Brief Communication

MOLECULAR METABOLISM

Hepatic GRK2 is dispensable for glucose homeostasis and other key metabolic parameters in mice

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ABSTRACT

Objective: G-protein-coupled receptor (GPCR) kinases (GRKs) abrogate GPCR signaling by promoting receptor desensitization and internalization. Accumulating evidence suggests that GRK2 represents an important regulator of GPCR-mediated effects on systemic glucose metabolism, obesity, and insulin resistance. Despite the key role of the liver in maintaining euglycemia, the potential metabolic relevance of hepatic GRK2 has yet to be examined. Thus, the goal of this study was to explore the potential role of hepatic GRK2 in maintaining glucose homeostasis and other key metabolic functions.

Methods: To address this question, we generated mice that showed a ~90% reduction in GRK2 protein expression selectively in hepatocytes (Hep-GRK2-KO mice) and subjected these mice, together with their control littermates, to systematic metabolic phenotyping studies.

Results: We found that Hep-GRK2-KO mice maintained on regular chow did not differ significantly from their control littermates in glycemia, glucose tolerance, insulin sensitivity, in vivo gluconeogenesis, and glucagon-induced hyperglycemia. We obtained similar findings when we analyzed Hep-GRK2-KO mice and control littermates consuming an obesogenic high-fat diet. Likewise, plasma levels of insulin, glucagon, free fatty acids, and ketone bodies remained unaffected by the lack of hepatocyte GRK2. The same was true when we examined the expression levels of key genes regulating hepatic glucose and fatty acid metabolism.

Conclusion: In summary, our data suggest that hepatocyte GRK2 is dispensable for systemic glucose homeostasis and other key metabolic functions in both lean and obese mice. This finding suggests that drug development efforts aimed at inhibiting GRK2 to improve impaired glucose homeostasis and insulin sensitivity need to focus on other metabolically important tissues.

Keywords G protein-coupled receptor; Liver; Glucose tolerance; Knockout mice; Metabolism

1. INTRODUCTION

G protein-coupled receptors (GPCRs) are cell surface receptors that regulate a vast array of physiological functions, including glucose homeostasis and many other important metabolic parameters [1]. GPCRs represent excellent therapeutic targets, as indicated by the fact that about one-third of all FDA-approved drugs act on one more GPCR subtypes [2]. The activity of most GPCRs is regulated by GPCR-associated proteins that play important roles in affecting the strength and duration of the physiological outcome [3,4].

GPCR kinases (GRKs) are GPCR-associated intracellular proteins that ensure that agonist-induced GPCR signaling does not continue unabated [4–6]. GRKs are able to bind to activated GPCRs and phosphorylate specific serine and threonine residues located within specific intracellular receptor domains [4–6]. Subsequently, the GRK-phosphorylated receptors are recognized by a group of cytosolic proteins known as arrestins (arrestin-1 to -4), which terminate GPCR interactions with G proteins via steric hindrance [4–7]. The receptor-associated arrestins also promote GPCR internalization via clathrin-coated pits [4–7]. While the metabolic functions of arrestins, in particular β-arrestin-1 and -2 (arrestin-2 and -3, respectively), have been studied in considerable detail [8,9], only a few studies have explored the metabolic relevance of GRKs under physiological and pathophysiological conditions, except for a vast body of research focusing on the important role of GRK2 in the cardiovascular system [10].

The GRK family consists of seven members (GRK1-7). GRK1 and GRK7 are exclusively expressed in the eye in rod and cone cells of the retina, respectively [4–6]. GRK4, 5, and 6 belong to the GRK4 subfamily of GRKs and, except for GRK4 which shows a very limited expression pattern, are expressed ubiquitously [4–6].

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The GRK2 family consists of GRK2 and GRK3 (alternative name: β-adrenergic kinases), which are also expressed in virtually all cell types [4–6]. Since GRK2 was the first non-visual GRK to be cloned more than three decades ago [11], it has been the focus of many molecular, physiological, and structural studies [4–6,12,13]. Interestingly, besides its canonical role in the desensitization of numerous GPCRs, GRK2 affects many other cellular pathways by phosphorylating non-GPCR substrates or by directly binding to various signaling proteins [14–16].

While whole-body GRK2 knockout (KO) mice die during embryogenesis [17], studies with heterozygous GRK2 mutant mice and other experimental approaches suggest that GRK2 plays a role in the development of various metabolic disorders including impaired glucose homeostasis and insulin resistance [12,18]. So far, it remains unclear which specific tissues or cell types mediate the metabolic effects of GRK2 on glucose homeostasis and related metabolic functions. However, such knowledge may prove useful for developing novel drugs or strategies to target GRK2 for therapeutic purposes.

The liver is the hub of glucose and lipid metabolism and expresses many GPCRs known to regulate glucose homeostasis, including the glucagon receptor [19,20]. Previous studies suggest that GRK2 plays a role in modulating several important functions of the liver. For example, in vitro studies demonstrated that GRK2 inhibits basal and insulin-stimulated glycogen synthesis [21]. In vivo studies showed that reduction of GRK2 expression throughout the body prevented hepatic steatosis in mice [22]. Moreover, mutant mice with partial GRK2 deficiency are resistant to the development of nonalcoholic steatohepatitis (NASH) [23].

The present study was designed to explore the potential metabolic role of hepatic GRK2 by selectively reducing the expression of GRK2 in mouse hepatocytes in vivo in an inducible fashion. Surprisingly, systematic metabolic phenotyping studies demonstrated that hepatocyte GRK2 is dispensable for glucose homeostasis and other important metabolic processes in lean and obese mice.

2. METHODS

2.1. Generation of Hep-GRK2-KO mice and high-fat diet feeding

The generation of floxed *Grk2* mice has been described previously [17]. For our studies, we used $Grk2^{fl/fl}$ mice that had been maintained on a C57BL/6 genetic background. Eight-week-old $Grk2^{fl/fl}$ mice were injected, via the tail vein, with an adeno-associated virus (AAV) coding for either Cre-recombinase (AAV8.TBG.PI.Cre.rBG; abbreviated name: AAV-TBG-CRE) (Addgene 107787-AAV8) [24] or eGFP (pAAV.TBG.PI.eGFP.WPRE.bGH; abbreviated name: AAV-TBG-eGFP) (Addgene, 105535-AAV8) at 2×10^{11} genomic copies per mouse [24] to generate hepatocyte-specific GRK2 KO mice (Hep-GRK2-KO mice) and control littermates, respectively. Another set of $Grk2^{fl/fl}$ mice was injected with PBS to generate additional control mice. Mouse phenotyping studies were initiated three weeks after virus administration. Unless stated otherwise, all experiments were conducted in group-housed mice, maintained on a 12-h light, 12-h dark cycle (lights off at 6 pm).

One cohort of mice was maintained on a regular chow diet (3.4 kcal/g, 24.7% kcal from protein, 62.1% carbohydrate, and 13.2% fat; Purina 5053). The second cohort of mice consumed a high-fat diet (HFD) (60% kcal fat; D12492, Research Diets Inc.) after reaching 8 weeks of age. Body composition (lean versus fat mass) was determined using EchoMRI (EchoMRI100H; EchoMRI LLC).

All animal experiments were conducted according to the US National Institutes of Health Guidelines for Animal Research and were approved by the NIDDK Institutional Animal Care and Use Committee.

2.2. Physiological studies

Metabolic tests were performed with Hep-GRK2-KO mice and control littermates (age range: 10–24 weeks). Intraperitoneal (i.p.) glucose tolerance tests (ipGTT) were conducted in mice fasted for 6 h. Mice consuming regular chow received a glucose dose of 2 g/kg body weight, while HFD mice were injected with 1 g glucose/kg body weight, respectively. Blood glucose levels were measured in tail vein blood using the Contour Next glucometer (Bayer) immediately prior to injection of the glucose bolus and at defined post-injection time points. Insulin tolerance tests (ITT) and glucagon challenge tests (GCT) were performed after a 4 h fast. For ITT, chow-fed lean mice or HFD-fed obese mice were injected i.p. with 0.75 U/kg or 1.5 U/kg of human insulin (HumulinR, Eli Lilly), respectively. For GCT, mice received an i.p. injection of glucagon (16 μg/kg). During ITT and GCT, blood glucose levels were measured at defined post-injection time points. For pyruvate tolerance tests (PTT), mice were fasted overnight for ∼12 h and then injected i.p. with sodium pyruvate (2 g/kg), followed by monitoring of blood glucose levels.

To study epinephrine-induced increases in blood glucose levels, mice were fasted for 4 h, followed by an initial i.p. injection of 0.5 mg/kg of epinephrine bitartrate (Sigma #PHR1509) [25]. Two hours later, the mice received a second dose of epinephrine (0.5 mg/kg i.p.), followed by the monitoring of blood glucose levels.

Blood ketone body levels in freely fed and 24-h-fasted mice were measured with a blood ketone monitoring kit (KetoBM). Blood samples were collected from the tail vein into EDTA-coated tubes and centrifuged for plasma collection (3,000 g, 5 min, 4 °C). Plasma insulin and glucagon levels were measured via ELISA (Crystal Chem #90080 and #81518, respectively). Non-esterified fatty acids (NEFA) were measured via a colorimetric assay, according to manufacturer's protocol (Wako Diagnostics). After a 6 h fast, plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST) (Sigma MAK052 and MAK055, respectively), and leptin (Crystal Chem #90030) levels were measured using commercially available kits.

Daily food consumption was measured using single-housed male mice, by directly weighing food pellets. Food intake during the first 2 days (acclimatization period) was excluded from the analysis.

2.3. Histological staining

Hematoxylin & Eosin (H&E) and Sirius Red staining were performed on 5 μm liver slices on Superfrost glass slides (Fisher Scientific). Initially, liver tissues were fixed in 4% paraformaldehyde overnight and then processed using ethanol and xylene, followed by embedding into paraffin blocks. For H&E staining, liver slices were stained for 10 min in Mayer hematoxylin solution and for 10 s in eosin Y solution, respectively. For Sirius Red staining, liver slices were incubated in the following solutions: 1) phosphomolybdic acid for 2 min; 2) picrosirius red F3BA staining solution for 60 min; and 3) 0.1 N HCl for 2 min. The stained slides were rinsed in 75% ethanol, dehydrated, and air-dried. Representative images were acquired with a light microscope.

2.4. Isolation of primary mouse hepatocytes, RNA isolation, and quantitative RT-PCR

Primary mouse hepatocytes were isolated from the livers of mice at least 8 weeks old using a two-step collagenase perfusion protocol, as

previously described [24]. Mice were fasted for 6 h prior to tissue or primary hepatocyte isolation. qRT-PCR was used to confirm the lack of *Grk2* expression in mouse primary hepatocytes or liver tissue from Hep-GRK2-KO mice. Total RNA was isolated from mouse tissues using TRIzol reagent (Life Technologies). Isolated RNA (1 μg) was used to synthesize cDNA by reverse transcription using a high-capacity cDNA reverse transcription kit (Vazyme, #R333-01). Gene expression analysis was performed by quantitative PCR with Taq Pro Universal SYBR qPCR Master Mix (Vazyme, Q712-02). Gene expression data were normalized to mRNA levels of *36b4*, a housekeeping gene. Primer sequences are listed in Supplementary Table 1.

2.5. Western blotting

Total proteins were isolated from whole liver and isolated hepatocytes using RIPA lysis buffer (Sigma), containing proteinase inhibitors (Roche). Proteins (20 μg) were separated via SDS-PAGE and blotted onto a nitrocellulose membrane, followed by probing with a rabbit polyclonal GRK2 primary antibody (Sigma #G0296). A rabbit monoclonal antibody for β-actin (Cell Signaling #4970) served as loading control. Goat anti-rabbit IgG (Cell signaling #7054) was used as the secondary antibody. Protein quantification was performed using ImageJ.

2.6. Statistics

Data are expressed as mean ± SEM for the indicated number of observations. Data were assessed for statistical significance by 2-way-ANOVA tests, followed by the indicated post-hoc tests, or by using a two-tailed unpaired Student's t-test, as appropriate. A p-value of less than 0.05 was considered statistically significant. The specific statistical tests that were used are indicated in the figure legends.

3. RESULTS

3.1. Lack of GRK2 in hepatocytes does not affect glucose homeostasis and other important metabolic parameters in mice consuming regular chow

To generate mice selectively lacking GRK2 in hepatocytes (Hep-GRK2−KO mice), we injected floxed *Grk2*$^{fl/fl}$ mice [17] into the tail vein with an AAV coding for Cre recombinase under the control of the hepatocyte-specific thyroxine-binding globulin (TBG) promoter (AAV-TBG-Cre) [24]. *Grk2*$^{fl/fl}$ mice injected with the AAV-TBG-eGFP virus [24] served as control littermates throughout all experiments (Figure 1A). Two weeks after AAV injections, *Grk2*$^{fl/fl}$ mice that had been treated with the Cre-encoding AAV showed a pronounced reduction (by ~80%) in *Grk2* expression in the liver but not in other metabolically important tissues (Figure 1B). In contrast, the hepatic mRNA levels of *Grk3, 4, 5,* and *6* were not significantly affected by the treatment of *Grk2*$^{fl/fl}$ mice with AAV-TBG-Cre (Figure 1C). Very similar results were obtained with primary hepatocytes obtained from *Grk2*$^{fl/fl}$ mice injected with the two different AAVs. Primary hepatocytes from mice treated with AAV-TBG-Cre showed a ~90% reduction of *Grk2* expression, as compared to control hepatocytes (Figure 1D). As observed with whole liver RNA, *Grk3, 4, 5,* and *6* expression levels determined in primary hepatocytes were not significantly affected by AAV-TBG-Cre treatment

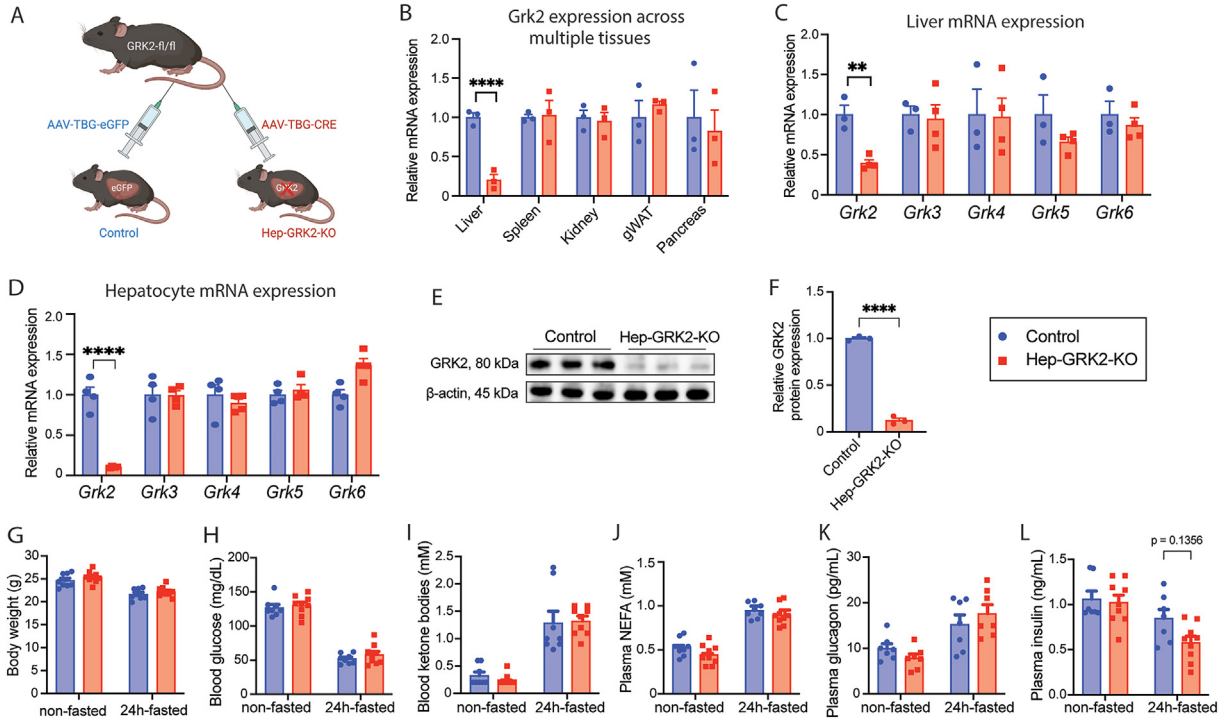

Figure 1: The lack of hepatocyte *Grk2* expression does not affect the blood or plasma levels of key metabolites and hormones under both fed and fasting conditions. (A) Scheme illustrating the generation of Hep-GRK2-KO mice. (B) Pronounced reduction of *Grk2 expression* in the liver after treatment of *GRK2*$^{fl/fl}$ mice with the AAV-TBG-CRE virus. gWAT, gonadal white adipose tissue. (C, D) Relative *Grk2-6* expression levels in liver (C) and primary hepatocytes (D) of Hep-GRK2-KO mice and control littermates. (E, F) Western blot showing a very pronounced reduction in GRK2 protein expression in primary hepatocytes from Hep-GRK2-KO mice. (G) Body weights of fed and fasted (for 24 h) Hep-GRK2-KO and control mice. (H–L) Inactivation of hepatocyte *Grk2* does not affect fed and fasting levels of blood glucose (H), blood ketone bodies (I), plasma non-esterified fatty acids (NEFA) (J), plasma glucagon (K), and plasma insulin (L). Experiments were carried out with male mice aged 10–18 weeks. Data are shown as means ± SEM. n = 3 or 4 mice per group for Figure 1B–F (**p < 0.001, ****p < 0.00001; Student's t-test). n = 7 or 8 mice per group for Figure 1G–L.

of *Grk2*$^{fl/fl}$ mice (Figure 1D). In agreement with the gene expression data, western blotting studies showed that the expression of GRK2 protein in primary hepatocytes was strongly reduced (by ~90%) in *Grk2*$^{fl/fl}$ mice injected with the Cre-encoding AAV (Figure 1E, F). To rule out the possibility that hepatic GRK2 expression was affected by the virus treatment, we injected *Grk2*$^{fl/fl}$ mice with either PBS, AAV-TBG-GFP, or AAV-TBG-Cre. As expected, only mice treated with the Cre-encoding AAV showed a significant reduction in hepatic GRK2 mRNA and protein expression levels (Supplementary Figure 1A–C). These findings clearly indicate that AAV-TBG-Cre treatment of *Grk2*$^{fl/fl}$ mice led to a very pronounced and selective reduction of GRK2 expression in mouse hepatocytes. For the sake of simplicity, we refer to these mutant mice as Hep-GRK2-KO mice throughout the text (note that these mice still retain residual GRK2 expression in hepatocytes).

Initially, we carried out a series of metabolic studies with Hep-GRK2-KO mice and control littermates maintained on a regular chow

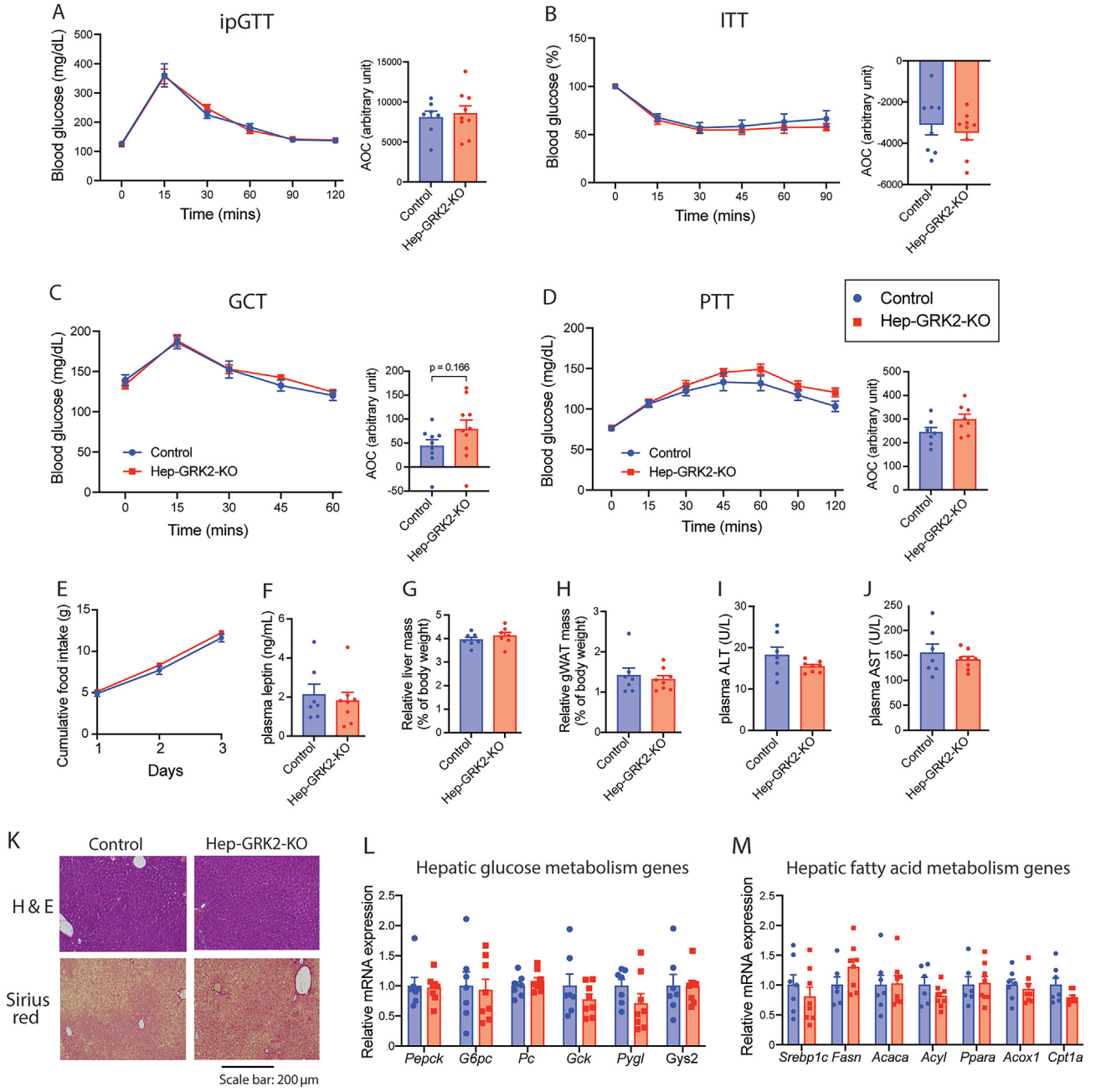

Figure 2: Lack of hepatocyte *Grk2* expression has no effect on key metabolic functions and markers of liver damage in mice maintained on regular chow. (A–D) I.p. glucose tolerance (A), insulin tolerance (B), glucagon challenge (C), and pyruvate tolerance (D) tests. AOC (area of the curve) values are shown to the right in each panel. (E, F) Cumulative food intake measured daily for 3 days (E) and plasma leptin levels (F). (G, H) Relative liver mass (G) and relative mass of gWAT (gonadal white adipose tissue) (H) of Hep-GRK2-KO and control littermates. (I, J) Plasma levels of ALT (I) and AST (J), two markers of liver damage. (K) Representative images of liver sections stained with H&E or Sirius Red. No changes in overall morphology and collagen deposition were observed between the two groups of mice. (L, M) Hepatic expression of key genes regulating glucose (L) and fatty acid (M) metabolism in control and Hep-GRK2-KO mice. Data in panels F–M are from mice sacrificed after a 6-h fast. Experiments were carried out with 10–18-week old mice. Data are shown as means ± SEM, n = 7 or 8 mice per group.

diet. Body weight did not differ between the two groups of mice under fed conditions or after a 24 h fast (Figure 1G). As shown in Figure 1H–L, the two groups of mice showed similar levels of blood glucose, blood ketone bodies (a marker for elevated fat metabolism), plasma non-esterified fatty acids (NEFA), plasma glucagon, and plasma insulin under both fed and fasting conditions (24 h fast). Fasting plasma insulin levels trended to be decreased in Hep-GRK2-KO mice, but this effect failed to reach statistical significance (Figure 1L).

To explore the potential effects of hepatocyte GRK2 deficiency on systemic glucose homeostasis in more detail, we next performed glucose tolerance (Figure 2A), insulin tolerance (Figure 2B), glucagon challenge (Figure 2C), and pyruvate tolerance (Figure 2D) tests. We found that Hep-GRK2-KO mice and control littermates showed similar glucose tolerance and insulin sensitivity (Figure 2A, B). In the glucagon tolerance test, blood glucose levels tended to be elevated in Hep-GRK2-KO mice, but this effect failed to reach statistical significance (Figure 2C). Finally, in vivo hepatic gluconeogenesis (determined in pyruvate tolerance tests) did not differ significantly between the two groups of mice (Figure 2D).

We also subjected Hep-GRK2-KO mice and control littermates to successive injections of epinephrine (0.5 mg/kg i.p.), an adrenergic receptor agonist, to monitor the effect of hepatic GKR2 deficiency on adrenergic receptor desensitization in vivo. It is well established that epinephrine administration causes hyperglycemic responses in experimental animals via stimulation of adrenergic receptors expressed by hepatocytes [25,26]. Following the first dose of epinephrine, both groups of mice showed pronounced increases in blood glucose levels (Supplementary Figure 1D). Two hours later, when blood glucose levels had returned to baseline, the mice received a second dose of epinephrine. This second dose resulted in significantly reduced hyperglycemic responses, as compared to those observed after the first injection of epinephrine (Supplementary Figure 1D–F), indicative of adrenergic receptor desensitization. Importantly, the magnitude of this diminished effect was similar in Hep-GRK2-KO mice and control littermates (Supplementary Figure 1F), suggesting that hepatic GRK2 does not play a role in the desensitization of epinephrine-induced hyperglycemia.

Similar to male mice, female Hep-GRK2-KO mice and control littermates showed no significant differences in body weight, glycemia, glucose tolerance, and glucagon-induced hyperglycemia (Supplementary Figure 1G–J).

We also found that food consumption (measured over 3 days) did not differ between Hep-GRK2-KO mice and control littermates (Figure 2E). Similarly, plasma leptin levels were similar between the two groups of mice (Figure 2F). Hep-GRK2-KO mice and control littermates also did not differ in liver mass (Figure 2G) or the mass of gonadal white adipose tissue (gWAT) (Figure 2H). Moreover, plasma levels of ALT and AST (Figure 2I, J) did not differ between the two groups of mice, indicating that the lack of hepatic GRK2 does not result in liver damage. H&E and Sirius Red staining studies showed that livers from Hep-GRK2-KO mice were histologically normal, without signs of steatosis or collagen deposition, a marker for liver fibrosis (Figure 2K).

We next examined the hepatic expression levels of key genes involved in regulating glucose and fatty acid metabolism in control and Hep-GRK2-KO mice. In agreement with the lack of any metabolic phenotypes displayed by the GRK2 mutant mice, hepatocyte GRK2 deficiency had no significant effect on the expression levels of any of the genes examined in this study (Figure 2L, M).

3.2. Hepatic GRK2 deficiency has no effect on glucose homeostasis and other important metabolic parameters in obese mice

We next investigated whether Hep-GRK2-KO mice showed any phenotypes under metabolically challenging conditions. We initiated metabolic phenotyping studies after maintaining Hep-GRK2-KO mice and control littermates on a high-fat diet (HFD) for 8 weeks. Mice were kept on the HFD for a total duration of 12 weeks. Male mice (genetic background: C57BL/6) consuming a HFD for at least 8 weeks are known to exhibit rapid body weight gain and adiposity, associated with severe metabolic deficits, including hyperglycemia, glucose intolerance, and insulin resistance [27]. Interestingly, under the same experimental conditions, female C57BL/6 mice do not develop glucose intolerance, insulin resistance, and related metabolic deficits [28,29]. During HFD feeding, body weight gain was very similar in Hep-GRK2-KO mice and control littermates (Figure 3A). Likewise, we observed no

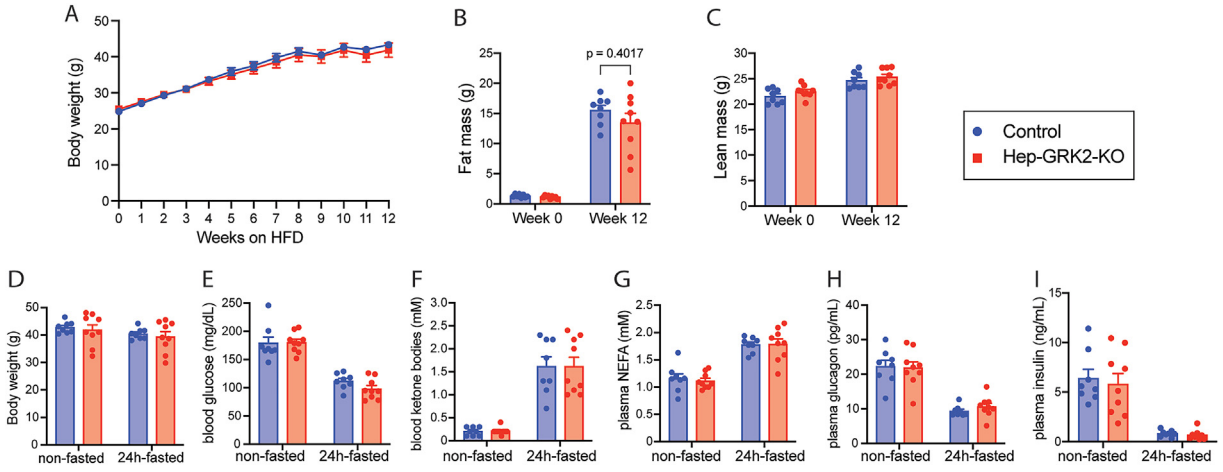

Figure 3: Hep-GRK2-KO mice and control littermates maintained on an obesogenic high-fat diet (HFD) show similar weight gain, body composition, and other metabolic parameters. (A) Weekly body weight on HFD. (B, C) Fat mass (B) and lean mass (C) before and after 12 weeks on HFD. (D) Body weight under fed and fasting conditions. (E–I) Hepatocyte GRK2 deficiency does not affect fed and fasting levels of blood glucose (E), blood ketone bodies (F), plasma non-esterified fatty acids (NEFA) (G), plasma glucagon (H), and plasma insulin (I). Mice were put on the HFD when they were ~11 weeks old. All experiments were performed after 8–12 weeks of HFD feeding. Data are shown as means ± SEM (n = 8 or 9 mice per group).

significant differences in lean or fat mass between the two mouse strains under these conditions (Figure 3B, C). A 24-h fast caused no significant changes in body weight (Figure 3D), but led to significantly reduced blood glucose (Figure 3E), plasma glucagon (Figure 3H), and plasma insulin (Figure 3I) levels. On the other hand, the 24-h fast resulted in significant increases in blood ketone bodies (Figure 3F) and plasma NEFAs (Figure 3G). Importantly, these fasting-induced changes in blood/plasma glucose, metabolite, and hormone levels did not differ significantly between obese Hep-GRK2-KO mice and obese control littermates.

Similarly, the lack of hepatocyte GRK2 in obese mice had no significant effect on the outcome of glucose tolerance (Figure 4A), insulin

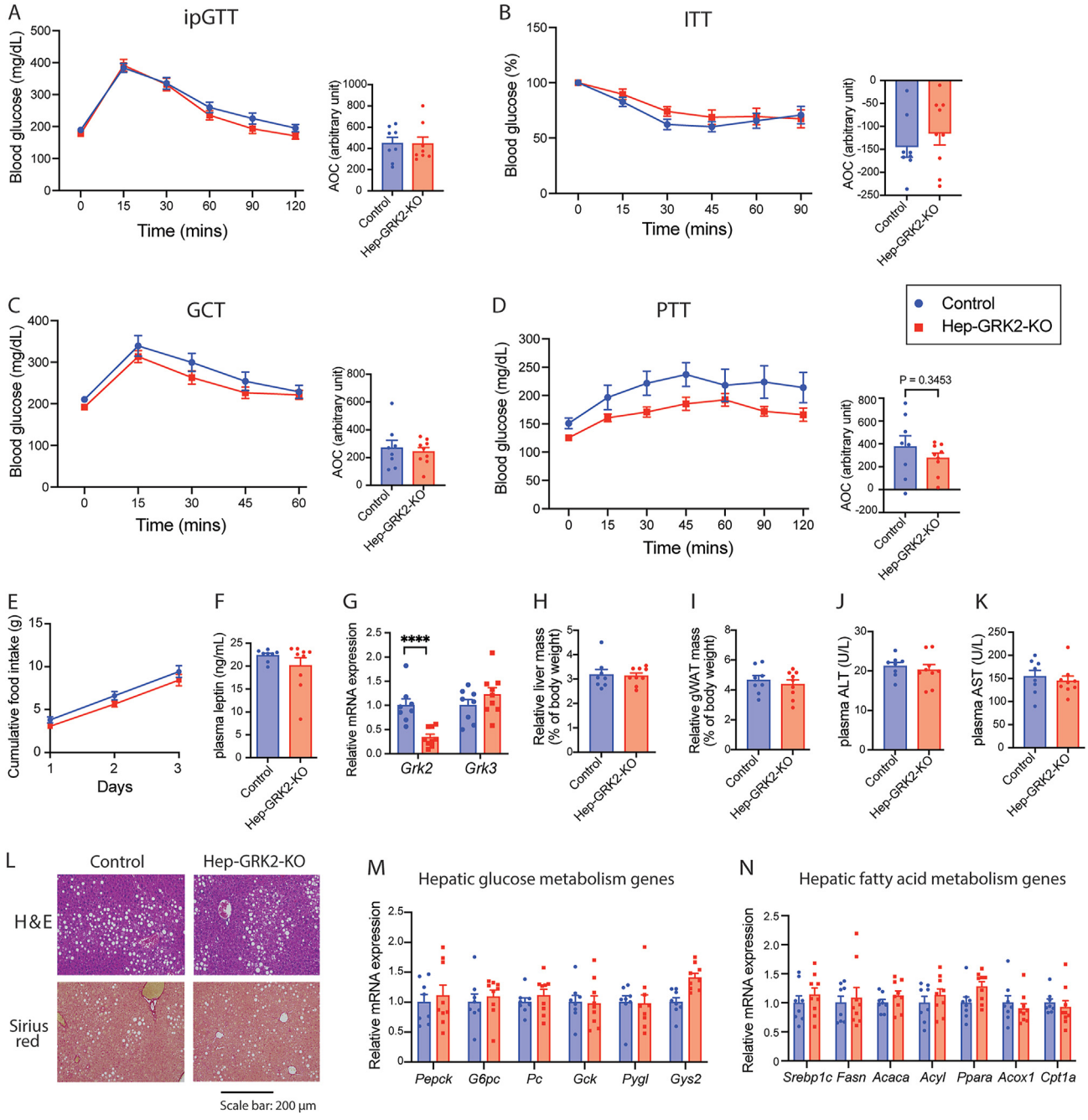

Figure 4: Hepatocyte GRK2 deficiency does not affect the outcome of key metabolic assays or metabolic parameters in obese mice. Mice were maintained on a HFD for at least 8 weeks. (A—D) I.p. glucose tolerance (A), insulin tolerance (B), glucagon challenge (C), and pyruvate tolerance (D) tests. AOC (area of the curve) values are shown to the right in each panel. (E, F) Cumulative food intake measured daily for 3 days (E) and plasma leptin levels (F). (G) Hepatocyte GRK2 deficiency does not affect *Grk3* expression under obesogenic conditions. (H, I) Relative liver mass (G) and relative mass of gWAT (gonadal white adipose tissue) (I) of Hep-GRK2-KO and control littermates. (J, K) Plasma levels of ALT (J) and AST (K), two markers of liver damage. (L) Representative images of liver sections stained with H&E or Sirius Red. No changes in overall morphology and collagen deposition were observed between the two groups of mice. (M, N) Hepatic expression of key genes regulating glucose (M) and fatty acid (N) metabolism in control and Hep-GRK2-KO mice. Data in panels F—M are from mice sacrificed after a 6-h fast. Experiments were performed with 10—18-week old mice. Data are shown as means ± SEM (n = 8 or 9 mice per group; ****p < 0.00001, Student's t-test).

tolerance (Figure 4B), glucagon challenge (Figure 4C), and pyruvate tolerance (Figure 4D) tests. Additionally, food intake (Figure 4E) and plasma leptin levels (Figure 4F) did not differ between the two groups of obese mice. Gene expression studies with liver RNA obtained from obese mice confirmed that hepatocyte *Grk2* deficiency (~60% reduction in liver) did not affect the expression levels of *Grk3*, the second member of the *Grk2* gene family (Figure 4G). HFD control mice showed similar hepatic *Grk2* mRNA expression levels as lean control mice consuming regular chow (Supplementary Figure 1K). However, both HFD control and Hep-GRK2-KO littermates showed significantly increased expression levels of several genes (*Mcp1*, *Tnfa*, and *Il6*) that are considered markers of hepatic inflammation, as compared to the corresponding hepatic expression levels observed with lean mice (Supplementary Figure 1L).

As observed with chow-fed mice, liver and gWAT mass (Figure 4H, I) as well as plasma levels of ALT and AST (Figure 4J, K) did not differ significantly between the two groups of HFD mice. H&E staining studies showed that the consumption of the HFD caused a similar degree of liver steatosis in the two groups of mice (Figure 4L). Likewise, Sirius Red staining studies did not reveal any collagen deposition in either GRK2-KO or control livers (Figure 4L). As observed with mice maintained on regular chow, studies with liver RNA obtained from obese Hep-GRK2-KO mice and control littermates demonstrated that the expression levels of key genes regulating glucose metabolism (Figure 4M) or fatty acid metabolism (Figure 4N) were not affected by the lack of hepatocyte GRK2.

4. DISCUSSION

GRK2 plays a key role in regulating numerous physiological and pathophysiological processes [10,12,18]. For example, increased GRK2 levels and/or enhanced GRK2 activity are of critical importance for the development of heart failure [10]. For this reason, pharmacological inhibitors of cardiac GRK2 activity are predicted to prove useful as cardioprotective drugs [10]. Additional studies have shown that changes in GRK2 activity can also affect systemic glucose homeostasis and insulin sensitivity of peripheral tissues, suggesting that GRK2 inhibitors could be beneficial for treating type 2 diabetes and related metabolic disorders [12,18].

Given the central role of the liver in regulating glucose and lipid homeostasis, this study was designed to investigate the potential role of hepatocyte GRK2 in regulating glucose homeostasis and other key metabolic functions. The liver contains dozens of GPCRs predicted to regulate various important hepatic functions [20,24,30]. Considerable evidence suggests that the function of these GPCRs is regulated by GRK2 and other members of the GRK family [4–6]. For these reasons, we hypothesized that mice lacking GRK2 in their hepatocytes (Hep-GRK2-KO mice) might display significant changes in glucose homeostasis and other key metabolic functions.

Surprisingly, we found that Hep-GRK2-KO mice did not differ significantly from control littermates in glucose tolerance and insulin sensitivity. Moreover, glucagon-induced hyperglycemia and in vivo gluconeogenesis were not affected by hepatocyte GRK2 deficiency. In line with these observations, hepatic tissue from Hep-GRK2-KO mice showed no signs of liver damage and appeared histochemically normal. Likewise, the lack of hepatocyte GRK2 had no significant effect on the expression levels of key genes regulating glucose and fatty acid metabolism. Importantly, these findings were obtained independent of the diet that the mice consumed, either regular chow or an obesogenic HFD.

Since we used an AAV8-Cre-based strategy that involved the selective inactivation of *Grk2* in hepatocytes of adult mice, it is unlikely that compensatory changes in hepatic function are responsible for the observation that Hep-GRK2-KO mice did not display any metabolic phenotypes. In agreement with this concept, the hepatic expression levels of *Grk3*, the second member of the *Grk2* gene family, remained unaffected by the lack of hepatocyte *Grk2* in both lean and obese mice. The same was true for other members of the *Grk* family expressed in mouse liver/hepatocytes (*Grk4, 5*, and *6*). However, it should be noted in this context that AAV8 transduces predominantly hepatocytes near central veins and yields lower transduction levels in hepatocytes in periportal regions, a phenomenon that may affect metabolic zonation [31].

Our data suggest that the loss of a single member of the GRK family (GRK2) is insufficient to trigger significant changes in liver metabolism and whole-body glucose heomeostasis and that other members of the GRK family can maintain normal hepatocyte function. Our findings also indicate that the changes in insulin resistance caused by altered GRK2 activity [12,18] are likely to involve other metabolically important tissues and cell types, including skeletal muscle cells, adipocytes, and cells of the endocrine pancreas [32] (e.g., insulin-producing β-cells). Clearly, the potential functional role of GRK2 in these cell types needs to be explored in detail in future studies.

Previous work has implicated GRK2 in regulating several important hepatic functions.

For example, studies with a mouse liver cell line strongly suggested that GRK2 negatively regulates basal and insulin-stimulated glycogen synthesis [21]. We found that hepatic *Grk2* mRNA expression levels were not significantly different between lean and obese mice (Supplementary Figure 1K). However, these data do not rule out possible changes in GRK2 protein expression levels, since GRK2 protein stability may be affected under specific experimental conditions.

Interestingly, tamoxifen-induced genetic depletion of GRK2 in all body cells prevented hepatic steatosis in mice and lowered the expression of pro-inflammatory marker genes in the liver [22], suggesting that GRK2 may play a role in hepatic lipid deposition and inflammatory responses. However, in the present study, the lack of GRK2 in hepatocytes did not result in significant differences in the expression levels of marker genes known to be involved in liver inflammation (Supplementary Figure 1L), suggesting that the phenotypes observed by Vila-Bedmar et al. [22] most likely depend on GRK2 activities in other cell types (non-hepatocytes). Cruces-Sande et al. [23] recently reported that mutant mice with partial GRK2 deficiency are resistant to the development of NASH. It should be of interest to study this phenomenon in greater detail in a follow-up study using the Hep-GRK2-KO mice analyzed in the present study.

In conclusion, we made the unexpected observation that hepatic GRK2 deficiency has no effect on glucose homeostasis, insulin resistance, and other important metabolic parameters in both lean and obese mice. Our study provides a rational basis for re-directing research efforts on the potential metabolic benefits of GRK2 inhibitors to other metabolically important tissues.

AUTHOR CONTRIBUTIONS

AO and JW designed the study. AO, SP, and YQ carried out experiments, and AO analyzed and interpreted the experimental data. AK provided backcrossed *Grk2*$^{fl/fl}$ mice. AO generated all figures. AO and JW wrote the manuscript.

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DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

DATA AVAILABILITY

Data will be made available on request.

APPENDIX A. SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://doi.org/10.1016/j.molmet.2023.101866.

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