicates significant differences in liver *Gck*^{w/-} mice vs age-matched *Gck*^{w/w} mice. *P<0.05.

Figure 8.

Changes in the mRNA levels of genes in the adipose tissue of liver-specific *Gck* knockout mice at young age. (A) Relative amounts of mRNA in the adipose tissue of *Gck^{w/w}* mice at 8 weeks in fasted state determined by real-time PCR and corrected with beta actin as the internal standard. Real-time PCR results of *HkI* (B), *HkII* (C), *Glut4* (D), *IR* (E), leptin (F) and resistin (G) in the adipose tissue of liver *Gck^{w/-}* mice and *Gck^{w/w}* mice in fasted state at different ages. n=5–8. Means±S.E. Asterisk indicates significant differences in liver *Gck^{w/-}* mice vs age-matched *Gck^{w/w}* mice. *P<0.05.

Figure 9.

Changes in the mRNA levels of genes in the liver and pancreas tissue of liver-specific *Gck* knockout mice in glucose-stimulated state at 8 weeks old. (A) Real-time PCR results of *Gck*, *Hk1*, *Hk2*, *Glut2* and *IR* in the liver of liver *Gck^{w/-}* mice and *Gck^{w/w}* mice in glucose-stimulated state at 8 weeks old. (B) Real-time PCR results of *Gck*, *Glut2*, *IR*, insulin and glucagon in the pancreas of liver *Gck^{w/-}* mice and *Gck^{w/w}* mice in glucose-stimulated state at 8 weeks old. n=5–8. Means±S.E. Asterisk indicates significant differences in liver *Gck^{w/-}* mice vs age-matched *Gck^{w/w}* mice. *P<0.05.

Figure 10.

Changes in the mRNA levels of genes in the liver and pancreas tissue of liver-specific *Gck* knockout mice in glucose-stimulated state at 4 weeks old. (A) Real-time PCR results of *Gck*, *HkI*, *HkII*, *Glut2* and *IR* in the liver of liver *Gck*^{w/-} mice and *Gck*^{w/w} mice in glucose-stimulated state at 4 weeks old. (B) Real-time PCR results of *Gck*, *Glut2*, *IR*, insulin and glucagon in the pancreas of liver *Gck*^{w/-} mice and *Gck*^{w/w} mice in glucose-stimulated state at 4 weeks old. n=5–8. Means±S.E. Asterisk indicates significant differences in liver *Gck*^{w/-} mice vs age-matched *Gck*^{w/w} mice. *P<0.05.

Table 1

primers for real-time PCR analysis of gene expression

Gene	primer	Tm(°C)
Glucokinase (<i>GCK</i>)	F- CAACTGGACCAAGGGCTTCAA	58
	R- TGTGGCCACCGTGTCATT	
Hexokinase I (<i>HkI</i>)	F- ATGATCGCCGCGCAACTAC	64
	R- AGAGCCGCATGGCATAACAGA	
Hexokinase (<i>HkII</i>)	F- CCGTGGACTGGACAACCTCA	64
	R- CGTCACATTTCCGAGCCAGA	
GLUT2	F- ATCAACATGATCTTCACGGCTGT	64
	R- TGGCAGTCATGCTCACGTAACCTC	
GLUT4	F- CTGTAACTTCATTGTCCGCGCATGG	64
	R- AGGCAGCTGAGATCTGGTCAAAC	
Insulin receptor (<i>IR</i>)	F- CAGCTCGAAACTGCATGGTTG	64
	R- GGTGACATCCACCTCACAGGAA	
Insulin	F- TGGCTTCTTCTACACCCAAG	58
	R- ACAATGCCACGCTTCTGCC	
Glucagon	F- TTCGCAGCTCAGGCTCACA	64
	R- GCTGCCTTGCACCAGCATT	
Resistin	F- CATCGATGAAGCCATCGACAA	64
	R- GGAGCAGCTCAAGACTGCTGTG	
Leptin	F- ACACACGCAGTCGGTATCC	64
	R- GAGTAGAGTGAGGCTTCCAGG	
ApoB	F- GGCACTGTGGGTCTGGAT	55
	R- TTCTTCTCTGGAGGGGACTG	
ApoE	F- CCTGGAGGCTAAGGACTTGTTTC	64
	R- GCCCACAGAGCCTTCATCTTC3	
HMG-CoA reductase	F- TGTGGTTTGTGAAGCCGTCAT	60
	R- CGTCAACCATAGCTTCCGTAGTT	
SREBP-1C	F- CCGAGATGTGCGAACTGGA	64
	R- GAAGTCACTGTCTTGGTTGTTGATG	
Beta actin	F- CATCCGTAAAGACCTCTATGCCAAC	58
	R- ATGGAGCCACCGATCCACA	