

# Role of KLF15 in Regulation of Hepatic Gluconeogenesis and Metformin Action

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**OBJECTIVE**—An increase in the rate of gluconeogenesis is largely responsible for the hyperglycemia in individuals with type 2 diabetes, with the antidiabetes action of metformin being thought to be achieved at least in part through suppression of gluconeogenesis.

**RESEARCH DESIGN AND METHODS**—We investigated whether the transcription factor KLF15 has a role in the regulation of gluconeogenesis and whether KLF15 participates in the antidiabetes effect of metformin.

**RESULTS**—Here we show that KLF15 regulates the expression of genes for gluconeogenic or amino acid–degrading enzymes in coordination with the transcriptional coactivator peroxisome proliferator–activated receptor  $\gamma$  coactivator 1 $\alpha$ . Liver-specific ablation of KLF15 in diabetic mice resulted in downregulation of the expression of genes for gluconeogenic or amino acid catabolic enzymes and in amelioration of hyperglycemia. Exposure of cultured hepatocytes to metformin reduced the abundance of KLF15 through acceleration of its degradation and downregulation of its mRNA. Metformin suppressed the expression of genes for gluconeogenic or amino acid–degrading enzymes in cultured hepatocytes, and these effects of metformin were attenuated by restoration of KLF15 expression. Administration of metformin to mice inhibited both the expression of KLF15 and glucose production in the liver, the latter effect also being attenuated by restoration of hepatic KLF15 expression.

**CONCLUSIONS**—KLF15 plays an important role in regulation of the expression of genes for gluconeogenic and amino acid–degrading enzymes and that the inhibitory effect of metformin on gluconeogenesis is mediated at least in part by downregulation of KLF15 and consequent attenuation of the expression of such genes. *Diabetes* 59:1608–1615, 2010

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An increase in the rate of gluconeogenesis is one of the most important pathological disorders in individuals with diabetes. Regulation of gluconeogenesis in the liver is thought to be achieved through control of the expression of genes for gluconeogenic enzymes such as phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase) (1). A variety of transcription factors, including cAMP-responsive element-binding protein (CREB), forkhead factor O1, and signal transducer and activator of transcription 3, as well as transcriptional coactivators such as CREB-binding protein (CBP), CREB-regulated transcription coactivator 2 (CRTC2, previously known as transducer of regulated CREB activity 2), and peroxisome proliferator–activated receptor  $\gamma$  coactivator 1 $\alpha$  (PGC1 $\alpha$ ) have been shown to participate in the hormonal regulation of genes for gluconeogenic enzymes in the liver (2–8).

We have previously shown that the hepatic abundance of Krüppel-like factor 15 (KLF15), a transcription factor that is highly expressed in the liver, is upregulated in fasted or diabetic mice and that forced expression of KLF15 in cultured hepatocytes increased the expression of the PEPCK gene (9), suggesting that KLF15 contributes to the regulation of gluconeogenesis in the liver. Mice deficient in KLF15 were subsequently found to manifest lower blood glucose levels in the fasted state as well as a smaller increase in blood glucose concentration when challenged by gluconeogenic substrates compared with control animals (10), confirming the notion that KLF15 plays an important role in gluconeogenesis. KLF15-deficient mice also manifested a decrease in the expression of genes for enzymes that mediate amino acid degradation, including those for alanine aminotransferase 1 (ALT1), 4-hydroxyphenylpyruvate dioxygenase (HPD), proline dehydrogenase (ProDH), and tryptophan 2,3-dioxygenase (TDO2) (10). Given that amino acids are major precursors for gluconeogenesis and must be catabolized before they can be used in gluconeogenic reactions, the suppression of the expression of genes for amino acid–degrading enzymes probably contributes to the downregulation of gluconeogenesis in KLF15-deficient mice.

We have now further investigated the role of KLF15 in the regulation of gluconeogenesis. We also examined whether KLF15 might participate in the antidiabetes action of metformin, a drug that suppresses hepatic glucose production through inhibition of gluconeogenesis (11). We found both that KLF15 regulates the hepatic expression of genes for amino acid–degrading and gluconeogenic enzymes in coordination with the transcriptional coactivator PGC1 $\alpha$  and that KLF15 plays an important role in the suppression of hepatic glucose production by metformin.

## RESEARCH DESIGN AND METHODS

**Recombinant adenoviruses.** The nucleotide sequence corresponding to nucleotides 1,105–1,123 of mouse KLF15 mRNA (NM\_023184) was synthesized as complementary antiparallel oligomers with a loop sequence (ACGTGTGCTGCCGT): 5'-gtttGCGGTAAGATGTACATCAAACGTGTGCTGCCGT TTGGTGTACATCTTCTGCTTTT-3' (forward) and 5'-atgcAAAAAGCAG CAAGATGTACACCAACGACAGCAGCACAGCTTTGATGTACATCTTACCGC-3' (reverse). The forward and reverse oligonucleotides were annealed and then ligated into pcPURmU6cassette (Takara Bio, Ohtsu, Japan), which contains the mouse U6 gene promoter. For production of adenoviral vectors encoding the KLF15 short hairpin RNA (shRNA) or containing the U6 promoter alone, the DNA sequence corresponding either to the shRNA construct together with the U6 promoter or to the U6 promoter alone was excised from the pcPURmU6cassette vector and ligated into the pAxcwitt cosmid cassette (Takara Bio). Adenoviruses encoding KLF15 shRNA (AxshKLF15) or containing the U6 promoter alone (AxU6, control) were then generated with the use of an Adenovirus Expression Vector Kit (Takara Bio, Shiga, Japan) as described (12). For production of an adenoviral vector encoding Flag-tagged KLF15, mouse KLF15 cDNA was generated by PCR and ligated into pCMV-tag2A (Stratagene, La Jolla, CA), which contains the DNA sequence for the Flag tag. The DNA sequence for Flag-KLF15 was then excised from the pCMV-tag2A vector, and an adenovirus encoding the tagged protein was generated with the use of an Adenovirus Expression Vector Kit as described previously (12). Adenoviral vectors for mouse PGC1 $\alpha$  and for  $\beta$ -galactosidase were as described previously (5,9).

**Gene expression analysis.** The expression of different genes was analyzed by RT and real-time PCR analysis with the use of a Sequence Detector (model 7900; Applied Biosystems, Carlsbad, CA) and with 36B4 mRNA as the invariant control, as described previously (5). The primers for mouse and rat G6Pase, PEPCK, and PGC1 $\alpha$  as well as for mouse Glut2, glucokinase, and pyruvate kinase were as described previously (5,9,13,14), and those for mouse and rat KLF15, mouse and rat HPD, mouse ALT1, mouse ProDH, mouse TDO2, rat CREB, and rat CRT2 are described in the supplementary Methods (available in an online appendix at <http://diabetes.diabetesjournals.org/cgi/content/full/db09-1679/DC1>).

**Immunoblot and ubiquitination analyses.** Polyclonal antibodies to KLF15 were generated as described previously (9). All other antibodies were from commercial sources, with details available on request. Nuclear extracts of mouse liver were prepared as described previously (15).

**Cells and liver-specific depletion of KLF15 in mice.** Rat hepatocytes (HL1c) (16) were provided by D.K. Granner, and primary cultured rat hepatocytes were prepared as described previously (9). HL1c cells or primary rat hepatocytes were exposed to the permeable cAMP analog 8-(4-chlorophenylthio)-cAMP (8-CPT-cAMP) or dexamethasone, as indicated, to induce KLF15 expression. All animal experiments were approved by the animal experimentation committee of Kobe University Graduate School of Medicine. Eight-week-old male C57BL/6 or *db/db* mice were injected via the tail vein with AxshKLF15 or AxU6 ( $1.0 \times 10^9$  plaque-forming units [pfu]) and were subjected to experiments at the indicated times thereafter. For pyruvate challenge, pyruvate (0.5 g/kg body mass) was injected as described previously (17).

**Clamp analysis.** An intravenous catheter was implanted into the cervical vein of 8-week-old male C57BL/6 mice, 4 days after which the mice were infected with an adenoviral vector encoding Flag-KLF15 or  $\beta$ -galactosidase via the tail vein at a dose of  $2.4 \times 10^8$  pfu. Four days after virus injection, the mice were deprived of food for 16 h and then subjected to clamp analysis. The animals were primed with [ $^3$ -H]glucose (0.05  $\mu$ Ci/min) for 90 min, and the euglycemic clamp was initiated by intravenous infusion of metformin (5 mg  $\cdot$  kg $^{-1}$   $\cdot$  min $^{-1}$ ), somatostatin (3  $\mu$ g  $\cdot$  kg $^{-1}$   $\cdot$  min $^{-1}$ ), and sufficient glucose to maintain the blood glucose concentration at 80–110 mg/dl. The clamp was maintained for 4 h, and hepatic glucose production (HGP) during the clamp was determined as described (18,19). To calculate the percent change in HGP, we subtracted the value at the end of the clamp from the value before the infusion of metformin and then divided the difference by the value before the infusion of metformin.

**Statistical analysis.** Data are presented as means  $\pm$  SEM and were compared between or among groups by a two-tailed unpaired Student *t* test or by one-way ANOVA followed by a Fisher least significant difference test. *P* < 0.05 was considered statistically significant.

## RESULTS

### Role of KLF15 in regulation of gluconeogenic genes.

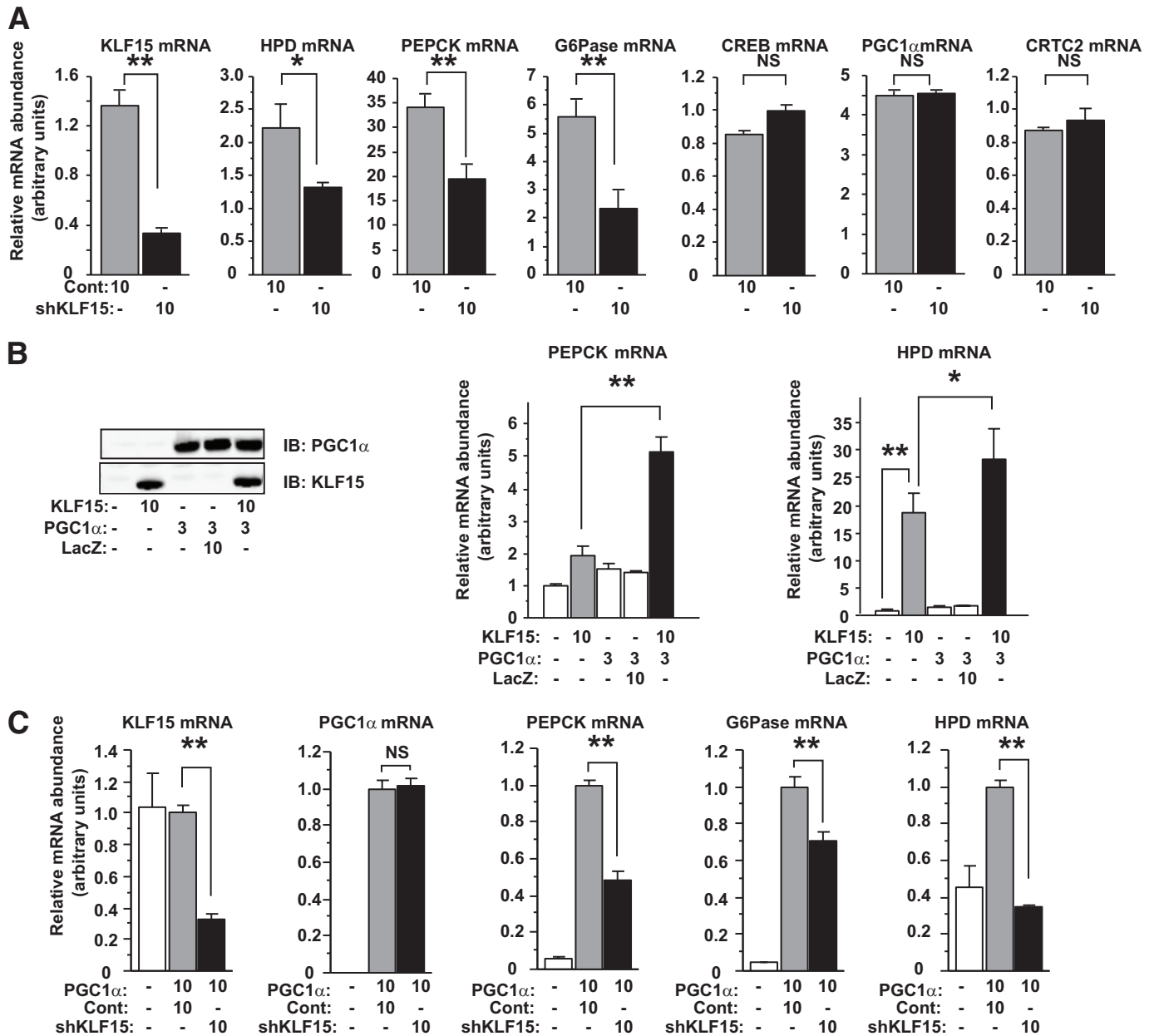
We have previously shown that forced expression of KLF15 in cultured hepatocytes increased both the expression of the PEPCK gene and the activity of the PEPCK gene promoter (9). The KLF class of transcription factors bind to a DNA region that conforms to CACCC (20). With

the use of a chromatin immunoprecipitation assay, we have now shown that KLF15 binds to a region of the PEPCK gene that contains this consensus binding motif (supplementary Fig. 1, available in the online appendix). Furthermore, KLF15 did not increase the activity of a modified form of the PEPCK gene promoter harboring mutations in this motif (supplementary Fig. 1), suggesting that KLF15 directly binds to and regulates the gene for PEPCK. We next examined the effect of KLF15 depletion by RNA interference (RNAi) on the expression of PEPCK and G6Pase genes in cultured hepatocytes. Infection of the cells with an adenoviral vector encoding a shRNA specific for KLF15 mRNA (AxshKLF15) resulted in a decrease in the abundance of KLF15 mRNA (Fig. 1A). Although the amounts of mRNAs for the amino acid-degrading enzymes ALT1 and ProDH in these cells were too small for quantitative evaluation (data not shown), that of HPD mRNA was decreased by depletion of KLF15. Infection with AxshKLF15 also reduced the abundance of PEPCK and G6Pase mRNAs without affecting that of CREB, PGC1 $\alpha$ , or CRT2 mRNAs (Fig. 1A), the products of all of which contribute to the regulation of gluconeogenic genes (2,3,7).

Given that PGC1 $\alpha$  is thought to play an important role in the regulation of gluconeogenesis in the liver, we next examined whether this transcriptional coactivator contributes to the regulation of gluconeogenic genes by KLF15. Consistent with previous observations (9,10), forced expression of KLF15 in cultured hepatocytes resulted in an increase in the abundance of HPD and PEPCK mRNAs (Fig. 1B). Forced expression of PGC1 $\alpha$  together with KLF15 increased the abundance of PEPCK and HPD mRNAs in a synergistic manner. Coimmunoprecipitation analysis revealed that ectopically expressed or endogenous KLF15 and PGC1 $\alpha$  form a complex in cultured hepatocytes (supplementary Fig. 2). These results thus suggested that KLF15 regulates the expression of genes for gluconeogenic or amino acid-degrading enzymes in coordination with PGC1 $\alpha$ . Forced expression of PGC1 $\alpha$  alone increased the abundance of PEPCK, G6Pase, and HPD mRNAs, and depletion of KLF15 by RNAi attenuated these effects (Fig. 1C), indicating the importance of KLF15 in PGC1 $\alpha$ -dependent expression of the genes for gluconeogenic or amino acid-degrading enzymes. However, the effects of KLF15 on the expression of these genes appear not to be totally dependent on PGC1 $\alpha$ , given that forced expression of KLF15 alone increased the abundance of PEPCK and HPD mRNAs in HL1c cells (Fig. 1B), which do not express endogenous PGC1 $\alpha$  (Fig. 1C).

### Effects of acute depletion of KLF15 in mouse liver.

We next investigated the effects of acute depletion of KLF15 in the liver of living animals. Systemic infusion of adenoviral vectors encoding shRNAs results in specific ablation of target proteins in the liver (3,7,21). Injection of C57BL/6 mice with AxshKLF15 resulted in a reduction in the hepatic abundance of KLF15 mRNA as well as in that of PEPCK and G6Pase mRNAs, whereas the amounts of mRNAs for PGC1 $\alpha$ , GLUT2, glucokinase, and pyruvate kinase, all of which contribute to hepatic glucose metabolism, were not affected (Fig. 2A). Infection with AxshKLF15 also significantly reduced the hepatic abundance of ALT1 and TDO2 mRNAs and tended to reduce the amounts of HPD and ProDH mRNAs. Insulin-induced phosphorylation of the insulin receptor, Akt, and forkhead factor O1 as well as the abundance and phosphorylation of CREB in the liver were not affected by infection with AxshKLF15

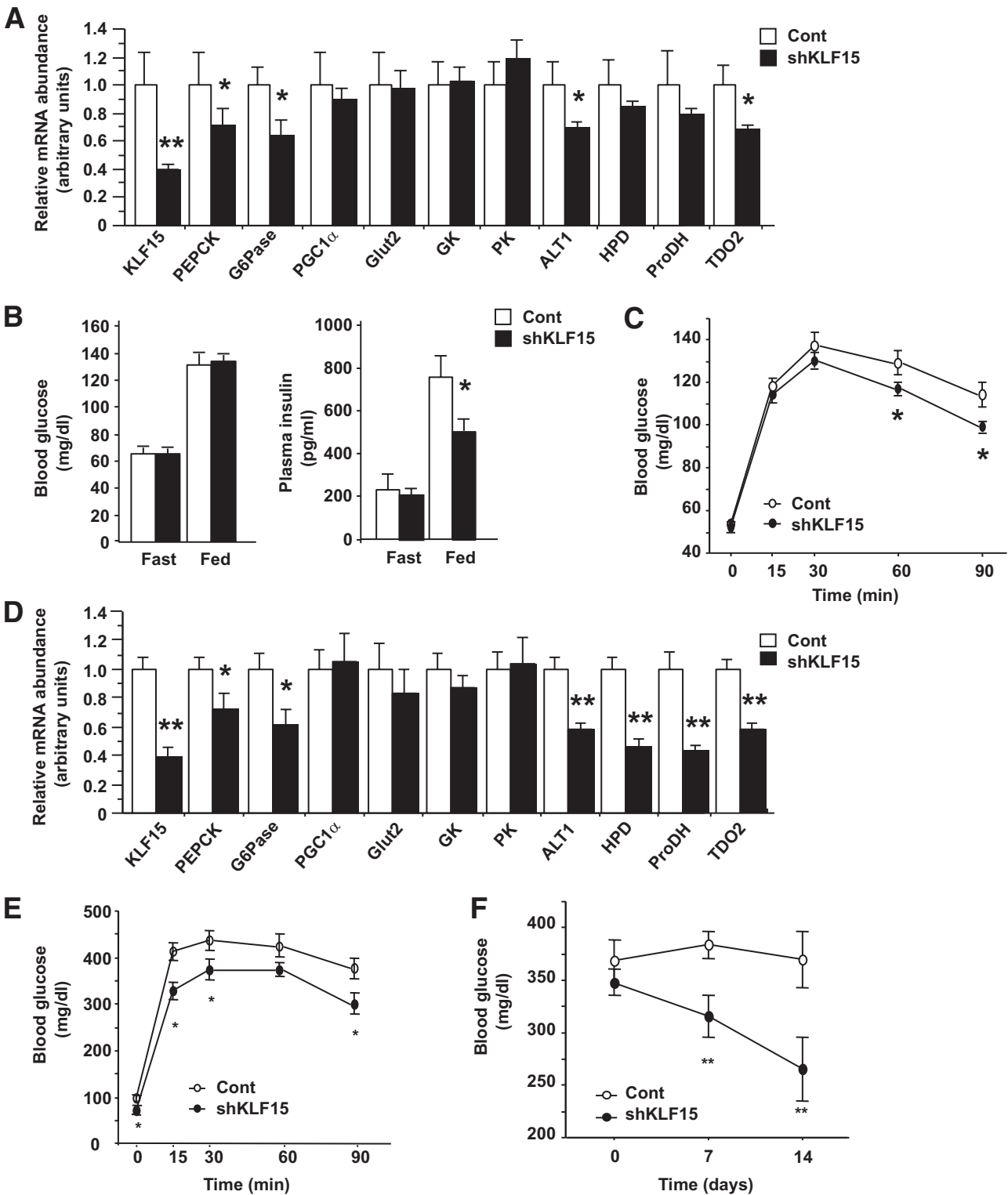


**FIG. 1.** Regulation by KLF15 of genes related to gluconeogenesis or amino acid degradation. *A*: Rat primary cultured hepatocytes that had been infected with an adenovirus encoding KLF15 shRNA (shKLF15) or with a control virus containing the U6 gene promoter alone (Cont) at a multiplicity of infection (MOI) of 10 pfu per cell were incubated for 6 h in the presence of the cell-permeable cAMP analog 8-CPT-cAMP at 100  $\mu$ M. The abundance of the indicated mRNAs was then determined by RT and real-time PCR analysis. *B*: HL1c rat hepatocytes that had been infected with adenoviruses encoding KLF15, PGC1 $\alpha$ , or LacZ at the indicated MOIs (plaque-forming units per cells) were subjected to immunoblot (IB) analysis of PGC1 $\alpha$  and KLF15 (*left panel*) or to RT and real-time PCR analysis of PEPCK and HPD mRNAs (*middle and right panels*). *C*: HL1c cells that had been infected (or not) with adenoviruses either encoding KLF15 shRNA, containing the U6 promoter alone (Cont), or encoding PGC1 $\alpha$  (each at an MOI of 10 pfu/cell) were subjected to RT and real-time PCR analysis of the indicated mRNAs. All quantitative data are means  $\pm$  SEM from three independent experiments. \* $P$  < 0.05, \*\* $P$  < 0.01.

(supplementary Fig. 3, available in an online appendix). Blood glucose and plasma insulin levels were not altered in mice injected with AxshKLF15 in the fasted state (Fig. 2B), suggesting that gluconeogenesis was not significantly reduced in the fasted state. However, plasma insulin concentration in the fed state was reduced in these animals, possibly reflecting suppression of gluconeogenesis in the fed state. Pyruvate is a substrate for gluconeogenesis, and the increase in blood glucose level induced by pyruvate challenge is dependent on the activity of gluconeogenic enzymes (17). The increase in the blood glucose level during a pyruvate challenge was attenuated in

mice injected with AxshKLF15 (Fig. 2C). These results thus suggested that acute depletion of KLF15 in the liver of mice results in the suppression both of hepatic gluconeogenesis and of the expression of genes for gluconeogenic and amino acid-degrading enzymes.

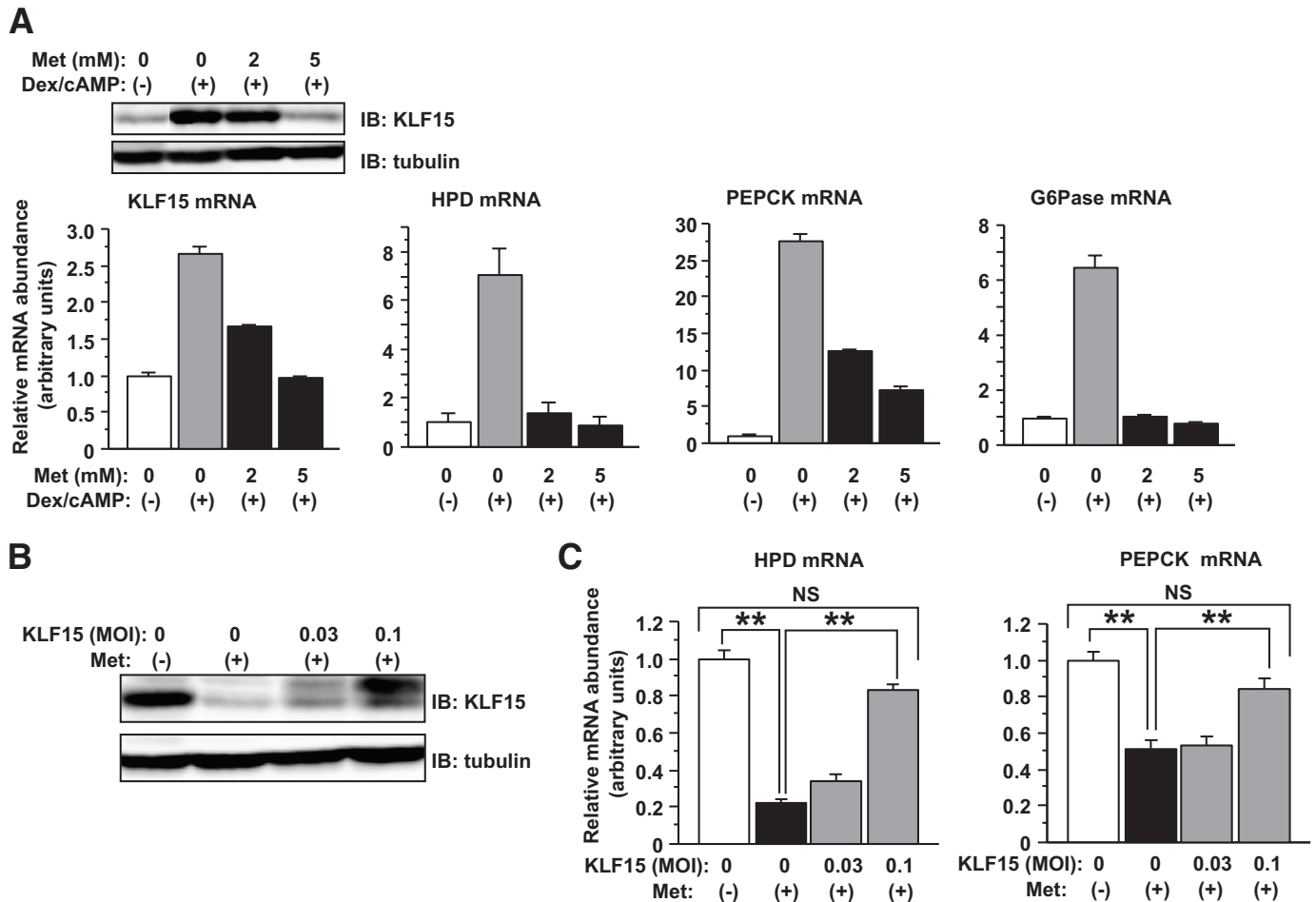
We have previously shown that the hepatic abundance of KLF15 is increased in *db/db* mice (9), which lack functional leptin receptors and develop obesity and diabetes. We next tested the effects of acute depletion of hepatic KLF15 in *db/db* mice. Downregulation of the hepatic abundance of KLF15 by infection with AxshKLF15 reduced hepatic expression of both the genes for the glu-



**FIG. 2.** Effects of acute depletion of KLF15 in mouse liver on hepatic gene expression as well as on blood glucose and plasma insulin concentrations. **A–C:** C57BL/6 mice were injected with an adenovirus encoding KLF15 shRNA or a control adenovirus containing the U6 promoter alone (Cont). The hepatic abundance of the indicated mRNAs ( $n = 6$ ) (**A**), blood glucose and plasma insulin concentrations in the fasted or randomly fed states ( $n = 6$ ) (**B**), and blood glucose concentration during pyruvate challenge ( $n = 8$  and 12 for Cont and shKLF15, respectively) (**C**) were determined 7 days after virus injection. **D–F:** *db/db* mice injected with the same adenoviral vectors were examined for the hepatic abundance of the indicated mRNAs ( $n = 9$  and 11 for Cont and shKLF15, respectively) (**D**) or for blood glucose concentration during pyruvate challenge ( $n = 8$ ) (**E**) at 7 days after virus injection. Blood glucose concentration in the randomly fed state was also determined at the indicated times after virus injection ( $n = 9$  and 11 for Cont and shKLF15, respectively) (**F**). All data are means  $\pm$  SEM. \* $P < 0.05$ , \*\* $P < 0.01$  vs. the corresponding Cont value.

coneogenic enzymes PEPCK and G6Pase and those for the amino acid-degrading enzymes ALT1, HPD, and ProDH in *db/db* mice (Fig. 2D). The abundance of PGC1 $\alpha$ , GLUT2,

glucokinase, and pyruvate kinase mRNAs was not affected. The insulin-induced phosphorylation and the abundance of different signaling molecules in the liver of *db/db*



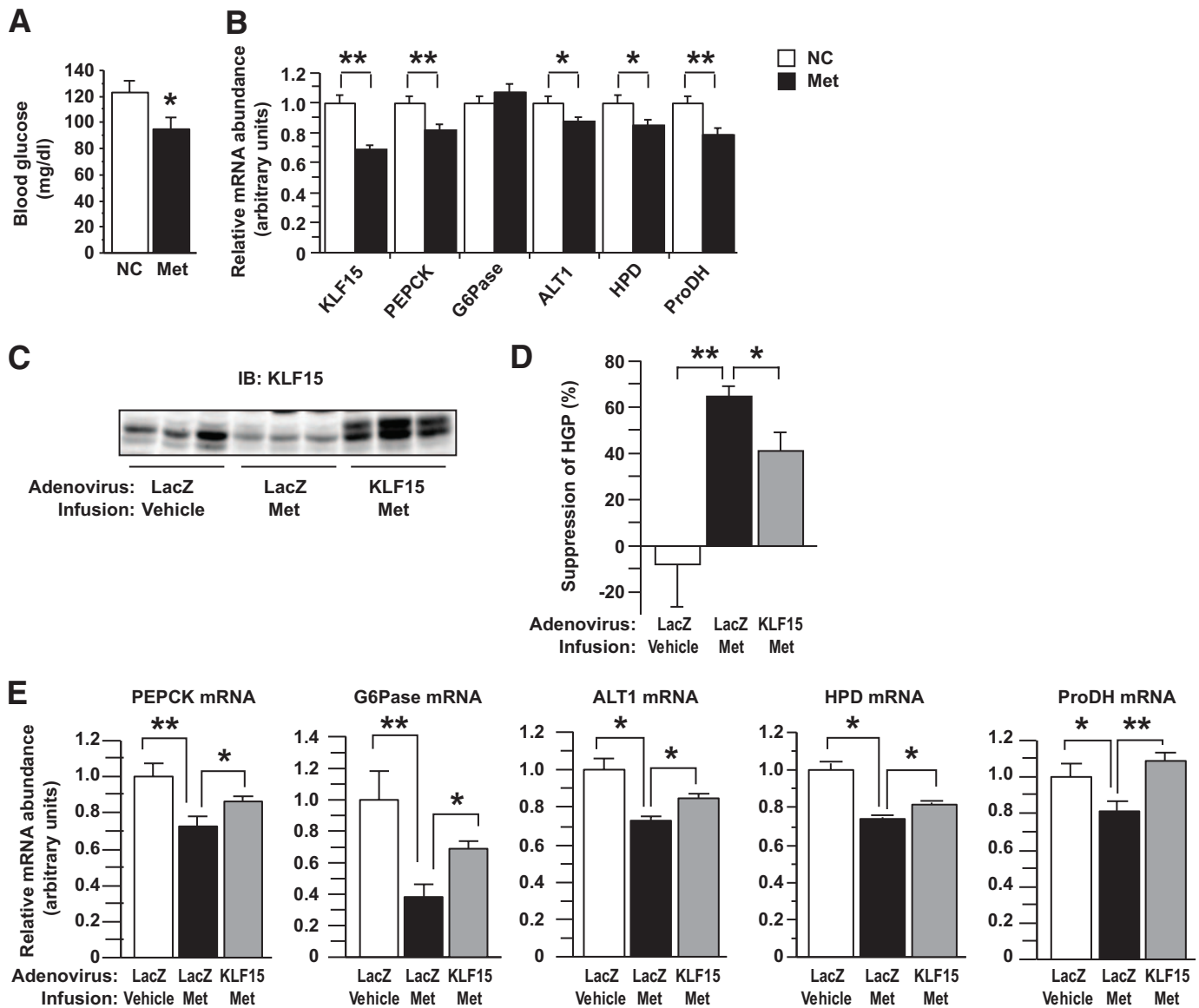
**FIG. 3.** Role of KLF15 in metformin-induced suppression of the genes for gluconeogenic or amino acid-degrading enzymes. **A:** HL1c cells were incubated for 6 h in the absence or presence of 100  $\mu\text{mol/l}$  8-CPT-cAMP and 500 nmol/l dexamethasone (Dex/cAMP) as well as with the indicated concentrations of metformin (Met). The amounts of KLF15 (upper panel) and of KLF15, HPD, PEPCK, and G6Pase mRNAs (lower panels) were then determined by immunoblot (IB) and RT and real-time PCR analyses, respectively. **B** and **C:** HL1c cells that had been infected with an adenoviral vector encoding Flag epitope-tagged KLF15 at the indicated multiplicity of infection (MOI) (plaque-forming units per cell) were incubated for 6 h with 500 nmol/l dexamethasone in the absence or presence of 5 mmol/l metformin. The cells were then subjected to immunoblot (IB) analysis with antibodies specific for KLF15 or for  $\alpha$ -tubulin (loading control) (**B**) or to RT and real-time PCR analysis of HPD and PEPCK mRNAs (**C**). All RT-PCR data are means  $\pm$  SEM from three or four independent experiments. **\*\*** $P < 0.01$ . Immunoblots are representative of at least three experiments.

mice were again not affected by infection with AxshKLF15 (supplementary Fig. 3). Blood glucose concentrations either during pyruvate challenge (Fig. 2E) or in the randomly fed state (Fig. 2F) were decreased in *db/db* mice injected with AxshKLF15 compared with those in animals injected with a control virus. Neither food consumption nor body mass differed between *db/db* mice injected with AxshKLF15 and those injected with the control virus (data not shown). These results thus suggested that KLF15 is a physiological regulator of gluconeogenesis and is a potential therapeutic target in diabetes. Blood glucose level in the randomly fed state was not decreased in C57BL/6 mice infected with AxshKLF15 (Fig. 2B) despite the similar reduction of KLF15 and gluconeogenic mRNAs (Fig. 2A and D). It is possible that hepatic gluconeogenesis is enhanced in *db/db* mice and, therefore, the glucose-lowering effects of AxshKLF15 were greater in these mice than in C57BL/6 mice.

**Role of KLF15 in metformin action.** The antidiabetes drug metformin inhibits hepatic glucose production through suppression of gluconeogenesis (11). We therefore investigated whether KLF15 contributes to the action of metformin. Metformin inhibited expression of the

PEPCK and G6Pase genes in cultured hepatocytes (Fig. 3A), consistent with previous results (22). It also inhibited the expression of endogenous KLF15 at the mRNA and protein levels as well as that of HPD at the mRNA level. Furthermore, metformin downregulates the abundance of ectopic KLF15 protein and increased the amount of ectopic KLF15 mRNA in cultured hepatocytes (supplementary Fig. 4). Treatment of the cells with cycloheximide, an inhibitor of protein synthesis, resulted in a reduction in the amount of KLF15 protein, and this effect was accelerated by metformin (supplementary Fig. 4). The metformin-induced loss of KLF15 protein was prevented by the proteasome inhibitor MG132, and metformin promoted the ubiquitination of KLF15 in cultured cells (supplementary Fig. 4), suggesting that metformin induces the proteasomal degradation of KLF15 by promoting its ubiquitination.

To evaluate the relevance of KLF15 to metformin action, we restored the expression of KLF15 in metformin-treated cells. Forced expression of KLF15 inhibited the downregulation of HPD and PEPCK mRNAs by metformin (Fig. 3B and C). Indeed, restoration of the abundance of KLF15 to a level similar to that of cells not treated with metformin virtually abolished the effects of the drug on the amounts



**FIG. 4.** Suppression of HGP by metformin through downregulation of KLF15. *A* and *B*: *db/db* mice (male, 7 weeks old) were fed normal chow (NC) or chow containing metformin (Met, 0.5%) for 7 days. They were then deprived of food for 10 h, after which blood glucose level (*A*) and the hepatic abundance of the indicated mRNAs (*B*) were determined ( $n = 12$  and 14 for normal chow and Met, respectively). *C–E*: C57BL/6 mice that had been injected with an adenoviral vector encoding either Flag-tagged KLF15 or LacZ were subjected to euglycemic clamp analysis with continuous infusion of metformin or vehicle. The abundance of KLF15 in nuclear extracts of the liver was determined by immunoblot (IB) analysis at the end of the clamp (*C*), and the suppression of HGP during the clamp ( $n = 4, 12,$  and 10 for LacZ+vehicle, LacZ+Met, and KLF15+Met, respectively) was measured (*D*). The amounts of the indicated mRNAs in the liver were also determined by RT and real-time PCR analysis ( $n = 10, 12,$  and 13 for LacZ+vehicle, LacZ+Met, and KLF15+Met, respectively) at the end of the clamp (*E*). All quantitative data are means  $\pm$  SEM. \* $P < 0.05$ , \*\* $P < 0.01$ .

of HPD and PEPCK mRNAs, indicating that downregulation of KLF15 is necessary for the inhibitory effects of metformin on the expression of genes for gluconeogenic and amino acid-degrading enzymes.

Feeding of *db/db* mice with chow containing metformin reduced blood glucose concentration (Fig. 4*A*) as well as hepatic expression of the genes for KLF15, PEPCK, ALT1, HPD, and ProDH (Fig. 4*B*), supporting the notion that KLF15 contributes to metformin action in living animals. To validate this notion, we evaluated the effects of metformin in mice by euglycemic clamp analysis in which metformin was continuously infused. Metformin infusion reduced the abundance of KLF15 in the liver (Fig. 4*C*) and suppressed HGP (Fig. 4*D*). Restoration of KLF15 expression in the liver by adenoviral infection attenuated the

metformin-induced suppression of HGP (Fig. 4*C* and *D*). Metformin infusion also stimulated the rate of glucose disappearance, which reflects glucose disposal by peripheral organs, but the restoration of KLF15 expression did not affect metformin-induced glucose disappearance ( $8.03 \pm 0.467$ ,  $11.20 \pm 0.41$ , and  $11.27 \pm 0.59$   $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  for  $\beta$ -galactosidase (LacZ)+vehicle, LacZ+metformin, and KLF15+metformin, respectively; means  $\pm$  SEM), indicating that restoration of KLF15 expression did not influence glucose disposal by peripheral organs.

Finally, we found that the hepatic abundance of PEPCK, G6Pase, ALT1, HPD, and ProDH mRNAs was decreased by metformin in a manner sensitive to restoration of KLF15 expression in the liver (Fig. 4*E*). These results thus suggested that the downregulation of KLF15 by metformin

contributes to the regulation of HGP and of the expression of genes related to this process by this drug. Given that the attenuation of the effects of metformin on HGP and gene expression in the liver by restoration of KLF15 expression was only partial, molecules in addition to KLF15 probably also contribute to metformin action.

## DISCUSSION

We have shown that depletion of KLF15 by RNAi resulted in downregulation of the expression of genes for gluconeogenic enzymes such as PEPCK and G6Pase in cultured hepatocytes. Our results suggest that KLF15 binds directly to the promoter region of the PEPCK gene and regulates the expression of this gene in coordination with the transcriptional coactivator PGC1 $\alpha$ . Moreover, the acute ablation of KLF15 specifically in the liver resulted in suppression of gluconeogenic gene expression in mice. These results thus indicate that KLF15 contributes to the regulation of genes for gluconeogenic enzymes. Mice with genetic KLF15 deficiency, however, were previously found to exhibit reduced hepatic expression of genes for amino acid catabolic enzymes but not of those for PEPCK and G6Pase (10). This apparent discrepancy with our present results might be attributable to a secondary effect of inborn deficiency of KLF15 in the entire body that leads to compensation for the lack of the transcription factor. In this regard, phenotypes associated with acute downregulation or genetic disruption of certain genes have previously been found to differ (7,23,24). Given that pharmacological interventions rarely lead to the complete loss of function of a target molecule, it is important to investigate the effects of partial downregulation, as opposed to complete deficiency, of specific gene products to evaluate their relevance as therapeutic targets. We have shown that an ~60% decrease in the abundance of KLF15 mRNA in the liver of *db/db* mice ameliorated their hyperglycemia. Given that the hepatic expression of KLF15 is increased in *db/db* mice (9), KLF15 is not only a potential therapeutic target for diabetes but also contributes to the disease pathogenesis in this animal model.

We found that exposure of cultured hepatocytes to metformin resulted in downregulation of the expression of genes not only for PEPCK and G6Pase but also for the amino acid-degrading enzyme HPD. Administration of metformin to mice, either orally or intravenously, attenuated the hepatic expression of genes for amino acid catabolic enzymes including ALT1, HPD, and ProDH, which are implicated in the regulation of gluconeogenesis through control of the availability of gluconeogenic substrate (10). It is thus possible that the effect of metformin on HGP is mediated at least in part through the downregulation of this class of enzymes. The relevance of KLF15 to metformin-induced downregulation of the genes for gluconeogenic and amino acid-degrading enzymes was underscored by the suppression of this action by forced restoration of KLF15 expression. However, this suppression of metformin action in mouse liver by restoration of KLF15 was only partial, suggesting that molecules in addition to KLF15 probably also contribute to the effect of metformin on gluconeogenesis. Transcriptional regulators including CRT2, small heterodimer partner, and CBP have been implicated in metformin action (6,8,22). The residual activity of metformin apparent after restoration of hepatic KLF15 expression might thus be mediated by one or more of these molecules.

Metformin suppressed the expression of endogenous KLF15 at the mRNA level. The abundance of KLF15 mRNA in cultured hepatocytes is increased by cAMP (9). It is thus possible that CREB, together with its coactivators CBP or CRT2, participates in the regulation of KLF15 gene expression. Given that the activities of both CBP and CRT2 are suppressed by metformin (6,8), these proteins might contribute to the metformin-induced downregulation of KLF15 mRNA. We also showed that metformin accelerates the degradation of KLF15 protein, probably through the promotion of its ubiquitination. Although the mechanism by which metformin stimulates the ubiquitination of KLF15 remains to be elucidated, this drug efficiently downregulates the abundance of KLF15 in cells by both suppression of its mRNA and degradation of its protein.

Overall, our present results suggest that KLF15 is an important target of metformin in the glucose-lowering effect of the drug and that the downregulation not only of gluconeogenic enzymes but also of amino acid catabolic enzymes in the liver contributes to the suppression of HGP by metformin. Further investigation of the mechanisms by which KLF15 is regulated in cells might provide additional insight into metformin action as well as a basis for the development of new antidiabetes drugs.

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