*Original Article*

# Restoration of Glucokinase Expression in the Liver Normalizes Postprandial Glucose Disposal in Mice With Hepatic Deficiency of PDK1

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**Phosphoinositide-dependent kinase-1 (PDK1) is implicated in the metabolic effects of insulin as a key mediator of phosphoinositide 3-kinase–dependent signaling. Here we show that mice with liver-specific PDK1 deficiency manifest various defects in the metabolic actions of insulin in the liver as well as a type 2 diabetes–like phenotype characterized by marked hyperinsulinemia and postprandial hyperglycemia. The hepatic abundance of glucokinase, an important determinant of glucose flux and glucose-evoked signaling in hepatocytes, was substantially reduced in these mice. Restoration of hepatic glucokinase expression, with the use of an adenoviral vector, induced insulin-like effects in the liver and almost completely normalized the fasting hyperinsulinemia and postprandial hyperglycemia in these animals. These results indicate that, if the hepatic abundance of glucokinase is maintained, ingested glucose is normally disposed of even in the absence of acute activation of proximal insulin signaling, such as the activation of Akt, in the liver. *Diabetes* 56:1000–1009, 2007**

The liver is one of the most important target organs of insulin and thus plays an essential role in nutrient metabolism (1,2). Such effects of insulin in the liver are mediated by a phosphoinositide 3-kinase (PI3K)-dependent signaling pathway. Mice in which hepatic PI3K activity is inhibited by a dominant negative form of the enzyme (3) or those lacking regulatory subunits of PI3K in the liver (4) manifest multiple defects in the metabolic actions of insulin and phenotypes that resemble type 2 diabetes. Moreover, the metabolic changes apparent in these animals are similar to those observed in mice with liver-specific knockout of the insulin receptor (LIRKO) (5), confirming the notion that PI3K-dependent signaling plays a central role in insulin action in the liver.

Downstream of PI3K, 3-phosphoinositide–dependent kinase-1 (PDK1) and its activation of members of the AGC family of protein kinases are implicated in the effects of insulin (6). Mora et al. (7) recently generated mice with liver-specific deficiency of PDK1 by breeding animals that harbor "floxed" *Pdk1* alleles with those that express Cre under the control of the albumin gene promoter with α-fetoprotein gene enhancer (Alfp-*Cre*). The activation of several signaling molecules, regulation of genes involved in nutrient metabolism, and induction of glycogen accumulation by insulin were affected in the liver of these mice. However, these animals also developed prominent edema and liver damage, and they died between 4 and 16 weeks of age (7). The metabolic role of hepatic PDK1 in living animals has thus not been sufficiently investigated with these animals.

We have now generated mice with liver-specific PDK1 deficiency achieved by the excision of floxed alleles of *Pdk1* with Cre expressed under the control of the albumin gene promoter (Alb-*Cre*). Unlike the mice with liver-specific PDK1 deficiency previously described (7), the mice characterized in the present study exhibited neither liver damage nor premature death. We were thus able to scrutinize the role of PDK1 in nutrient metabolism in normally developed animals and show that hepatic deficiency of PDK1 in mice results in various defects in the regulation of nutrient metabolism, among which a reduction in the abundance of glucokinase is largely responsible for an impairment of postprandial glucose disposal.

## RESEARCH DESIGN AND METHODS

**Mice and adenoviral vectors.** The study was approved by the Animal Experimentation Committee of Kobe University. Mice that harbor a floxed *Pdk1* allele (*Pdk1*$^{flox/+}$ mice) (8) or a transgene for Alb-*Cre* (9) were previously described. A cDNA-encoding rat glucokinase was synthesized with PCR, and an adenoviral vector containing this cDNA (AxCA*Gck*) was generated as described (10). Mice were injected via the tail vein with adenoviral vectors, and experiments were performed 4 days after injection.

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ALT, alanine aminotransferase; AxCA*G*ck, adenoviral vector-encoding rat glucokinase; ERK, extracellular signal–related kinase; FAS, fatty acid synthase; G6P, glucose 6-phosphate; G6PC, glucose-6-phosphatase; GS, glycogen synthase; IGFBP1, IGF-1 binding protein; IRS, insulin receptor substrate; P13K, phosphoinositide 3-kinase; PCK-1, phosphoenolpyruvate carboxykinase; PDK1, phosphoinositide-dependent kinase-1; PFU, plaque-forming units; PGC1α, peroxisome proliferator–activated receptor-γ coactivator 1α; PK, pyruvate kinase insulin receptor substrate; SCD-1, stearoyl-CoA desaturase-1; SREBP, sterol regulatory element–binding protein.

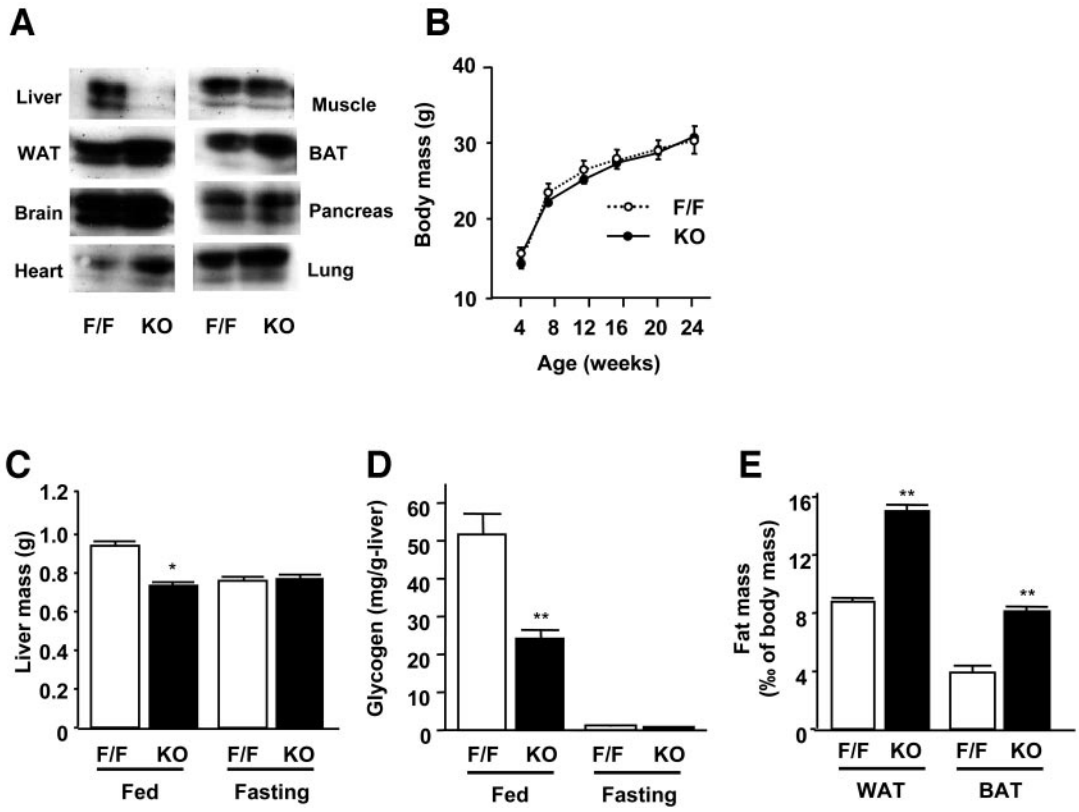


**FIG. 1. PDK1 expression, growth rate, liver and fat mass, and hepatic glycogen content in L-*Pdk1*KO mice. *A*: Immunoblot analysis of PDK1 in various tissues of L-*Pdk1*KO (KO) and *Pdk1*$^{flox/flox}$ (F/F) mice. Data are representative of at least three independent experiments. BAT, brown adipose tissue; WAT, white adipose tissue. *B*: Growth curves for L-*Pdk1*KO ($n$ = 13) and *Pdk1*$^{flox/flox}$ ($n$ = 9) mice. Data are means ± SEM. *C* and *D*: Liver mass ($n$ = 6–8) and hepatic glycogen content ($n$ = 3–5) of L-*Pdk1*KO and *Pdk1*$^{flox/flox}$ mice either in the randomly fed state of after food deprivation for 16 h. Glycogen content is expressed as milligrams per gram of liver tissue. Data are means ± SEM. *$P$ < 0.05, **$P$ < 0.01 (Student's $t$ test) vs. corresponding value for *Pdk1*$^{flox/flox}$ mice. *E*: Mass of subcutaneous white adipose tissue and interscapular brown adipose tissue of L-*Pdk1*KO and *Pdk1*$^{flox/flox}$ mice in the randomly fed state. Data are means ± SEM ($n$ = 4). **$P$ < 0.01 (Student's $t$ test) vs. corresponding value for *Pdk1*$^{flox/flox}$ mice.**

**Immunoblot analysis and assay of kinase activities.** For insulin or glucose treatment, mice deprived of food for 16 h were injected intraperitoneally with human regular insulin (5 units/kg) or glucose (2 g/kg) and were then killed after 5 or 30 min, respectively. Total homogenates were prepared from the liver and subjected to immunoblot analysis or to immunoprecipitation. Antibodies to PDK1 were kindly provided by F. Liu (University of Texas, Health Science Center, San Antonio, TX) (11). All other antibodies used for immunoblot analysis were commercially available, and the information of the antibodies will be provided on request. PI3K activity (3) and the activities of Akt and p70 S6K (12,13) in immunoprecipitates were assayed as described.

**Glucose and insulin tolerance tests and refeeding.** For the glucose tolerance test, mice deprived of food for 16 h were loaded intraperitoneally with glucose (2 g/kg). Glycogen and glucose 6-phosphate (G6P) contents as well as glycogen synthase (GS) activity were determined as described (3) with total liver homogenates prepared from mice killed 30 min after the initiation of a glucose tolerance test. For the insulin tolerance test, nonfasted mice were injected intraperitoneally with human regular insulin (0.75 units/kg). For assay of glucose concentration and hepatic gene expression during refeeding, mice were deprived of food for 24 h and then fed with a high-carbohydrate chow containing 70% sucrose.

**Hepatic gene expression.** Hepatic gene expression was evaluated by reverse transcription and real-time PCR analysis with 36B4 mRNA as the invariant control, as described (3). The primers used were as follows: glucokinase, 5′-CCCTGAGTGGCTTACAGTTCGK-3′ and 5′-ACGGATGTGAGTGTTGAAGC-3′; pyruvate kinase (PK), 5′-GAGAACCATGAAGGCGTGAA-3′ and 5′-CCGC CCGAGTTGGTC-3′; insulin receptor substrate (IRS)-2, 5′-GGAGAACCCAG ACCCTAAGCTACT-3′ and 5′-GATGCCTTTGAGGCCTTCAC-3′; glucose transporter 2, 5′-TAACCTTCCTAGCCCTGTTCTACTG-3′ and 5′-ACGCAAA ACCCGAAGTCTAATC-3′; fatty acid synthase (FAS), 5′-GCTGGCATTCGT GATGGAGTCGT-3′ and 5′-AGGCCACCAGTGATGATGTAACTCT-3′; stearoyl-CoA desaturase–1 (SCD-1), 5′-TCTCAGAAACACACGCCGACC-3′ and 5′-TAG CCTGTAAAAGATTTCTGCAAACC-3′; peroxisome proliferator–activated receptor-α, 5′-CCTGAACATCGAGTGTCGAATA-3′ and 5′-GGTCTTCTTCT GAATCTTGCAGCT-3′; and IGF-1 binding protein (IGFBP1), 5′-TTGCCAC TACTATCTACTCA-3′ and 5′-GCCAAGAAACAACAGTTAG-3′. The primers for the catalytic subunit of glucose-6-phosphatase (G6PC) (8), phosphoenolpyruvate carboxykinase (PCK-1) (14), peroxisome proliferator–activated receptor–γ coactivator 1α (PGC1α) and 36B4 (15), and sterol regulatory element–binding protein (SREBP)1c and SREBP1a (16) were as described.

**Statistical analysis.** Data are presented as means ± SEM and were compared between or among groups by Student's $t$ test or ANOVA.

## RESULTS

**Generation of liver-specific PDK1-deficient mice.** We bred *Pdk1*$^{flox/+}$ mice (8) with Alb-*Cre* mice (9) and then bred the resulting *Pdk1*$^{flox/+}$/Alb-*Cre* offspring with *Pdk1*$^{flox/+}$ mice. The genotypes of animals born from the latter cross conformed approximately to the expected ratio (data not shown). Given that the abundance of PDK1 in major organs including the liver was similar in *Pdk1*$^{+/+}$ mice and *Pdk1*$^{flox/flox}$ mice (data not shown), and that Alb-*Cre* mice did not exhibit metabolic abnormalities (9), we performed subsequent experiments with *Pdk1*$^{flox/flox}$/Alb-*Cre* (L-*Pdk1*KO) mice and *Pdk1*$^{flox/flox}$ mice generated by crossing *Pdk1*$^{flox/flox}$/Alb-*Cre* mice with *Pdk1*$^{flox/flox}$ mice; the offspring of such breeding were born in a Mendelian ratio (data not shown). We studied male mice at 2–3 months of age, unless otherwise indicated.

The expression of PDK1 in the liver of liver-specific PDK1-deficient (L-*Pdk1*KO) mice was almost completely abolished, whereas that in other various tissues did not differ between L-*Pdk1*KO mice and their *Pdk1*$^{flox/flox}$ littermates (Fig. 1*A*). The growth of L-*Pdk1*KO mice also did not

differ from that of their control littermates (Fig. 1*B*), and the gross appearance and histology of the liver appeared normal in the former animals (data not shown). The plasma concentration of alanine aminotransferase (ALT), a marker of liver damage, was within normal range in L-*Pdk1*KO mice and control littermates (31.6 ± 3.3 and 28.8 ± 1.1 IU/l, respectively; means ± SEM, $n = 3$). The liver mass of L-*Pdk1*KO mice in the randomly fed state was ~14% smaller than that of *Pdk1*$^{flox/flox}$ mice; however, in the fasted state, no significant difference was apparent (Fig. 1*C*), suggesting that the difference in the randomly fed state was attributable not to a defect in liver development but rather to a defect in nutrient metabolism. Consistent with this, the hepatic glycogen content of L-*Pdk1*KO mice in the randomly fed state was <50% of that in *Pdk1*$^{flox/flox}$ mice, whereas no significant difference was apparent in the fasted state (Fig. 1*D*). The mass of subcutaneous white adipose tissue or interscapular brown adipose tissue was increased in L-*Pdk1*KO mice (Fig. 1*E*). A similar increase in adipose tissue mass was observed in mice with liver-specific expression of a dominant negative form of PI3K (3) and in those lacking regulatory subunits of PI3K in the liver (4), which was likely attributable to enhanced lipogenesis and reduced lipolysis as a result of hyperinsulinemia in these mice.

**Insulin-induced activation of signaling molecules in L-*Pdk1*KO mice.** PI3K activity associated with IRS-1 or IRS-2 in the liver was increased in L-*Pdk1*KO mice (Fig. 2*A*), consistent with the notion that downstream components of PI3K contribute to negative feedback regulation at the level of IRS (13,17). The hepatic abundance of IRS-2, but not of IRS-1, was increased in L-*Pdk1*KO mice.

Threonine-308 of Akt is phosphorylated directly by PDK1, whereas phosphorylation of Akt on Ser$^{473}$ is mediated in a PDK1-independent manner (6). As expected, the insulin-induced phosphorylation of Akt on Thr$^{308}$ was abolished in the liver of L-*Pdk1*KO mice (Fig. 2*B*). The abundance of Akt, as assessed by analysis with antibodies to Akt1 or to Akt2, as well as the extent of immunoreactivity with antibodies to the phospho-Ser$^{473}$ form of Akt, were increased in the liver of these animals. The insulin-induced increase in the activity of Akt in immunoprecipitates prepared with antibodies to this kinase was almost completely abolished in L-*Pdk1*KO mice (Fig. 2*C*). The insulin-induced phosphorylation of GSK3β and that of Foxo1, both of which are mediated directly by Akt (18,19), were prevented in the liver of L-*Pdk1*KO mice (Fig. 2*B*). The phosphorylation of p70 S6K on Thr$^{389}$ (Fig. 2*B*) as well as the increase in p70 S6K activity (Fig. 2*C*) induced by insulin were inhibited in L-*Pdk1*KO mice. The insulin-induced phosphorylation of the ribosomal protein S6, an endogenous substrate of p70 S6K, was also not apparent in the liver of L-*Pdk1*KO mice (Fig. 2*B*). The amounts of GSK3β, p70 S6K, and S6 did not differ between the two genotypes. Although Foxo1 itself was not detected in total liver homogenates with antibodies to this protein, the abundance of Foxo1 mRNA in the liver did not differ between *Pdk1*$^{flox/flox}$ and L-*Pdk1*KO mice (data not shown). The phosphorylation of extracellular signal–related kinase (ERK) in the liver of L-*Pdk1*KO mice was increased even without administration of insulin and was not increased further by insulin treatment, whereas the abundance of ERK did not differ between the two genotypes (Fig. 2*B*). A similar increase in the phosphorylation of ERK was observed in embryonic stem cells lacking *Pdk1* (20). The insulin-induced increases in PI3K activity associated with IRS-1 as well as in the phosphorylation of Akt in skeletal muscle and epididymal fat tissue did not differ between L-*Pdk1*KO and *Pdk1*$^{flox/flox}$ mice (data not shown).

**Metabolic defects in L-*Pdk1*KO mice.** Blood glucose and plasma insulin concentrations in both fasted and randomly fed states were greater in L-*Pdk1*KO mice than in *Pdk1*$^{flox/flox}$ mice (Fig. 3*A*). In contrast to the slightly elevated blood glucose concentrations of L-*Pdk1*KO mice in the fasted or randomly fed states, the increase in blood glucose level after either glucose administration (Fig. 3*B*) or refeeding after fasting (Fig. 3*C*) was greatly exaggerated in these animals. The percent reduction of blood glucose concentration in response to exogenous administered insulin was 27.4 and 37.4% (Fig. 3*D*), and the absolute values of the reduction were 38.7 ± 6.2 and 46.2 ± 4.8 mg/dl in L-*Pdk1*KO mice and *Pdk1*$^{flox/flox}$ mice, respectively (Fig. 3*E*). LIRKO mice exhibit hyperinsulinemia and glucose intolerance at 2 months of age, but these conditions are ameliorated by 6 months of age by an unknown mechanism (5). In L-*Pdk1*KO mice, both the elevated plasma insulin concentration compared with that in control animals (23.5 ± 0.7 vs. 4.5 ± 0.9 ng/ml; means ± SEM, $n = 10$ and 9, respectively; $P < 0.05$) and the exaggerated increase in blood glucose concentration during a glucose challenge test (Fig. 3*F*) were still apparent at 6 months of age. Six-month-old LIRKO mice also exhibit abnormal hepatic histology (characterized by the presence of multiple nodule-like structures and destruction of hepatic lobules) as well as a marked increase in serum transaminase level (5). We observed neither such histological abnormalities of the liver (data not shown) nor an increase in plasma ALT concentration (31.0 ± 2.5 and 24.4 ± 1.0 IU/l in L-*Pdk1*KO and *Pdk1*$^{flox/flox}$ mice, respectively; means ± SEM, $n = 3$) in L-*Pdk1*KO mice at 6 months of age.

While the amounts of G6P and glycogen as well as the activity of GS in the liver of *Pdk1*$^{flox/flox}$ mice were increased in response to intraperitoneal glucose administration, these responses were greatly attenuated in the liver of L-*Pdk1*KO mice (Fig. 3*G–I*). The hepatic abundance of mRNAs known to be upregulated by insulin (including those for SREBP1c, FAS, SCD-1, PK, and glucokinase) and that of mRNAs known to be downregulated by insulin (including those for PCK-1, G6PC, PGC1α, and IRS-2) were decreased and increased, respectively, in the liver of randomly fed L-*Pdk1*KO mice (Fig. 4*A*). The amounts of mRNAs for glucose transporter 2, SREBP1a, and peroxisome proliferator–activated receptor-α in the liver did not differ significantly between L-*Pdk1*KO and *Pdk1*$^{flox/flox}$ mice. The mRNAs encoding PCK-1, PGC1α, G6PC, IRS-2, and IGFBP1 were downregulated during feeding for 6 h in the liver of *Pdk1*$^{flox/flox}$ mice; however, these effects of feeding were attenuated in L-*Pdk1*KO mice (Fig. 4*B*). The upregulation of glucokinase mRNA by feeding was also suppressed in the liver of L-*Pdk1*KO mice (Fig. 4*B*). Given that refeeding for 6 h had little effect on the hepatic abundance of SREBP1c, FAS, and SCD-1 mRNAs even in *Pdk1*$^{flox/flox}$ mice, we examined the expression of these genes after refeeding for 12 h. The abundance of hepatic SREBP1c mRNA after refeeding was similar between L-*Pdk1*KO mice and *Pdk1*$^{flox/flox}$ mice (Fig. 4*C*). The abundances of FAS and SCD-1 mRNAs were smaller in the livers of L-*Pdk1*KO mice after refeeding; however, the differences were not statistically significant. These results suggest that, although PDK-1 is important to maintain the expression of the lipogenic genes at steady states, the

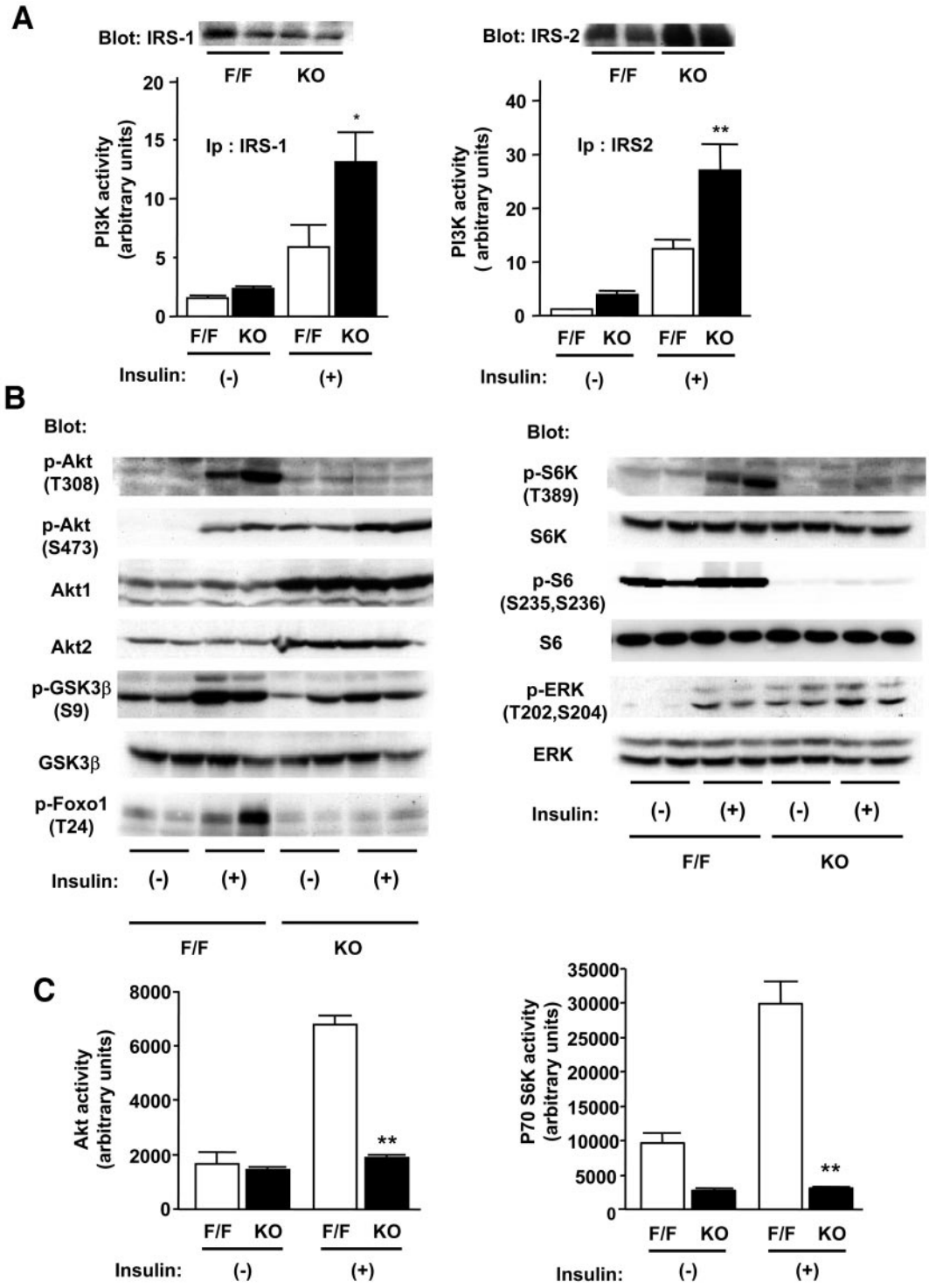


**FIG. 2. Inhibition of insulin-induced activation of various signaling molecules in the liver of L-*Pdk1*KO mice. *A*: IRS-1 (*left panel*) or IRS-2 (*right panel*) immunoprecipitates (Ip) prepared from the liver of L-*Pdk1*KO or *Pdk1*$^{flox/flox}$ mice that had been deprived of food for 16 h and then injected intraperitoneally with insulin (+) or vehicle (–) and were subjected to immunoblot analysis with the corresponding antibodies (*upper panels*) or assayed for PI3K activity (*lower panels*). Immunoblots are representative of at least three independent experiments. Activity data are means ± SEM ($n$ = 3). *$P$ < 0.05, **$P$ < 0.01 (Student's $t$ test) vs. corresponding value for *Pdk1*$^{flox/flox}$ mice. *B* and *C*: Immunoblot analysis of the phosphorylation (p) of various signaling molecules (*B*) and assay of the activities of Akt and p70 S6K (*C*) in the liver of L-*Pdk1* KO or *Pdk1*$^{flox/flox}$ mice that had been deprived of food for 16 h and then injected intraperitoneally with insulin (+) or vehicle (–). Immunoblots are representative of at least three independent experiments. Activity data are means ± SEM ($n$ = 4). **$P$ < 0.01 (Student's $t$ test) vs. corresponding value for *Pdk1*$^{flox/flox}$ mice.**

induction of those genes during refeeding is mediated also by a PDK1-independent pathway. In this regard, lipogenic genes were shown to be strongly induced by refeeding in the liver of insulin-deficient mice (21).

The plasma concentrations of free fatty acids (0.28 ± 0.02 vs. 0.44 ± 0.06 mmol/l; means ± SEM, $n$ = 11 and 10, respectively; $P$ < 0.05) and triglycerides (52.2 ± 9.5 vs. 119.0 ± 15.0 mg/dl; $n$ = 11 and 10, respectively; $P$ < 0.01)

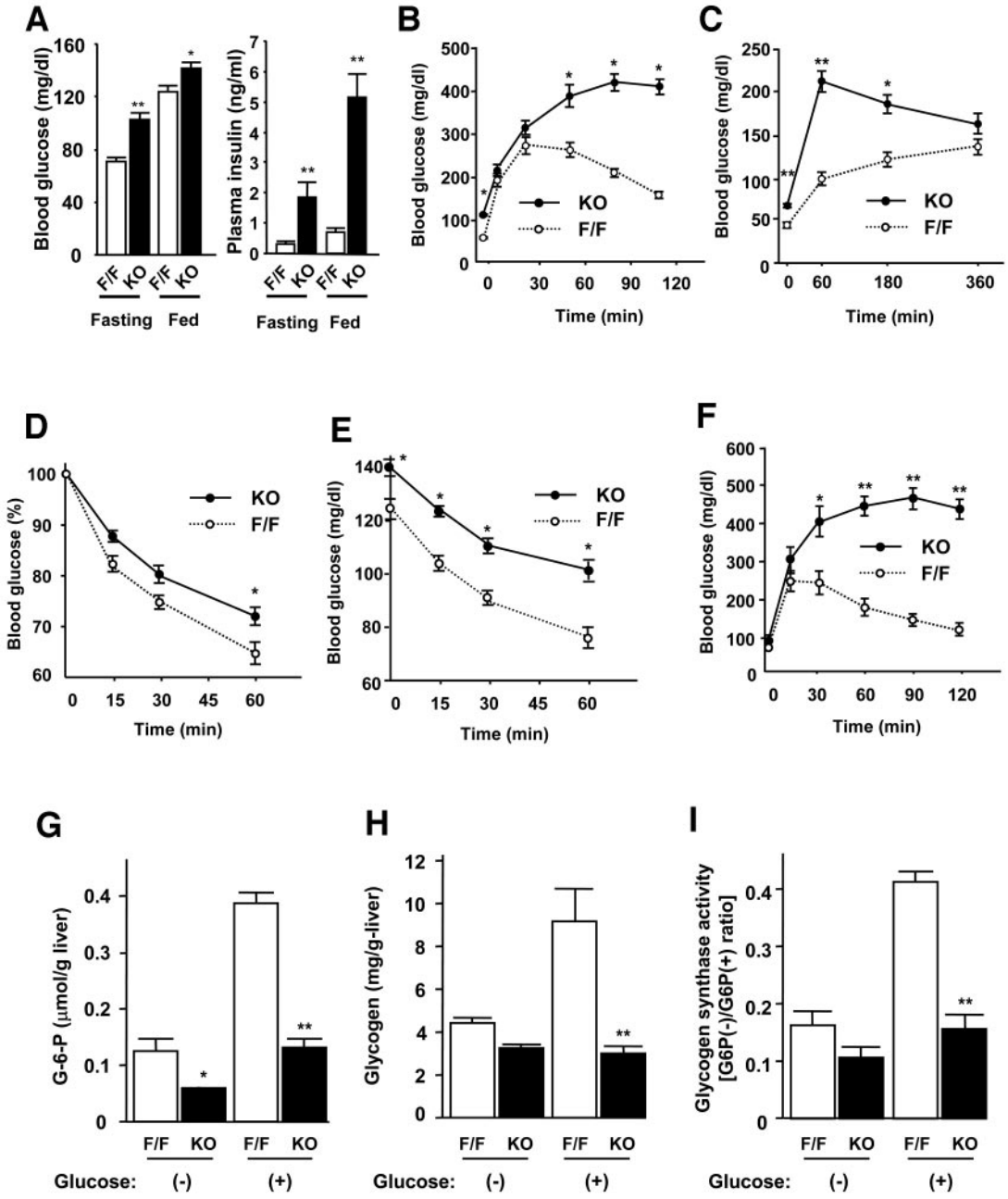


**FIG. 3. Metabolic defects in L-*Pdk1* KO mice. *A*: Blood glucose (*left*, $n = 14–20$) and plasma insulin (*right*, $n = 8–18$) concentrations of L-*Pdk1*KO and *Pdk1*$^{flox/flox}$ mice in the randomly fed state or after food deprivation for 16 h. *B–F*: Blood glucose concentration during a glucose challenge test (*B*, *F*), during refeeding after fasting (*C*), or during an insulin tolerance test (*D* and *E*) in L-*Pdk1*KO or *Pdk1*$^{flox/flox}$ mice at 2–3 months (*B–E*) or 6 months (*F*) of age. Percent reduction (*D*) and absolute values (*E*) of blood glucose concentrations were shown. Data are means ± SEM of values from 9 and 8 (*B*), 8 and 8 (*C*), 17 and 12 (*D* and *E*), and 9 and 8 (F) L-*Pdk1*KO and *Pdk1*$^{flox/flox}$ mice, respectively. *G–I*: Amounts of G6P (*G*) and glycogen (*H*) as well as the activity of GS (*I*) in the liver measured 30 min after administration of glucose (+) or vehicle (–) in L-*Pdk1*KO or *Pdk1*$^{flox/flox}$ mice ($n = 5$). GS activity is expressed as the ratio of that in the absence to that in the presence of GP6. In all panels (*A–I*), data are means ± SEM. *$P < 0.05$, ** $P < 0.01$ (Student's *t* test) vs. corresponding value for *Pdk1*$^{flox/flox}$ mice.**

in the fasted state were lower in L-*Pdk1*KO mice than in *Pdk1*$^{flox/flox}$ mice. A similar reduction in the circulating levels of these metabolites was previously observed in mice in which insulin signaling was specifically inhibited in the liver (3–5).

**Restoration of hepatic glucokinase reverses fasting hyperinsulinemia and postprandial hyperglycemia in L-*Pdk1*KO mice.** Glucokinase is thought to contribute to glucose-triggered metabolic actions by promoting glucose flux into the liver and generating glucose metabolites that might act as mediators of intracellular signaling. Given that the abundance of glucokinase (Fig. 4*A*) was markedly reduced in the liver of L-*Pdk1*KO mice, the metabolic defects of these animals are likely attributable to inhibition of both insulin- and glucose-triggered effects. We therefore investigated the impact of restoration of hepatic glucokinase on the metabolic alterations in L-*Pdk1*KO mice.

Injection of an adenoviral vector-encoding rat glucokinase (AxCA*Gck*) into L-*Pdk1*KO mice resulted in an increase in the abundance of this enzyme in the liver (Fig. 5*A*) but had no effect on that in other tissues, including

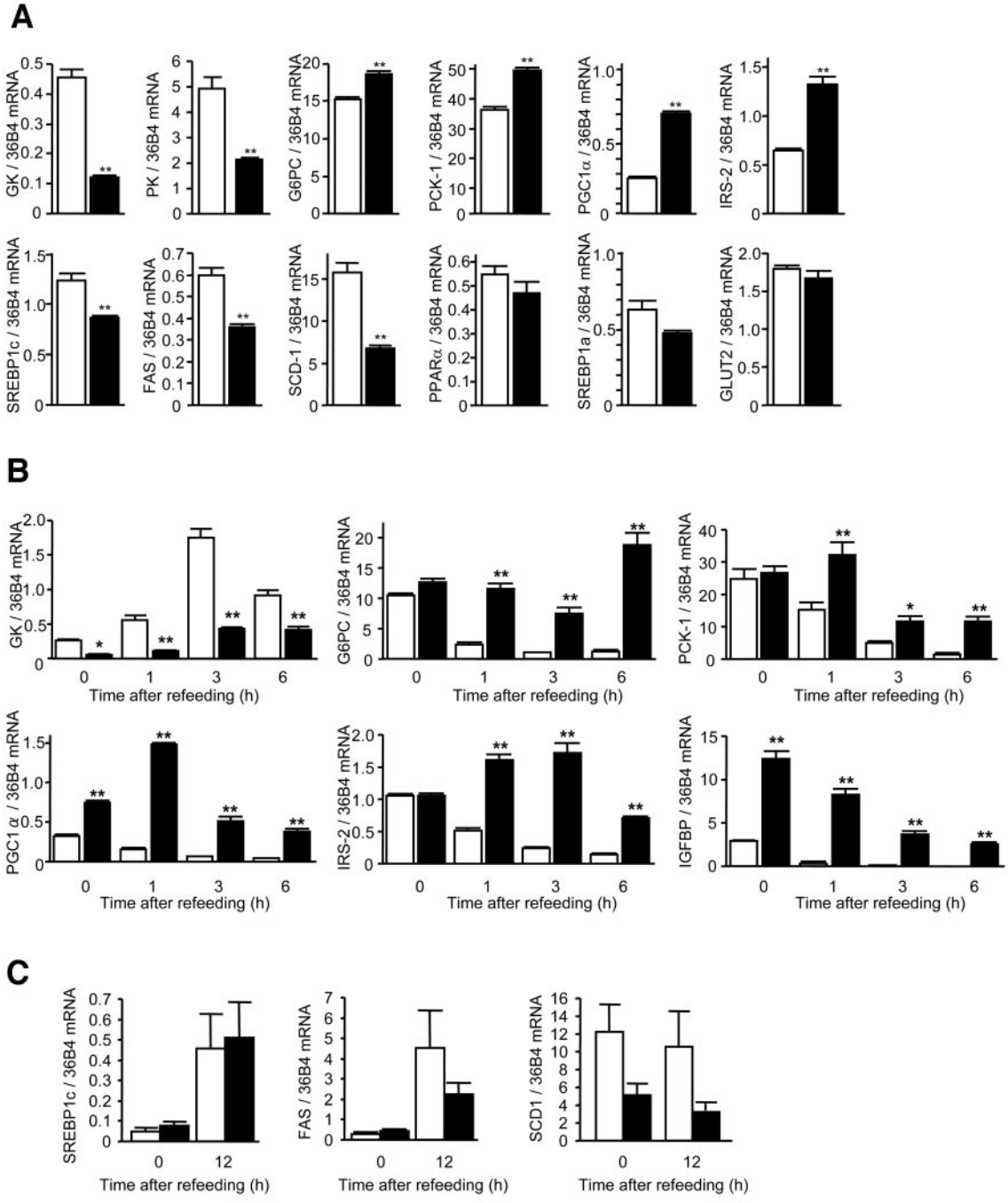


**FIG. 4. Changes in hepatic gene expression in L-*Pdk1*KO mice. The amounts of the indicated mRNAs in the liver of L-*Pdk1*KO (■) or *Pdk1*$^{flox/flox}$ (□) mice in the randomly fed state (*A*) or at the indicated times of refeeding after food deprivation for 24 h (*B* and *C*) were determined. GK, glucokinase. Data are means ± SEM ($n$ = 5–6 or $n$ = 4, respectively, in *A* and *B*, and $n$ = 4 in each genotype in *C*) and are expressed relative to the amount of 36B4 mRNA. *$P < 0.05$, **$P < 0.01$ (Student's $t$ test) vs. corresponding value for *Pdk1*$^{flox/flox}$ mice.**

skeletal muscle and adipose tissue (data not shown). The hepatic abundance of glucokinase in L-*Pdk1*KO mice that had been injected with AxCA*Gck* at a dose of $6 \times 10^7$ plaque-forming units (PFU) per animal was 80–90% of that in *Pdk1*$^{flox/flox}$ mice that had been injected with saline alone (Fig. 5*A*). Injection of a control virus (AxCALacZ) at $6 \times 10^7$ PFU per animal did not affect the amount of glucokinase in the liver of *Pdk1*$^{flox/flox}$ or L-*Pdk1*KO mice. Injection of the adenoviral vectors did not affect the plasma concentration of ALT (27.4 ± 0.5, 31.1 ± 1.8, and 28.5 ± 1.2 IU/l for *Pdk1*$^{flox/flox}$ or L-*Pdk1*KO mice injected with AxCALacZ or L-*Pdk1*KO mice injected with AxCA*Gck*, respectively; means ± SEM, $n$ = 9, 5, and 5, respectively). The plasma concentration of insulin in L-*Pdk1*KO mice was decreased by injection of AxCA*Gck*, reaching a level in the animals injected with this vector at $6 \times 10^7$ PFU similar to that apparent in *Pdk1*$^{flox/flox}$ mice injected with AxCALacZ at the same dose (Fig. 5*B*). Injection of L-*Pdk1*KO mice with AxCA*Gck* also ameliorated the exaggerated increase in blood glucose concentration apparent in these animals during a glucose challenge test (Fig. 5*C* and *D*) or during refeeding (Fig. 5*E*). The blood glucose concentrations after glucose administration or refeeding in L-*Pdk1*KO mice injected with

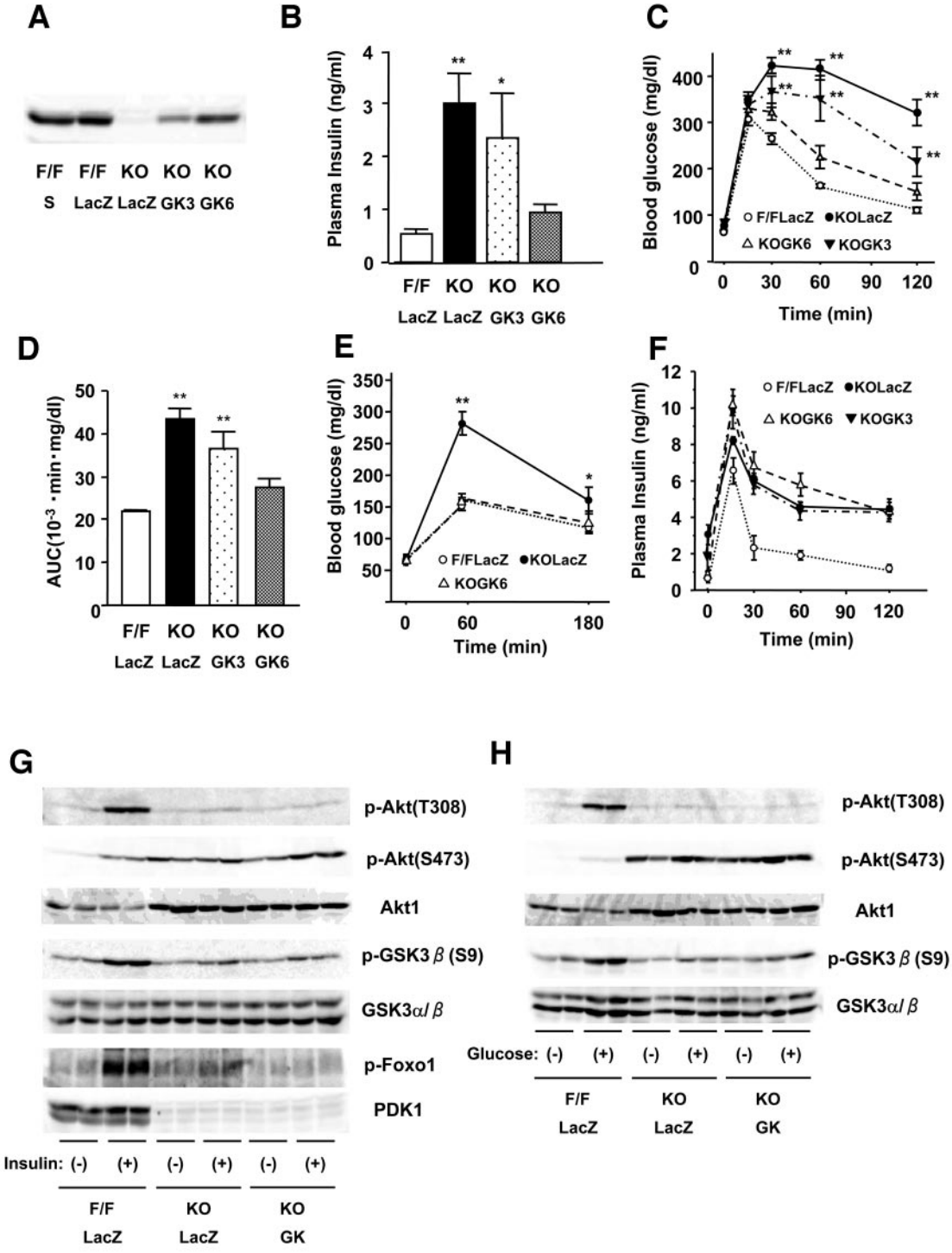


**FIG. 5. Effects of restoration of glucokinase expression in the liver on the metabolic defects of L-*Pdk1*KO mice. *A–F*: Immunoblot analysis of hepatic glucokinase in the randomly fed state (*A*); plasma insulin concentration in the fasted state (*B*); blood glucose level (*C*); the area under the curve (AUC) for blood glucose level (*D*) and plasma insulin concentration (*F*) during a glucose tolerance test; and blood glucose level after refeeding (*E*) in *Pdk1*$^{flox/flox}$ mice injected with saline or with AxCALacZ at 6 × 10$^7$ PFU (LacZ, ○) or in L-*Pdk1*KO mice injected with AxCALacZ at 6 × 10$^7$ PFU (LacZ, ●) or with AxCA*Gck* at 3 × 10$^7$ PFU (GK3, ▲) or 6 × 10$^7$ PFU (GK6, △). Data in *A* are representative of three independent experiments, and those in *B* and in *C*–F are means ± SEM of values from four or five or from five to eight animals, respectively. *$P$ < 0.05, **$P$ < 0.01 (Student's $t$ test) vs. corresponding value for *Pdk1*$^{flox/flox}$ mice injected with AxCALacZ (*B–E*). *F*: The values for L-*Pdk1*KO mice injected with AxCALacZ, AxCA*Gck* at 3 × 10$^7$ PFU, or AxCA*Gck* at 6 × 10$^7$ PFU were not significantly different from each other at 15, 30, 60, or 120 min after glucose challenge (ANOVA). *G* and *H*: Immunoblot analysis of the phosphorylation or abundance of Akt, GSK3β, Foxo1, and PDK1, with or without intraperitoneal administration of insulin (*G*) or glucose (*H*) in the liver of L-*Pdk1*KO or *Pdk1*$^{flox/flox}$ mice that had been injected with AxCALacZ at 6 × 10$^7$ PFU or AxCA*Gck* at 6 × 10$^7$ PFU (GK). Data are representative of at least three independent experiments.**

AxCA*Gck* at 6 × 10$^7$ PFU were similar to those in *Pdk1*$^{flox/flox}$ mice injected with AxCALacZ (Fig. 5*C* and *E*). The area under the curve for blood glucose concentration during the glucose challenge test did not differ significantly between L-*Pdk1*KO mice injected with AxCA*Gck* at 6 × 10$^7$ PFU and *Pdk1*$^{flox/flox}$ mice injected with AxCALacZ at the

same dose (Fig. 5*D*). In contrast to the marked amelioration of fasting hyperinsulinemia, the exaggerated increase in plasma insulin concentration during a glucose challenge test in L-*Pdk1*KO mice was not reversed by infection with AxCA*Gck* (Fig. 5*F*).

The loss of insulin-induced phosphorylation of Akt, GSK3β, and Foxo1 in the liver of L-*Pdk1*KO mice was not affected by restoration of hepatic glucokinase expression (Fig. 5*G*). The abundance of Foxo1 mRNA in the liver of L-*Pdk1*KO mice was also not altered by restoration of glucokinase (data not shown). Intraperitoneal administration of glucose also stimulated phosphorylation of Akt and GSK3β in the liver of *Pdk1*$^{flox/flox}$ mice, which was likely a result of the increase in endogenous insulin secretion, but these effects were not observed in L-*Pdk1*KO mice that had been injected with AxCA*Gck* (Fig. 5*H*).

Injection of L-*Pdk1*KO mice with AxCA*Gck* increased the hepatic content of G6P both in the fasted state and after glucose administration to values similar to those apparent in *Pdk1*$^{flox/flox}$ mice injected with the control virus (Fig. 6*A*), suggesting that the restoration of hepatic glucokinase increased glucose flux into the liver of L-*Pdk1*KO mice. The activity of GS in the liver of L-*Pdk1*KO mice after glucose administration was increased by restoration of hepatic glucokinase but did not reach the level apparent in glucose-treated *Pdk1*$^{flox/flox}$ mice injected with AxCALacZ (Fig. 6*B*). The hepatic content of glycogen in L-*Pdk1*KO mice was increased by the restoration of glucokinase to values greater than those in *Pdk1*$^{flox/flox}$ mice injected with the control virus (Fig. 6*C*).

The increases in the abundance of mRNAs for PGC1α and PCK-1, but not that in the amount of G6PC mRNA, in the liver of L-*Pdk1*KO mice were attenuated by restoration of hepatic glucokinase (Fig. 6*D*). The decrease in the mRNA of FAS in the liver of L-*Pdk1*KO mice was significantly recovered by the expression of glucokinase, and the tendency of recovery was apparent in mRNA of PK, suggesting that glucose-evoked signaling such as that mediated by carbohydrate response element–binding protein (22) plays an important role in the regulation of these genes. On the other hand, little effects were apparent on the genes for SREBP1c and SCD-1 by the restoration of glucokinase, suggesting that glucose-evoked signaling is not sufficient and insulin signaling is important for the regulation of these genes.

The serum concentrations of triglycerides and free fatty acids were restored fully and partially, respectively, by the hepatic expression of glucokinase in L-*Pdk1*KO mice (Fig. 6*E*), whereas adipose tissue mass was not affected by this treatment (Fig. 6*F*). It is possible that biochemical alterations independent of glucokinase are responsible for the change in adipose tissue mass. It is also possible that the restoration of hepatic glucokinase only for 4 days was not sufficient to restore the increase in adipose tissue mass.

## DISCUSSION

Given that disruption of *Pdk1* throughout the body results in embryonic death (23), mice lacking this gene in specific organs have been generated in order to characterize the functions of PDK1 in living animals (7,8,24,25). Mice in which *Pdk1* is excised by Alfp-*Cre* (26) exhibit liver-specific PDK1 deficiency together with severe liver damage and premature death (7). We now show that L-*Pdk1*KO mice, in which *Cre* is driven by the promoter of the albumin gene (Alb-*Cre*), do not manifest morphological abnormalities or dysfunction of the liver. Given that α-fetoprotein is expressed in the liver at an early stage of development, it is possible that Alfp-*Cre* is induced in the liver at an earlier phase of development than is the authentic albumin gene. It is thus likely that PDK1 plays an important role in liver development at an early stage, but is not essential at later stages of life to maintain the normal morphology of the liver. LIRKO mice, in which Cre is also driven by the promoter of the albumin gene, develop age-dependent abnormalities in liver morphology and function (5). It is therefore likely that, even in mature hepatocytes, insulin signaling that is independent of the PDK1 pathway contributes to maintenance of liver morphology and function. The size of the body and organs, including that of the liver, was smaller in mice homozygous for a hypomorphic *Pdk1* allele than in wild-type animals (23), indicating that PDK1 contributes to the regulation of organ size. Liver mass of L-*Pdk1*KO mice was not decreased in the fasted state, however, suggesting that PDK1 influences organ size at a stage before the onset of albumin gene expression during development.

Dysregulation of hepatic glucose production correlates with the increase in fasting glucose levels in individuals with type 2 diabetes. Although hepatic metabolic actions of insulin appeared to be abolished in L-*Pdk1*KO mice, the increase in fasting blood glucose concentration in these animals was relatively small, suggesting that, even in the absence of hepatic insulin action, the defect in glucose metabolism in the fasting condition is substantially (but not completely) compensated for, probably by the associated hyperinsulinemia. In contrast, the increase in blood glucose level in response to feeding or to glucose administration was markedly exaggerated in these animals. Similar metabolic changes were observed in mice in which hepatic PI3K signaling was prevented (3,4). It is thus likely that the PI3K-PDK1 signaling pathway in the liver is crucial for normal glucose disposal, especially in the postprandial state.

Unexpectedly, restoration of glucokinase in the liver almost completely normalized the postprandial hyperglycemia of L-*Pdk1*KO mice. The restoration of glucokinase did not affect the inhibition of PDK1-dependent signaling in the liver of these animals. These results suggest that, if the hepatic abundance of glucokinase is appropriately maintained, living animals are able to dispose of ingested glucose in the absence of acute activation of proximal insulin signaling, such as the activation of Akt, in the liver. In this regard, hepatic overexpression of glucokinase has been shown to ameliorate hyperglycemia of insulin-deficient animals (27). In hepatocytes, glucose induces insulin-like effects, including the suppression of PCK-1 gene expression, the upregulation of glycolytic genes, the activation of GS, and the accumulation of glycogen (28). Evidence suggests that such glucose-triggered effects require metabolism of glucose by glucokinase (22,29–32). Indeed, the restoration of hepatic glucokinase in L-*Pdk1*KO mice partially attenuated the suppression of GS activity along with the increased and decreased expression of certain gluconeogenic and lipogenic genes, respectively, in the liver of these animals. These data indicate that, whereas signaling evoked as a result of the metabolism of glucose by glucokinase contributes to the regulation both of GS activity and of the expression of some genes involved in nutrient metabolism, insulin signaling is required for normal regulation of these parameters. The

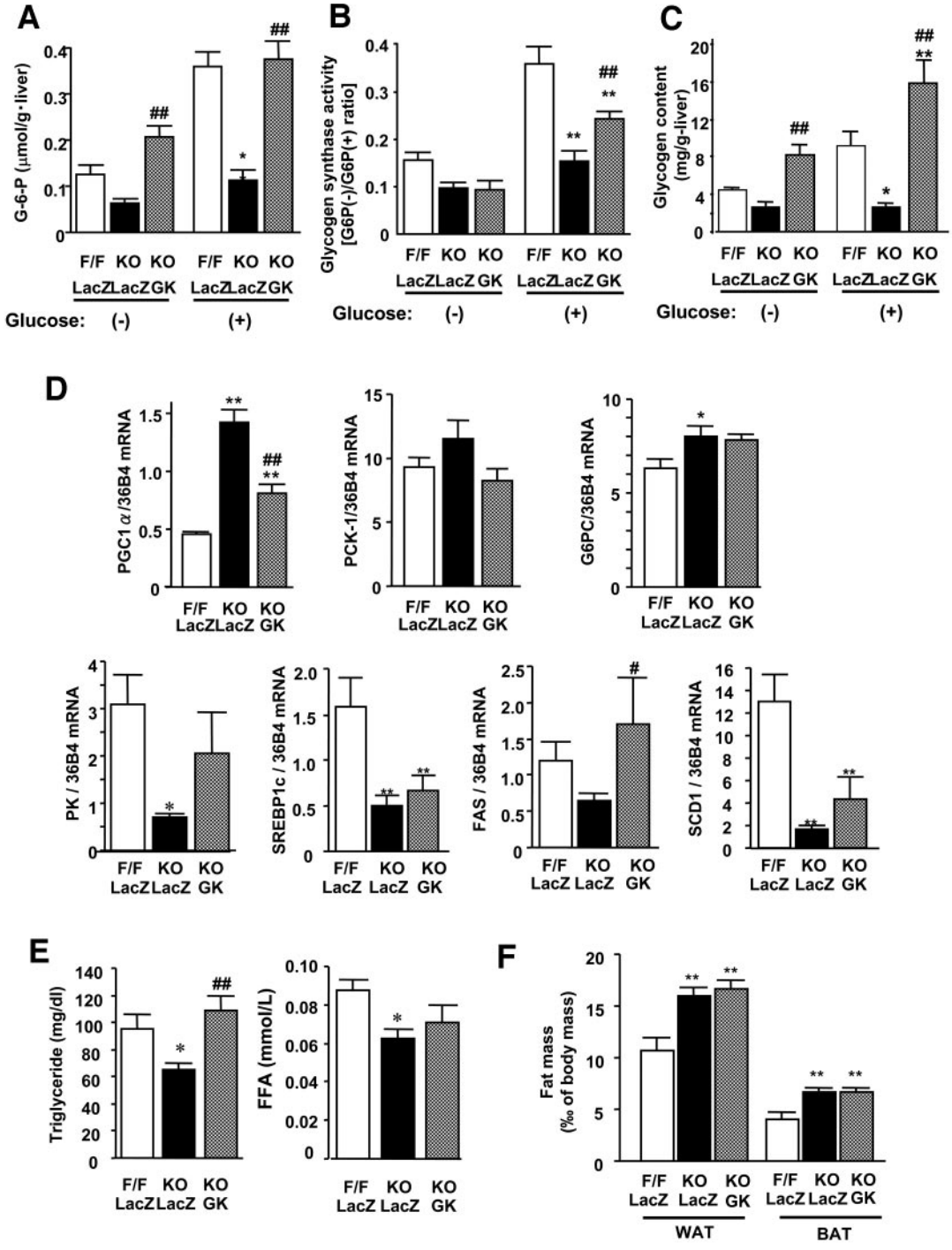


**FIG. 6. Effects of restoration of hepatic glucokinase expression on metabolic changes in the liver of L-*Pdk1*KO mice. The amount of G6P (*A*) or glycogen (*C*) and the activity of GS (*B*) with or without intraperitoneal administration of glucose in the liver of L-*Pdk1*KO or *Pdk1*$^{flox/flox}$ mice that had been injected with AxCALacZ (LacZ) or AxCA*Gck* (GK) at $6 \times 10^7$ PFU per animal. Data are means ± SEM ($n$ = 3–5 or 4–6, respectively). Hepatic abundance of PGC1α, PCK-1, and G6PC mRNAs (in the fasted state) and of PK, SREBP1c, FAS, and SCD-1 mRNAs (in the randomly fed state) (*D*); serum concentrations of triglycerides and FFA (in the randomly fed state) (*E*); and mass of subcutaneous white adipose tissue (WAT) and interscapular brown adipose tissue (BAT) (*F*) of L-*Pdk1*KO or *Pdk1*$^{flox/flox}$ mice that had been injected with AxCALacZ or AxCA*Gck* as in *A*–*C*. Data are means ± SEM ($n$ = 3). *$P$ < 0.05, **$P$ < 0.01 (Student's $t$ test) vs. corresponding value for *Pdk1*$^{flox/flox}$ mice injected with AxCALacZ and #$P$ < 0.05, ##$P$ < 0.01 (Student's $t$ test) vs. corresponding value for L-*Pdk1*KO mice injected with AxCALacZ.**

rate of glucose flux into hepatocytes is determined by the difference between the intra- and extracellular concentrations of glucose. Glucokinase plays a key role in maintenance of this glucose gradient by catalyzing the conversion of glucose to G6P, thereby reducing the intracellular glucose level. The restoration of glucokinase in the liver of L-*Pdk1*KO mice thus likely normalizes postprandial glucose disposal by reinstating both the signaling triggered by glucose and the flux of glucose into hepatocytes.

In summary, we have shown that restoration of hepatic glucokinase expression to a physiological level normalized postprandial hyperglycemia in mice with liver-specific PDK1 deficiency. Our results suggest that, among the various effects evoked by insulin in the liver, the mainte-

nance of glucokinase at an appropriate level is largely responsible for normal disposal of ingested glucose in the postprandial state. Agents that activate glucokinase hold promise as a novel treatment for diabetes (33). The findings of the present study may provide helpful information on which to base the clinical application of such agents.

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