Glucokinase Links Krüppel-Like Factor 6 to the Regulation of Hepatic Insulin Sensitivity in Nonalcoholic Fatty Liver Disease

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The polymorphism, KLF6-IVS1-27A, in the Krüppel-like factor 6 (KLF6) transcription factor gene enhances its splicing into antagonistic isoforms and is associated with delayed histological progression of nonalcoholic fatty liver disease (NAFLD). To explore a potential role for KLF6 in the development of insulin resistance, central to NAFLD pathogenesis, we genotyped KLF6-IVS1-27 in healthy subjects and assayed fasting plasma glucose (FPG) and insulin sensitivities. Furthermore, we quantified messenger RNA (mRNA) expression of KLF6 and glucokinase (GCK), as an important mediator of insulin sensitivity, in human livers and in liver tissues derived from a murine Klf6 knockdown model (DeltaKlf6). Klf6 overexpression studies in a mouse hepatocyte line were utilized to mechanistically link KLF6 with Gck promoter activity. KLF6-IVS1-27Gwt (i.e., less KLF6 splicing) was associated with stepwise increases in FPG and insulin and reduced hepatic insulin sensitivity. KLF6 binds to the liver-specific Gck promoter and activates a GCK promoter-reporter, identifying GCK as a KLF6 direct transcriptional target. Accordingly, in DeltaKlf6 hepatocytes Gck expression was reduced and stable transfection of Klf6 led to up-regulation of Gck. GCK and KLF6 mRNAs correlate directly in human NAFLD tissues and immunohistochemistry studies confirm falling levels of both KLF6 and GCK in fat-laden hepatocytes. In contrast to full-length KLF6, splice variant KLF6-SV1 increases in NAFLD hepatocytes and inversely correlates with glucokinase regulatory protein, which negatively regulates GCK activity. Conclusion: KLF6 regulation of GCK contributes to the development of hepatic insulin resistance. The KLF6-IVS1-27A polymorphism, which generates more KLF6-SV1, combats this, lowering hepatic insulin resistance and blood glucose. (Hepatology 2012;55:1083-1093)

The prevalence of nonalcoholic fatty liver disease (NAFLD), the hepatic manifestation of the metabolic syndrome, is rising dramatically.1 The factors conferring a risk of progression to cirrhosis include advanced age, increased body mass index (BMI), and an elevated fasting plasma glucose (FPG) associated with insulin resistance (IR).2 In addition to metabolic and anthropometric factors, genetic factors,
such as polymorphisms in adiponutrin (PNPLA3), are associated with the progression risk of NAFLD. Among these we have identified the Krüppel-like factor 6 (KLF6) genotype as a predictor of NAFLD histological stage. KLF6 is a ubiquitously expressed transcription factor established as an immediate early gene in activated hepatic stellate cells (HSCs) after liver injury and as a tumor suppressor gene in a range of tissues including the liver. A functional intron 1 single nucleotide polymorphism (SNP), KLF6-IVS1-27G>A (rs3750861), creates a novel binding site for the splicing factor, SRp40, and promotes alternative splicing of KLF6 into antagonistic, truncated isoforms. Among these isoforms, KLF6 splice variant 1 (KLF6-SV1) promotes cell growth while reducing differentiation in cancer cells and tumors in vivo. The roles of KLF6 and KLF-SV1 in nonmalignant cellular homeostasis, however, are unknown.

To elucidate the mechanisms underlying KLF6’s contribution to NAFLD, we explored the association of KLF6-IVS1-27G>A with metabolic factors predictive of disease progression. Here we report a significant and independent association of the KLF6-IVS1-27A allele with lower FPG and increased hepatic insulin sensitivity. Glucokinase (GCK) is a major determinant of hepatic glucose metabolism and is closely associated with hepatic glucose disposal and production in response to glucose and insulin. Further, we suggest that there is a mechanistic link between KLF6 isoforms and the regulation of GCK and the glucokinase regulatory protein (GCKR).

Materials and Methods

Characterization of Human Subjects. A total of 1,276 healthy subjects were recruited from the RISC (Relationship between Insulin Sensitivity and Cardiovascular Disease) cohort, as approved by the local Ethics Committees. Subjects with diabetes, hypertension, dyslipidemia, or a history of liver disease were excluded. Subjects underwent detailed anthropometric assessment and a 75 g oral glucose tolerance test (OGTT). Mouse Model. Experiments were approved and conform to regulations of the Institutional Animal Care and Use Committee (IACUC). Mice with a floxed Klf6 targeting vector (C57BL/6;129Sv, Genentech, San Francisco, CA) were crossed with mice expressing Cre recombinase (Cre) under control of the albumin promoter (B6.Cg-Tg(Alb-cre)21Mgn/J; Jackson Laboratories, Bar Harbor, ME). After backcrossing, male offspring expressing Cre with two floxed KLF6 alleles were used as the experimental group (“DeltaKlf6”). Mice with two floxed alleles and no Cre expression were used as controls (wildtype, WT). Temperature, humidity, and light-dark cycle conditions were controlled, mice were allowed food and water ad libitum, and were euthanized at 3 months following an overnight fast. Caval blood was drawn and glucose quantified with a handheld device (LifeScan, Milpitas, CA). Liver tissue was conserved for histology, RNA, and protein isolation. Serum insulin and colorimetric glycogen were quantified using commercial enzyme-linked immunosorbent assay (ELISA) kits (Biovision, Mountain View, CA), whereas GCK was by IF-staining and...
western blot with an anti-GCK antibody (H-88) (Santa Cruz Biotechnology, Santa Cruz, CA).

**Supporting Methods.** Supporting methods describing stable Klf6 expression in mouse hepatocytes, liver GCK promoter characterization, real-time polymerase chain reaction (PCR) analyses, studies on NAFLD liver tissues, and statistical analyses are detailed as online Supporting information.

**Results**

**KLF6-IVS1-27G>A Is Associated with Lower Fasting Plasma Glucose and Fasting Plasma Insulin.** We first sought to expand our previous study on the KLF6-IVS1-27G>A SNP in NAFLD in an independent cohort. In normal subjects there was a highly significant association between the KLF6 genotype and fasting blood glucose (FPG) based on linear trend analysis of the genotype groups (AA, AG, and GG) (Table 1A). Additionally, fat mass was lower in the KLF6-IVS1-27G>A group by univariate analysis (AA versus AG+GG). Importantly, the association between FPG and KLF6 genotype was significant even when corrected for fat mass or BMI, as well as age, sex, and recruitment center. Trends in fasting plasma insulin (FPI) levels were also significant and paralleled those of FPG.

**KLF6-IVS1-27G>A Is Associated with Enhanced Insulin Sensitivity and Insulin Clearance.** We next sought to elucidate the mechanisms underlying the protective effect of KLF6-IVS1-27G>A on glucose homeostasis. Insulin sensitivity assessed by the hyperinsulinemic clamp (M/I), a measure primarily of extrahepatic glucose disposal in euglycemic conditions, was not significantly different in the three genotypes. Because hepatic glucose disposal is markedly dependent on the route of glucose delivery and its concentration in the portal vein,19 the OGTT, and insulin sensitivity based on the OGTT data (OGIS),16 provides greater insight into hepatic insulin sensitivity. The glucose profile during the OGTT was identical in all three KLF6 genotype groups (Fig. 1A). Remarkably, however, the initial insulin plasma level was significantly lower in the KLF6-IVS1-27AA group (30-min; \( P = 0.006 \) relative to GG; Fig. 1B) and there was a trend toward lower insulin levels at later timepoints.

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**Table 1. KLF6 Genotype NAFLD-Related Associations in Normal Individuals**

<table>
<thead>
<tr>
<th>KLF6</th>
<th>AA (1249)</th>
<th>GA (1234)</th>
<th>GG (1234)</th>
<th>Linear Trend</th>
<th>AA vs. AG + GG</th>
<th>AA + AG vs. GG</th>
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</thead>
<tbody>
<tr>
<td>Number</td>
<td>12</td>
<td>155</td>
<td>1082</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>42.08±2.95</td>
<td>45.74±0.68</td>
<td>43.57±0.25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex (M:F)*</td>
<td>6:6</td>
<td>7:17:21</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>23.72±1.29</td>
<td>25.97±0.33</td>
<td>25.53±0.12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat mass (kg/m²)</td>
<td>15.43±2.37</td>
<td>22.12±0.75</td>
<td>20.94±0.27</td>
<td>0.023</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>4.79±0.20</td>
<td>5.01±0.05</td>
<td>5.08±0.02</td>
<td>0.003</td>
<td>ns</td>
<td>0.009</td>
</tr>
<tr>
<td>FPI (pmol/l)</td>
<td>20.21±3.47</td>
<td>31.36±1.51</td>
<td>32.86±0.68</td>
<td></td>
<td>0.022</td>
<td>(0.050)</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.95±0.14</td>
<td>1.37±0.07</td>
<td>1.36±0.03</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OGIS</td>
<td>486.1±19.7</td>
<td>442.8±5.11</td>
<td>441.5±2.04</td>
<td></td>
<td>0.042</td>
<td>ns</td>
</tr>
<tr>
<td>M/1 μmol min⁻¹ (kg fat-free mass)⁻¹ nmol⁻¹</td>
<td>159.8±25.6</td>
<td>143.5±5.48</td>
<td>137.7±2.21</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>icl_OGTT (/min/m²)</td>
<td>2.20±0.29</td>
<td>1.65±0.65</td>
<td>1.62±0.56</td>
<td></td>
<td>(0.051)</td>
<td>KW</td>
</tr>
<tr>
<td>TG (mmol/l)</td>
<td>79.00±7.92</td>
<td>110.05±6.09</td>
<td>107.40±2.23</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>WC (cm)</td>
<td>82.41±4.76</td>
<td>87.70±1.01</td>
<td>86.58±0.38</td>
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</table>

**Table 1A includes variables classed by genotype for the cohort of 1249 individuals, while Table 1B presents subset data for those in which endogenous glucose production (EGP) was measured. Nonparametric comparisons for quantitative variables was by Mann-Whitney testing. Linear trend analyses were corrected for differences in age, sex, center, and BMI, as were combined group comparisons using the General Linear Model (GLM).**

BMI, body mass index; HOMA-IR, homeostatic model assessment of insulin resistance; OGIS, oral glucose insulin sensitivity index; M/I, whole-body insulin sensitivity measured by hyperinsulinemic clamp; FPI, Fasting Plasma Insulin; TG, triglycerides; WC, waist circumference; GCR_b, Basal glucose clearance (production/concentration); icl_OGTT, endogenous prehepatic clearance during OGTT. ns: not significant; na: not appropriate; KW, Kruskal Wallis test.
Insulin secretion was the same across the genotype groups (data not shown), indicating that lower plasma insulin levels (Fig. 1B) for the KLF6-IVS1-27AA genotype were not due to insulin secretion. This suggests a greater hepatic clearance of insulin, resulting in a lower peripheral level. Endogenous “hepatic” clearance of insulin (icl-OGTT) has been estimated from insulin secretion and concentration during the OGTT; this parameter was significantly higher in KLF6-IVS1-27AA individuals (Table 1A).

Despite the reduced insulin concentration attributed to increased insulin clearance in KLF6-IVS1-27AA individuals, the glucose concentrations during the OGTT were not increased, suggesting increased insulin sensitivity. Although the hyperinsulinemic clamp data reflecting extrahepatic glucose disposal showed no significant differences in peripheral insulin sensitivity, insulin sensitivity estimated by OGIS during the OGTT was significantly associated with KLF6 genotype (Table 1A). The association of KLF6 genotype with OGIS but not M/I is again suggestive of a difference in the hepatic insulin clearance and sensitivity, rather than peripheral reaction to insulin.

KLF6-IVS1-27G>A Is Associated with Increased Hepatic Insulin Sensitivity. To explore an association with hepatic insulin sensitivity, EGP was measured after an overnight fast by infusion of 6,6-2H₂-glucose in a subgroup of 367 patients from nine centers. EGP was identical in the KLF6 genotype groups (Table 1B). However, when EGP was corrected for the insulin concentration (EGP × fasting insulin), which is a measure of hepatic insulin resistance, there was a stepwise change indicating increased insulin sensitivity associated with the KLF6 IVS1-27<A allele (KLF6_AA 343 ± 102; KLF6_GA 422 ± 44; KLF6_GG 494 ± 19; P = 0.023). Thus, KLF6-IVS1-27AA individuals have both increased hepatic insulin sensitivity and insulin clearance. The hepatic insulin resistance index was correlated with the insulin clearance estimate (Spearman rho −0.44, P < 0.0001).

Glucokinase Expression Is Reduced in DeltaKlf6 Mouse Livers. GCK is the rate-limiting enzyme in glycolysis and its expression in the liver is closely associated with hepatic glucose metabolism in response to glucose and insulin. An hepatic GCK promoter polymorphism is associated with altered hepatic insulin resistance and underscores the potential role of GCK as a KLF6-regulated target. Moreover, GCK activity is closely linked to the expression of GCKR, which determines GCK activity, subcellular location, and GCK protein stability.

To explore the role of KLF6 in the regulation of GCK, we generated mice with reduced expression of Klf6 (DeltaKlf6) in hepatocytes by crossing Klf6 floxed mice with animals expressing albumin-Cre. Cre expression was gradually increased during the first 6 weeks and tissues were harvested at 11-12 weeks of age. Immunohistochemistry confirmed a clear reduction in hepatocyte expression of Klf6 (Fig. 2). There were no clear phenotypic differences (hematoxylin and eosin, Fig. 2), although there was a trend toward higher FBG, HOMA-IR, and triglyceride levels (Supporting Table 3). In whole liver lysates, Klf6 messenger RNA (mRNA) was not in fact reduced in the DeltaKlf6 mice (Fig. 3A) and this was attributed to the reduction in hepatocytes being masked by a relative increase in KLF6 protein in hepatic perisinusoidal cells, as evident from immunohistochemistry of liver sections (Fig. 2) and as reported for other genes targeted in the albumin-Cre model. In contrast, in hepatocytes isolated from DeltaKlf6 mice, Klf6 was
markedly reduced at mRNA and protein levels (Fig. 3D,F). Both in vivo and in hepatocytes in vitro, Gck mRNA and protein were markedly down-regulated in the DeltaKlf6 mice relative to WT littermates (Figs. 2, 3D,F). Notably, in whole tissues there were no significant changes in the expression of other transcription factors that regulate Gck, including Hnf4a, Hnf6, or Usf1, or in hexokinase 2, which is expressed at low levels in hepatocytes26 (Fig. 3B). In isolated hepatocytes, the negative regulator Gckr was reduced

![H&E (x40)](image1)

![Klf6(x40)](image2)

![Gck (x40)](image3)

**Fig. 2. Reduced Klf6 and glucokinase expression in vivo DeltaKlf6 mice.** Hematoxylin and eosin (H&E) stains for WT and DeltaKlf6 mice were similar with no apparent phenotypic differences at 3 months of age. KLF6 IHC revealed a decreased expression of KLF6 within hepatocytes (4.1-fold fewer positive nuclei by manual counting; \( P = 0.002 \)) rather than nonparenchymal cells. GCK immunofluorescent staining (1,300 ms exposure time, DAPI blue, GCK red) confirmed a relative abundance (1.92-fold by integrated density quantification, \( P = 0.038 \)) of hepatocyte Gck expression in WT versus DeltaKlf6 mice.

**Fig. 3. Reduced Klf6 and glucokinase expression in DeltaKlf6 mice.** Although expression of Klf6, Gck, and Gckr mRNA was not significantly suppressed in whole liver tissues in DeltaKlf6 mice compared to WT littermates (n = 7) (A), levels were markedly suppressed in primary hepatocytes isolated from DeltaKlf6 mice (n = 3) (D). Data are presented as fold change (FC) relative to WT littermates. Known transcriptional activators of Gck were not reduced in the presence of reduced Gck in deltaKlf6 mice (B,E). Western blot of both whole tissues and isolated hepatocytes confirmed decreased Gck protein in association with reduced Klf6 (*\( P < 0.05 \); **\( P < 0.005 \)).
(Fig. 3D,F), whereas significant increases in Hnf4a and Hnf6 mRNAs were observed (Fig. 3E). It is unlikely, therefore, that these factors contributed to the Gck decrease in the DeltaKlf6 mice.

**Klf6 Expression in Mouse Hepatocytes Increases Gck.** To further link Klf6 directly to Gck expression in hepatocytes, we examined the impact of stable Klf6 complementary DNA (cDNA) expression on Gck expression in the AML12 mouse hepatocyte cell line, which retains major hepatocyte characteristics.\(^{27}\) In cells stably expressing full-length Klf6 cDNA, an increase in Gck mRNA and protein was evident (Fig. 4A,C). Consistent with the observations in DeltaKlf6 mice, Hnf4a expression was significantly decreased in these cells, most likely as part of a counterregulatory mechanism (Fig. 4B). Immunofluorescence confirmed hepatocyte accumulation of GCK protein (Supporting Fig. 1).

**Gck Promoter Is a Direct Transcriptional Target of Klf6.** To test for direct transcriptional activation of the GCK promoter by Klf6, AML12 cells were cotransfected with the Klf6 cDNA and a Gck-luc promoter reporter containing candidate KLF6 binding sites.\(^{28}\) Cotransfection with Klf6 increased Gck promoter activity (Supporting Fig. 2A). Interaction of KLF6 with the endogenous Gck promoter at two defined sites containing putative KLF6 binding sites was confirmed (Supporting Fig. 2B) by chromosomal immunoprecipitation (ChIP).

**KLF6 and GCK Expression Are Directly Correlated in NAFLD Livers.** We analyzed KLF6 and GCK expression in 28 precirrhotic NAFLD biopsy samples classed as having mild (grade 1) or severe (grades 2 and 3) hepatocyte steatosis. Although we have previously shown increased KLF6 expression in NAFLD livers in association with inflammation and fibrosis,\(^{5}\) the data presented here demonstrate a significant reduction of the full-length isoform in association with increased steatosis (Pearson correlation KLF6-FL and steatosis: \(-0.445; P = 0.018; 1.34 \pm 0.126 \text{ versus } 0.91 \pm 0.122; t \text{ test } = 0.018, \text{ Fig. 5C}). This apparent discrepancy is clarified by immunohistochemistry (IHC) data which reveal differential KLF6 expression in distinct populations of liver cells. IHC studies in normal liver using KLF6 monoclonal 1A9 antibody (detecting all KLF6 isoforms) demonstrate predominantly nuclear KLF6 expression in hepatocytes, portal tract cells, and occasional sinusoidal cells (Fig. 6Ai). In more advanced disease (Fig. 6Ci), there is a dramatic increase in KLF6 expression, but this is notably in a mixed inflammatory cell population within portal tracts and in sinusoidal cells, rather than hepatocytes. Parallel IHC with a KLF6-SV1 isoform specific monoclonal antibody (2A2) shows the opposite, with no parallel increase in inflammatory cells, but with much more prominent expression in hepatocytes (Fig. 6Ci+ii). These data parallel isoform specific real-time PCR data in which, unlike KLF6-FL,
the KLF6-SV1 isoform is significantly increased with more advanced steatosis (Fig. 5C).

We explored the relationship between KLF6 and its target gene, GCK, in these NAFLD biopsies. Bivariate analysis of real-time PCR analyses of mRNA extracted from whole human liver confirmed a highly significant association between KLF6 and GCK (Pearson correlation 0.687; \( P < 0.0001 \)). By linear regression, corrected for other significantly associated variables (Supporting Table 4: center, fat and GCKR) the association between GCK and KLF6 remained independently significant (Fig. 5A). IHC studies in normal liver confirm GCK expression in hepatocytes, which is notably sequestered in the nucleus in association with nuclear GCKR. Although the role of KLF6-SV1 in NAFLD is presently unknown, our SNP association studies suggest a protective role in both chronic liver disease progression\(^5\) and enhanced hepatic sensitivity...
to insulin. It is interesting, therefore, that KLF6-SV1 is increasingly expressed in hepatocytes in the presence of steatosis, a marker not just of the severity of the insult, but possibly also of the liver’s ability to tolerate it. Further, there is a highly significant negative correlation between KLF6-SV1 and GCKR at mRNA levels (Fig. 5B). This is apparent also at the protein level, as when KLF6-SV1 expression is evident in the presence of increased steatosis, GCKR is not detectable (Fig. 6Cii–iv).

**Discussion**

Although NAFLD prevalence is increasing in Western societies, established predictors of disease progression to advanced stages are scarce. We previously demonstrated that the functional KLF6-IVS1-27G>A polymorphism correlates inversely with disease progression in NAFLD. Individuals with KLF6-IVS1-27G>A were less likely to have significant fibrosis when compared to those with the wildtype allele. In the work presented here, we have functionally linked KLF6 to components of the hepatic insulin resistance, which is central to the progression of NAFLD.

The association between the KLF6 genotype groups and FPG is novel and striking. Although the KLF6-IVS1-27A allele was also associated with a lower FPI, the euglycemic clamp data discount peripheral insulin resistance as the cause of the variation with genotype. Together, the OGIS and OGTT-derived data and absence of a difference in pancreatic beta cell insulin secretion support increased hepatic clearance of insulin and increased hepatic insulin sensitivity in KLF6-IVS1-27A individuals. This was subsequently confirmed by assessing endogenous glucose production relative to fasting insulin levels.

IR is an established key feature of the metabolic syndrome and increased resistance associated with fasting hyperglycemia is believed to contribute to hepatic steatosis in obese individuals. There is evidence, however, that insulin resistance is not essential for the development of steatosis. In hypobetalipoproteinemia, for example, fatty liver develops because of defective very low-density lipoprotein (VLDL) export in the absence of IR. Furthermore, whereas a fatty liver genome-wide association study (GWAS) identified the rs738409 C>G SNP in PNPLA3, which leads to a missense mutation (I148M), and is associated with increased liver fat, it is not associated with IR. Additional GWAS data in patients with fatty liver disease has further highlighted our incomplete understanding of the relationship between fatty liver disease and features of the metabolic syndrome. Although some of the steatosis-associated variations of candidate genes are associated with triglycerides and plasma LDL-cholesterol, for example, the opposite is true of other variants. Furthermore, the PP1R3B and GCKR polymorphisms are associated with a lower rather than a higher fasting glucose, in keeping with a relatively enhanced, rather than reduced, sensitivity to insulin. Therefore, whereas IR indisputably contributes to the progression of NAFLD to fibrosis and cirrhosis, its role in the development of steatosis is not as clear. Rather than being indicative of disease severity, in some individuals steatosis may instead be a biomarker of an enhanced ability to convert glucose to hepatic fat, conferring protection from an elevated blood glucose and reflecting enhanced insulin sensitivity.

Our own study focused on GCK, whose expression in the liver is closely associated with hepatic insulin sensitivity. In conjunction with GCKR, it is a major determinant of insulin responsive hepatic glucose metabolism, catalyzing the production of glucose-6-phosphate. Although the hepatic expression of GCK protein is reduced in cirrhosis attributed to both alcohol excess and primary biliary cirrhosis, its contribution to NAFLD has not previously been characterized. GCK’s vital role in determining blood glucose is underscored by the discoveries of over 600 hereditary mutations of GCK associated with glycemic disease. Subjects with a single inactivating mutant allele have a mild form of diabetes, associated with elevated FPG. Furthermore, recent data suggest a role for KLF6 in response to glucose stimulation. GCK activity is closely linked to the abundance of its regulator, GCKR, and small changes in the molar ratio of GCK/GCKR protein markedly impact hepatic glucose metabolism. In the fasting state GCK is sequestered in an inactive state in the nucleus, bound to GCKR. However, after a meal glucose and insulin act synergistically in causing rapid dissociation of GCK from GCKR and translocation to the cytoplasm.

Our *in vivo* and *in vitro* murine data demonstrate that down-regulation of Klf6 in hepatocytes is associated with reduced Gek, whereas Klf6 overexpression increases Gek. ChIP and reporter studies confirm direct transactivation of the Gek promoter by Klf6, leading us to propose Klf6 as an additional mediator of glucose homeostasis. The normal phenotype of DeltaKlf6 mice is consistent with published data from liver-specific Gek knockout mice as well as Gekr knockout mice, in which no differences in fasting glucose levels were detected. Biopsy studies from patients with histologically scored NAFLD confirm a significant
association between \textit{KLF6-FL} and \textit{GCK} mRNA expression in human tissues. Although the significant reductions of \textit{KLF6-FL} and \textit{GCK} in individuals with more advanced versus mild steatosis may simply be representative of more advanced disease, they may also represent effectors of the development of resistance of hepatocytes to insulin.

In human tissues, several alternative splice forms of \textit{KLF6} have been identified, and the presence of the \textit{KLF6-IVS1-27G}>A allele promotes their generation.\textsuperscript{5} To date, \textit{Klf6} splicing in the mouse has not been described, precluding our ability to study the effects of murine dominant negative splice variants on \textit{Gck} or \textit{Gckr} in an \textit{in vivo} model. The mechanism underlying the enhanced hepatic insulin sensitivity in human individuals with the \textit{KLF6-SV1}-promoting \textit{KLF6-IVS1-27G}>A SNP is presently unknown. However, our expression data identifying a highly significant negative correlation between \textit{KLF6-SV1} and \textit{GCKR} suggests that antagonism of \textit{GCKR}, the negative regulator of \textit{GCK}, is one potential mechanism. Here we are able to draw a link to the recently published data on SNPs in \textit{GCKR}. The \textit{GCKR} rs780094 GWAS identified SNP\textsuperscript{54,47} was previously associated with higher triacylglycerol, reduced insulin levels, and a reduced risk of type 2 diabetes.\textsuperscript{48,49} \textit{GCKR} rs780094 is commonly inherited with a \textit{GCKR} coding SNP, rs1260326 (Pro446Leu), which codes for a mutant and inactive form of \textit{GCKR}.\textsuperscript{50} The consequences include enhanced \textit{GCK} activity and reduced FPG, as well as increased \textit{de novo} lipogenesis attributed to enhanced production of malonyl coA, the substrate for fatty acid synthesis.\textsuperscript{51} Similar to this phenotypically altered \textit{GCKR} variant, we hypothesize that \textit{KLF6-SV1} contributes to the lowering of FPG as a result of increased \textit{GCK} activity brought about by the antagonism of \textit{GCKR}, as summarized in Fig. 7.

In conclusion, we propose \textit{KLF6} as an additional regulator of fasting plasma glucose and hepatic insulin sensitivity. Understanding the interactions between \textit{KLF6} and \textit{KLF6-SV1}, as well as their roles in regulation of expression of \textit{GCK} and \textit{GCKR}, may help us to clarify the role(s) of NAFLD in the metabolic syndrome, as well as its progression to more advanced disease.

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ing protein-1c, peroxisome proliferator-activated receptor gamma, and odd heterodimer partner in the transcriptional regulation of glucoki-


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