Original article

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

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A B S T R A C T

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+/-) 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+/- 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.

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1. 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 [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 [5–7]. 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 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.

2. Materials and methods

2.1. Animals

Liver-specific Gck<sup>−/−</sup> 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 8:00 AM to 14:00 PM on the day of sacrifice to generate a fasted state and sacrificed at 14:00 PM. To obtain mice with a fed status, mice were first fasted for 8 hours and then injected with a 20% glucose solution at a dose of 2 g/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.

2.2. 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).

### Table 1

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer</th>
<th>Tm (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucokinase (GCK)</td>
<td>F- CAACCTGACCAAAGCCCTTCAA</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>R- TGGGCCCCCCTGCTCATTC</td>
<td></td>
</tr>
<tr>
<td>Hexokinase 1 (Hk1)</td>
<td>F- ATGATTGCGCCGAACACTAC</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>R- AGACGCCCAGGTCATACAGA</td>
<td></td>
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<tr>
<td>Hexokinase (Hk2)</td>
<td>F- CCGTGCAACTGACAACTTCA</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>R- CGTACATTTCGGGACACCAGA</td>
<td></td>
</tr>
<tr>
<td>GluT2</td>
<td>F- ATCAACATTGCTACCGCTTTGTC</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>R- TGGCCAGTACGCTCACTGTAACCTC</td>
<td></td>
</tr>
<tr>
<td>GluT4</td>
<td>F- CTGTAACCTTACCTGCGGAGGGC</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>R- AGGCGACGTCAGACTTGGTACAA</td>
<td></td>
</tr>
<tr>
<td>Insulin receptor</td>
<td>F- CAGCTGAAAATGGTGGTTGG</td>
<td>64</td>
</tr>
<tr>
<td>(IR)</td>
<td>R- GGTGATCACCACCTCACAGGAA</td>
<td></td>
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<tr>
<td>Insulin</td>
<td>F- TGGCTTCTTACTACACCCCAAGG</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>R- ACAATGCGACGGTTTCTTGC</td>
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<tr>
<td>Glucagon</td>
<td>F- TTGGACGCTAGCGCTTCA</td>
<td>64</td>
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<tr>
<td></td>
<td>R- GCTGGCCTTGAGCACCAGTA</td>
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<tr>
<td>Resistin</td>
<td>F- CATGACAGCCGTTAGCGCAAA</td>
<td>64</td>
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<tr>
<td></td>
<td>R- GGACGAGCTCCAAAGTGAGTGG</td>
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</tr>
<tr>
<td>Leptin</td>
<td>F- ACAACACCAAGTCCGGATTCC</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>R- GAGTAACTGAGGCTTACCGG</td>
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</tr>
<tr>
<td>ApoB</td>
<td>F- GGCACTGTGGCCGCTSGAT</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>R- TTTTCTTCTGGAGGGACCT</td>
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<tr>
<td>ApoE</td>
<td>F- CTTGACGAGCAGAACGTGTTGC</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>R- GCCCCACAGCCTTATACCC</td>
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</tr>
<tr>
<td>HMG-CoA reductase</td>
<td>F- TGCACTGTGGGACGCTAT</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>R- CGTCAACGATCTCTCGGTC</td>
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<tr>
<td>SREBP-1 C</td>
<td>F- CCAGACATTCGCGGAATGG</td>
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<tr>
<td></td>
<td>R- GAAATCATCTTCTTGTTGATG</td>
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<tr>
<td>Beta-actin</td>
<td>F- CATCGTAAACACTCTTACTGGCACAC</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>R- ATGGGACCCAAGCGATCACA</td>
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</tr>
</tbody>
</table>

China, F03PJA). Cholesterol, triglyceride, HDL-cholesterol and LDL-cholesterol (enzyme methods) were determined using a commercial kit (BIOSINO, China) and an automatic analyzer (HITACHI7080).

2.3. Hexokinase 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).

2.4. Isolation of total RNA and quantitative RT-PCR

Total RNA was isolated from ~50 mg of frozen liver, muscle and pancreatic tissue and ~150 mg of frozen adipose tissue using Trizol Reagent (Invitrogen) according to the manufacturer’s instructions. cDNA was synthesized from 2 μg 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 systemCFB-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.

2.5. Western blot

About 50 mg of liver or muscle tissues was homogenized in 500 μL of lysis buffer containing 50 mmol/L Tri-HCl (pH 8.0), 150 mmol/L NaCl, 0.02% NaN3, 0.1% SDS, 1 mmol/L EDTA, 100 mg/L PMSF, 1 mg/L leupeptin and 1% NP-40. The mixture incubated at
4°C for 30 min, 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 (pH 6.8), 4% SDS, 0.2% bromophenol blue, 20% glycerol and 200 mmol/LDTT 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 (2 h 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).

### 2.6. Statistical analysis

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

### 3. Results

#### 3.1. 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<sup>−/−</sup>) and wild-type mice (Gck<sup>+/+</sup>) 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<sup>−/−</sup> mice are significantly higher than those in age-matched Gck<sup>+/+</sup> mice. The change in blood glucose levels did not affect body weight as the weights of
Gck\textsuperscript{w/w} mice and age-matched Gck\textsuperscript{w/w} mice were not significantly different (Fig. 1B).

In Fig. 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\textsuperscript{w/w} and Gck\textsuperscript{w/w} mice at any ages (Fig. 2A), but serum glucagon levels of the Gck\textsuperscript{w/w} mice was higher than seen in the Gck\textsuperscript{w/w} mice at 8 weeks (Fig. 2B). Cholesterol levels of the Gck\textsuperscript{w/w} mice were higher than those of Gck\textsuperscript{w/w} mice at 8 weeks (Fig. 2C), which is due to the LDL-C rather than the HDL-C component (Fig. 2D and E). The triglyceride levels of the Gck\textsuperscript{w/w} mice were similar to those of the Gck\textsuperscript{w/w} mice at all ages (Fig. 2F).

3.2. Changes in the mRNA levels of genes expression in the liver of liver-specific Gck\textsuperscript{w/w} knockout mice at a young age

Since blood glucose levels in the liver-specific Gck\textsuperscript{w/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

Fig. 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\textsuperscript{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), HkI (C), HkII (D), Glut2 (E), Glut4 (F), IR (G), ApoB (H), ApoE (I), HMG-CoA (J) and SREBP-1c (K) in the liver of liver Gck\textsuperscript{w/w} mice and Gck\textsuperscript{w/w} mice in fasted state at different ages. n = 5–8. Means ± SE. Asterisk indicates significant differences in liver Gck\textsuperscript{w/w} mice vs age-matched Gck\textsuperscript{w/w} mice. *P < 0.05.
Fig. 4. Western blot analysis of glucokinase and insulin receptor levels in the liver of liver Gck<sup>−/−</sup> mice and Gck<sup>+/−</sup> mice in fasted state at different ages.

Principal glucose transporter, and insulin being an important hormonal regulator of carbohydrate and lipid metabolism. Heterozygous liver-specific Gck<sup>+/−</sup> mice expressed levels of Gck that were lower than age-matched Gck<sup>+/+</sup> 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 HkiI and HkiII transcripts did not show an increase at any age (Fig. 3C and D). Glut2 levels were lower in the Gck<sup>−/−</sup> mice, but only at 8 weeks of age did this decrease reach statistically 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<sup>+/−</sup> mice (Fig. 3C), and was also reflected in Western blots (Fig. 4).

Total cholesterol and LDL-C of Gck<sup>−/−</sup> 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 Fig. 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<sup>−/−</sup> 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).

3.3. Increased glucagon secretion by the pancreas of Gck<sup>−/−</sup> 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 Fig. 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 insulin and glucagon genes 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<sup>−/−</sup> mice are similar to the wild-type mice (Figs. 2A and 5E). However, Gck<sup>−/−</sup> 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<sup>−/−</sup> mice did not change with age, nor were they different from those in wild-type mice (Fig. 5C and D).

Fig. 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<sup>−/−</sup> 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<sup>−/−</sup> mice and Gck<sup>+/−</sup> mice in fasted state at different ages. n = 5–8. Means ± SE. Asterisk indicates significant differences in liver Gck<sup>−/−</sup> mice vs age-matched Gck<sup>++</sup> mice. *P < 0.05.
3.4. Early change in the muscle of liver-specific Gck knockout 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 isofrom in muscle tissue that participates in glucose metabolism.

No change in gene expression was detected for any gene until at age of 8 weeks (Fig. 6). At 8 weeks the levels of HKII mRNA in Gck<sup>−−</sup> 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<sup>−−</sup> 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 (Fig. 6D and E).

3.5. 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<sup>−−</sup> 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...
Fig. 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<sup>wt</sup>/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<sup>wt</sup> mice and Gck<sup>ko</sup> mice in fasted state at different ages. n = 5–8. Means ± S.E. Asterisk indicates significant differences in liver Gck<sup>ko</sup> mice vs age-matched Gck<sup>wt</sup> mice. *P < 0.05.

Fig. 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, Hki, HKII, Glut2 and IR in the liver of liver Gck<sup>wt</sup> mice and Gck<sup>ko</sup> 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<sup>wt</sup> mice and Gck<sup>ko</sup> mice in glucose-stimulated state at 8 weeks old. n = 5–8. Means ± S.E. Asterisk indicates significant differences in liver Gck<sup>ko</sup> mice vs age-matched Gck<sup>wt</sup> mice. *P < 0.05.
also added two key hormones that are secreted by adipose cells, leptin and resistin, in Gck\textsuperscript{−/−} and wild-type mice at 6 and 8 weeks of age. Fig. 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 for the insulin receptor. Both Hkl and HkII are expressed in adipose tissue, with HkII expressed at a level about three times greater than Hkl.

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

3.6. No change in gene expression profiles was seen in the liver and pancreas of Gck\textsuperscript{−/−} mice compared with 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 Figs. 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\textsuperscript{−/−} 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\textsuperscript{−/−} 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).

Figs. 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\textsuperscript{−/−} mice was similar to that of wild-type mice at young ages. Although the mRNA levels for glucagon in the Gck\textsuperscript{−/−} mice were decreased in the fasted state, no difference was seen between Gck\textsuperscript{−/−} and wild-type mice in the fed state (Figs. 9B and 10B).

4. 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\textsuperscript{−/−} mice, insulin levels stay at normal level in both the fasted and fed states (Figs. 2A, 5E, 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% to 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\textsuperscript{−/−} 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\textsuperscript{−/−} 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\textsuperscript{−/−} 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 transcriptional 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<sup>−/−</sup> mouse model, Glut2 expression in pancreatic beta cells was not changed 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<sup>−/−</sup> 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. 3).

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 occurring 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<sup>−/−</sup> 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 (Figs. 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<sup>−/−</sup> mice, which makes hyperglycemia more severe. It has been reported that SREBP-1 C 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-1 C levels in the muscle of liver-specific GCK deficient mice (Fig. 5I).

In summary, our findings suggest that in liver-specific Gck<sup>−/−</sup> mice, different peripheral tissues tackle hyperglycemia by different responses 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.

**Disclosure of interest**

The authors declare that they have no conflicts of interest concerning this article.

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**Appendix A. Supplementary data**


**References**


Matschinsky FM, Glucokinase as glucose sensor and metabolic signal genera


