Inhibition of the oxygen sensor PHD2 in the liver improves survival in lactic acidosis by activating the Cori cycle

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Loss of prolyl hydroxylase 2 (PHD2) activates the hypoxia-inducible factor-dependent hypoxic response, including anaerobic glycolysis, which causes large amounts of lactate to be released from cells into the circulation. We found that *Phd2*-null mouse embryonic fibroblasts (MEFs) produced more lactate than wild-type MEFs, as expected, whereas systemic inactivation of PHD2 in mice did not cause hyperlacticacidemia. This unexpected observation led us to hypothesize that the hypoxic response activated in the liver enhances the Cori cycle, a lactate–glucose carbon recycling system between muscle and liver, and thereby decreases circulating lactate. Consistent with this hypothesis, blood lactate levels measured after a treadmill or lactate tolerance test were significantly lower in *Phd2*-liver-specific knockout (*Phd2-LKO*) mice than in control mice. An in vivo ^{13}C-labeled lactate incorporation assay revealed that the livers of *Phd2-LKO* mice produce significantly more glucose derived from ^{13}C-labeled lactate than control mice, suggesting that blockade of PHD2 in the liver ameliorates lactic acidosis by activating gluconeogenesis from lactate. *Phd2-LKO* mice were resistant to lactic acidosis induced by injection of a lethal dose of lactate, displaying a significant elongation of survival. Moreover, oral administration of a PHD inhibitor improved survival in an endotoxin shock mice model. These data suggest that PHD2 is a potentially novel drug target for the treatment of lactic acidosis, which is a serious and often fatal complication observed in some critically ill patients.

hypoxic response | PHD inhibitor | hyperlactatemia | gluconeogenesis | sepsis

In metazoans, the cellular enzyme lactate dehydrogenase (LDH) converts pyruvate to lactate, which is then exported from cells. Lactate efflux is increased under hypoxic conditions, where available molecular oxygen is limited. The cellular response to hypoxia is mainly regulated by the heterodimeric transcription factor hypoxia-inducible factor (HIF), which consists of an unstable alpha subunit (HIFα) and a stable beta subunit (HIFβ) (1, 2). HIF prolyl hydroxylases called PHDs (PHD1–PHD3) (3) target HIFα for ubiquitin-proteasome-dependent protein degradation under normoxic conditions, lowering HIF levels and suppressing HIF signaling. Under hypoxic conditions, PHDs become inactive because they require molecular oxygen for enzymatic activity. This inactivation leads to HIFα stabilization and activation of HIF signaling. Activated HIF up-regulates genes involved in various events, including erythrocytosis, neovascularization, and anaerobic glycolysis. HIF activation enhances anaerobic glycolysis by up-regulating glucose transporters and glycolytic enzymes, including lactate dehydrogenase A (LDHA), and monocarboxylate transporters, leading to enhanced lactate secretion.

Results and Discussion

Inactivation of *Phd2* in the Liver Reduces the Blood Lactate Level. PHD2 is the dominant HIF-prolyl hydroxylase in vivo among all three PHDs (4), and inactivating PHD2 alone is sufficient to activate HIF (5), which would enhance lactate efflux from the cells. As expected, lactate efflux from *Phd2*$^{-/-}$ mouse embryonic fibroblasts (MEFs) was significantly higher than from *Phd2*$^{+/+}$ MEFs (Fig. 1*A*). We therefore expected that systemic inactivation of PHD2 would cause increased lactic acid production and hyperlacticacidemia. To test this, we studied mice in which *Phd2* can be conditionally inactivated systemically (*Phd2-SKO*) (5). We first confirmed that the expression levels of some mRNAs linked to glycolysis were significantly increased in skeletal muscles of *Phd2-SKO* mice compared to control mice (Fig. S1*A* and Table S1). Unexpectedly, however, blood lactate levels in *Phd2-SKO* were not higher, and in fact tended to be lower, than in the control mice, although this difference did not reach statistical significance (Fig. 1*B*). To examine the mechanisms responsible for this unexpected observation, we measured lactate levels in mice subjected to treadmill exercise. Blood lactate levels were significantly

Significance

When oxygen availability becomes limited, organs and cells activate the hypoxic response to generate energy. This response releases a large amount of lactate into the circulation as a result of anaerobic glycolysis. However, we found that activating the hypoxic response in the liver by inhibiting the oxygen sensor prolyl hydroxylase domain-containing protein 2 (PHD2) enhances the uptake of lactate for gluconeogenesis, also known as the Cori cycle, and ameliorates lactic acidosis. Our findings suggest that PHD2 serves as a viable drug target for the treatment of life-threatening lactic acidosis, which is frequent complication of severe infectious and ischemic diseases, as well as of biguanide treatment in patients with diabetes with renal failure.

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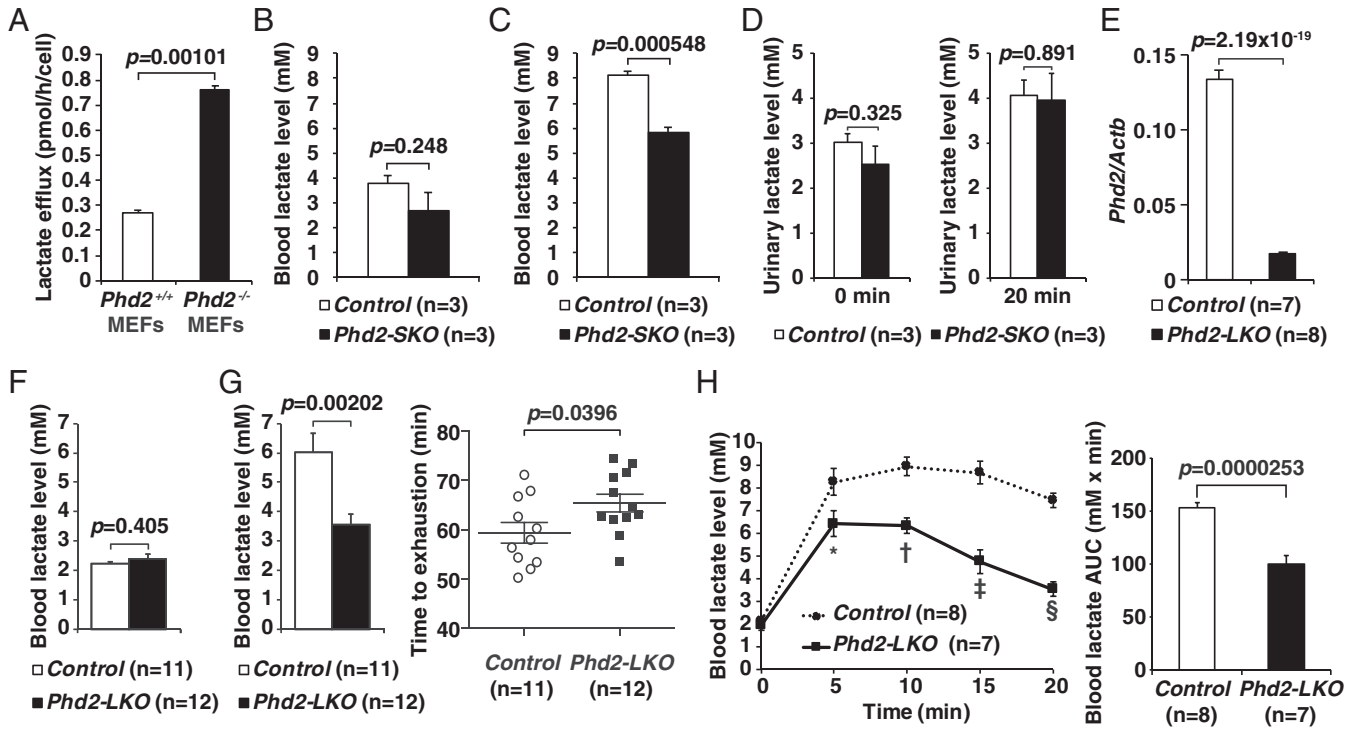

Fig. 1. Inactivation of *Phd2* in the liver reduces blood lactate levels. (*A*) Lactate efflux analysis in $Phd2^{+/+}$ and $Phd2^{-/-}$ MEFs. Values were from triplicated dishes. Error bars indicate 1 SEM. (*B*) The blood lactate level in *Control* and *Phd2-SKO* mice. Error bars, SEM. (*C*) The blood lactate level 50 min after the treadmill in *Control and Phd2-SKO* mice. Error bars, SEM. (*D*) Urinary lactate measurement. Lactate levels in urine were measured before (*Left*) and 20 min after (*Right*) the lactate injection in *Control* and *Phd2-SKO* mice. Error bars, SEM. (*E*) Real-time RT-PCR analysis of *Phd2 mRNA* in the livers of *Control* and *Phd2-LKO* mice. Error bars, SEM. (*F*) Blood lactate levels in *Control* and *Phd2-LKO* mice. Error bars, SEM. (*G*) Treadmill experiment in *Control* and *Phd2-LKO* mice. The blood lactate levels 50 min after the exercise (*Left*) and time to exhaustion (*Right*) are shown. Error bars, SEM. (*H*) Lactate tolerance test. 0.5 mg per gram body weight of lactate was intraperitoneally injected, and the blood lactate levels were measured at the indicated time (*Left*). Area under the curve (AUC) was also analyzed (*Right*). Error bars, SEM. **P* = 0.0448; †*P* = 0.000342; ‡*P* = 0.000118; §$P = 2.53 \times 10^{-7}$ versus *Control*.

lower in *Phd2-SKO* mice after treadmill exercise compared to control mice (Fig. 1*C*). This result suggested that systemic inactivation of *Phd2* increases lactate clearance, perhaps to compensate for enhanced lactate production. Notably, these treadmill experiments were performed a week after tamoxifen-induced *Phd2* inactivation, well before the induction of erythrocytosis (5, 6). Therefore, our results cannot be attributed to changes in red blood cell mass.

Urinary lactate levels did not differ between *Phd2-SKO* mice and control mice, suggesting the lower blood lactate levels in *Phd2-SKO* mice are not a result of enhanced lactate clearance by the kidneys (Fig. 1*D*). As circulating lactate can be taken up by the liver for gluconeogenesis (7), we hypothesized that the hypoxic response in hepatocytes contributes to systemic lactate clearance. To test this hypothesis, we crossed *Phd2*-conditional mice with an *Albumin-Cre* strain to generate *Phd2* liver-specific knockout (*Phd2-LKO*) mice (6, 8). Real-time RT-PCR confirmed that *Phd2 mRNA* expression was drastically decreased in the livers of *Phd2-LKO* mice (Fig. 1*E* and Table S1). The frequency of intact *Phd2* alleles was also analyzed by real-time PCR with genomic DNA obtained from various tissues, including the liver, kidney, heart, and muscle (Fig. S1*B* and Table S2) (5). The results confirmed that the *Phd2* gene was inactivated in the liver of *Phd2-LKO* mice, but not in other tissues, consistent with prior characterizations of this Cre strain (6, 8, 9). *Phd2-LKO* and control mice showed no differences in blood glucose levels under either ad libitum or fasted conditions (Fig. S1*C*). They also showed no difference in hematocrit levels (8).

Baseline blood lactate levels in *Phd2-LKO* mice were similar to those in control mice (Fig. 1*F*). However, blood lactate levels in the *Phd2-LKO* mice after 50 min of treadmill exercise were significantly lower than in the control mice (Fig. 1*G*, *Left*). Time to exhaustion was also significantly longer in *Phd2-LKO* mice than in control mice (Fig. 1*G*, *Right*), suggesting that inactivation of *Phd2* in the liver is sufficient to reduce blood lactate levels and enhance exercise performance. To further assess whether *Phd2* inactivation in the liver is able to overcome critical levels of hyperlacticacidemia, we performed lactate tolerance tests. After systemic lactate injection, blood lactate levels were significantly lower in *Phd2-LKO* mice than in control mice (Fig. 1*H*), suggesting the inactivation of *Phd2* in hepatocytes ameliorates hyperlacticacidemia.

Inactivation of *Phd2* in the Liver Activates Gluconeogenesis from Lactate. To discern how imported lactate was being used in hepatocytes, the expression levels of the enzymes involved in lactate import and metabolism were quantitated by real-time RT-PCR (Fig. 2*A*, Fig. S2*A*, and Table S1). Lactate is transported into hepatocytes through monocarboxylate transporters (MCTs). Among several MCTs, MCT2 (*Slc16a7*) has the highest affinity with lactate (10) and was significantly up-regulated in *Phd2-LKO* livers compared to control livers (Fig. 2*A*). Lactate is then converted to pyruvate by LDHA (*Ldha*), which was also up-regulated in *Phd2*-deficient livers (Fig. 2*A*), suggesting that both lactate import and its conversion to pyruvate are accelerated in *Phd2*-deficient livers.

Pyruvate is converted to acetyl CoA, a component of the tricarboxylic acid (TCA) cycle, by pyruvate dehydrogenase (PDH) in the mitochondria. PDH kinase 1 (PDK1), which is induced by HIF, negatively regulates the activity of PDH through inhibitory

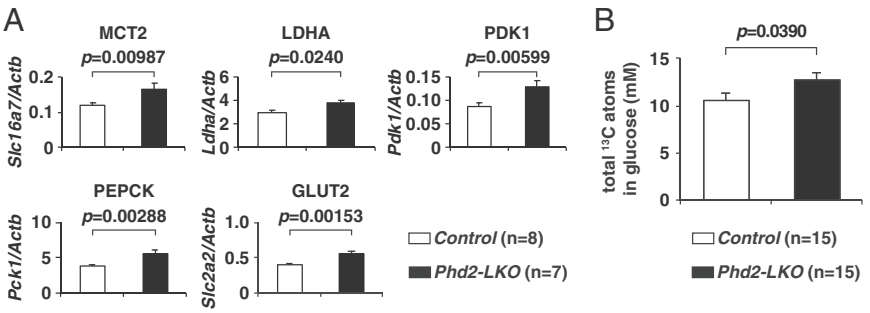

Fig. 2. Inactivation of *Phd2* in the liver activates gluconeogenesis from lactate. (*A*) Real-time RT-PCR analysis of transporters or enzymes involved in gluconeogenesis in the livers of *Control* and *Phd2-LKO* mice. Error bars, SEM. (*B*) LC/MS-based quantification of total ^{13}C atoms in glucose that were newly synthesized from $^{13}C_3$-lactate. Data were not corrected for the natural abundances of $^{13}C_x$-glucose (*Materials and Methods*). Error bars, SEM.

phosphorylation of its E1α subunit (11). Therefore, PDK1 is considered to be a gatekeeper of the TCA cycle. As expected, PDK1 (*Pdk1*) was up-regulated in *Phd2*-deficient livers, suggesting that PDH activity is inhibited by induced PDK1 and that the flux of pyruvate–acetyl CoA conversion is down-regulated in *Phd2*-inactivated livers. Therefore, fatty acid synthesis from pyruvate via acetyl CoA is not likely to be activated in *Phd2*-deficient livers.

Another possible metabolic pathway for the biotransformation of pyruvate under hypoxic conditions is gluconeogenesis. Pyruvate can be imported into mitochondria and converted to oxaloacetate (OAA) by pyruvate carboxylase (PC). OAA is converted to malate by mitochondrial malate dehydrogenase 2 (MDH2), exported from mitochondria, and converted back to OAA by cytoplasmic malate dehydrogenase 1 (MDH1). This cytoplasmic OAA is then converted to phosphoenolpyruvate by phosphoenolpyruvate carboxykinase (PEPCK), one of the rate-limiting enzymes of gluconeogenesis. *Pck1* mRNA, which encodes PEPCK, was significantly up-regulated in *Phd2*-deficient livers compared with control livers (Fig. 2*A*). In contrast, expression of glucose-6-phosphate (G6P) dehydrogenase (*G6pd*), the enzyme that triggers entry of G6P into the pentose phosphate pathway, was comparable between the *Phd2*-deficient and control livers (Fig. S2*A*).

The last stage of gluconeogenesis involves the dephosphorylation of G6P and excretion of the resulting glucose from hepatocytes via the glucose transporter GLUT2 (*Slc2a2*) (12), which was also up-regulated in *Phd2-LKO* livers. Taken together, inactivation of *Phd2* in the liver appears to turn on hypoxic responses that activate gluconeogenesis from lactate and decrease lactate use in the TCA cycle, fatty acid synthesis, and the pentose phosphate pathway. These responses contribute to the reduction in blood lactate levels (Fig. 2*A*).

To confirm that gluconeogenesis from lactate is indeed activated in *Phd2*-deficient liver, stable isotope-labeled lactate ($^{13}C_3$-lactate) was intraperitoneally injected into fasted mice, and plasma concentrations of ^{13}C-labeled glucose ($^{13}C_1$–$^{13}C_6$) were quantified using liquid chromatography–mass spectrometry (LC/MS) (Fig. 2*B* and Fig. S2*B*). Nonlabeled ($^{12}C_6$) or one- to three-labeled ($^{13}C_1$–$^{13}C_3$) glucose in the plasma were unchanged between two genotypes. In contrast, four- to full-labeled ($^{13}C_4$–$^{13}C_6$) glucose, as well as total ^{13}C atoms in glucose, were significantly elevated in the plasma of the *Phd2-LKO* mice compared to the control mice, providing further evidence that inactivation of *Phd2* in the liver activates gluconeogenesis from lactate.

In addition to lactate, alanine is another major gluconeogenic substrate that comes from the muscle to the liver (glucose–alanine cycle or Cahill cycle) (13). If global gluconeogenesis is activated upon *Phd2*-inactivation in the liver, alanine would be used as a source of gluconeogenesis, and blood glucose levels in *Phd2-LKO* mice might be higher than control mice under fasted conditions as a result of enhanced gluconeogenesis. Under such circumstances, however, blood glucose levels were unchanged between *Phd2-LKO* and control mice (Fig. S1*C*, *Right*). This suggests that only gluconeogenesis from lactate is enhanced in *Phd2*-inactivated liver, and global hepatic gluconeogenesis is not enhanced.

Detailed Quantitative Analysis of the Metabolites Derived from $^{13}C_3$-Lactate Confirms the Activation of Gluconeogenesis from Lactate in *Phd2*-Deficient Hepatocytes. Because liver contains multiple cell types, such as blood cells, sinusoidal cells, Kupffer cells, endothelial cells, stellate cells, and cholangiocytes, in addition to hepatocytes, crude liver extracts contain a variety of metabolites from cells that are not hepatocytes. To clarify the molecular mechanism by which *Phd2* inactivation activates gluconeogenesis from lactate in hepatocytes themselves, we perfused the livers of control mice and *Phd2-LKO* mice to isolate hepatocytes. Cultured hepatocytes were labeled with $^{13}C_3$-lactate for 5 min and then subjected to metabolome analysis (Fig. 3). The ^{13}C contents of each metabolite were quantified using capillary electrophoresis–mass spectrometry (CE/MS). The ^{13}C contents of lactate, pyruvate, and malate were significantly greater in *Phd2*-deficient hepatocytes than in control hepatocytes. In contrast, the ^{13}C contents of succinate were significantly lower in *Phd2*-deficient hepatocytes, suggesting that the pyruvate derived from $^{13}C_3$-lactate was less incorporated into the TCA cycle in *Phd2*-deficient hepatocytes compared with controls. The ^{13}C contents of G6P were significantly increased, whereas those of glucose-1-phosphate and ribose-5-phosphate were not increased in *Phd2*-inactivated hepatocytes compared with control hepatocytes. Although uncharged metabolites such as glucose cannot be quantified using CE/MS, our data support the idea that $^{13}C_3$-lactate-derived G6P is used primarily for gluconeogenesis rather than glycogen synthesis or the pentose phosphate pathway in *Phd2*-deficient hepatocytes (Fig. 3).

Previous studies reported that HIF2α activation caused by the loss of PHD3 enhanced insulin signaling in the liver and suppressed gluconeogenesis (14, 15). However, inactivating PHD2 alone induces PHD3, which is HIF-responsive and compensates for PHD2 loss, and thus might enhance gluconeogenesis. Although *Pck1 and Slc2a2* mRNAs were induced in *Phd2-LKO* mice, other gluconeogenic mRNAs such as the *Pcx* and *Fbp1* mRNAs were not (Fig. S2). Clearly, additional studies are needed to understand how, mechanistically, PHD2 regulates gluconeogenesis and the degree to which it does so through HIF versus other substrates.

Switching between glycolysis and gluconeogenesis might be controlled not only by the mRNA expression levels of rate-limiting enzymes, including bidirectional enzymes, linked to glycolysis and gluconeogenesis but also by posttranslational modification and allosteric regulation of those enzymes (16). For example, the rate-limiting enzymes of glycolysis (phosphofructokinase) and gluconeogenesis (fructose bisphosphatase) are both regulated by the abundance of fructose 2,6-bisphosphate (16). We

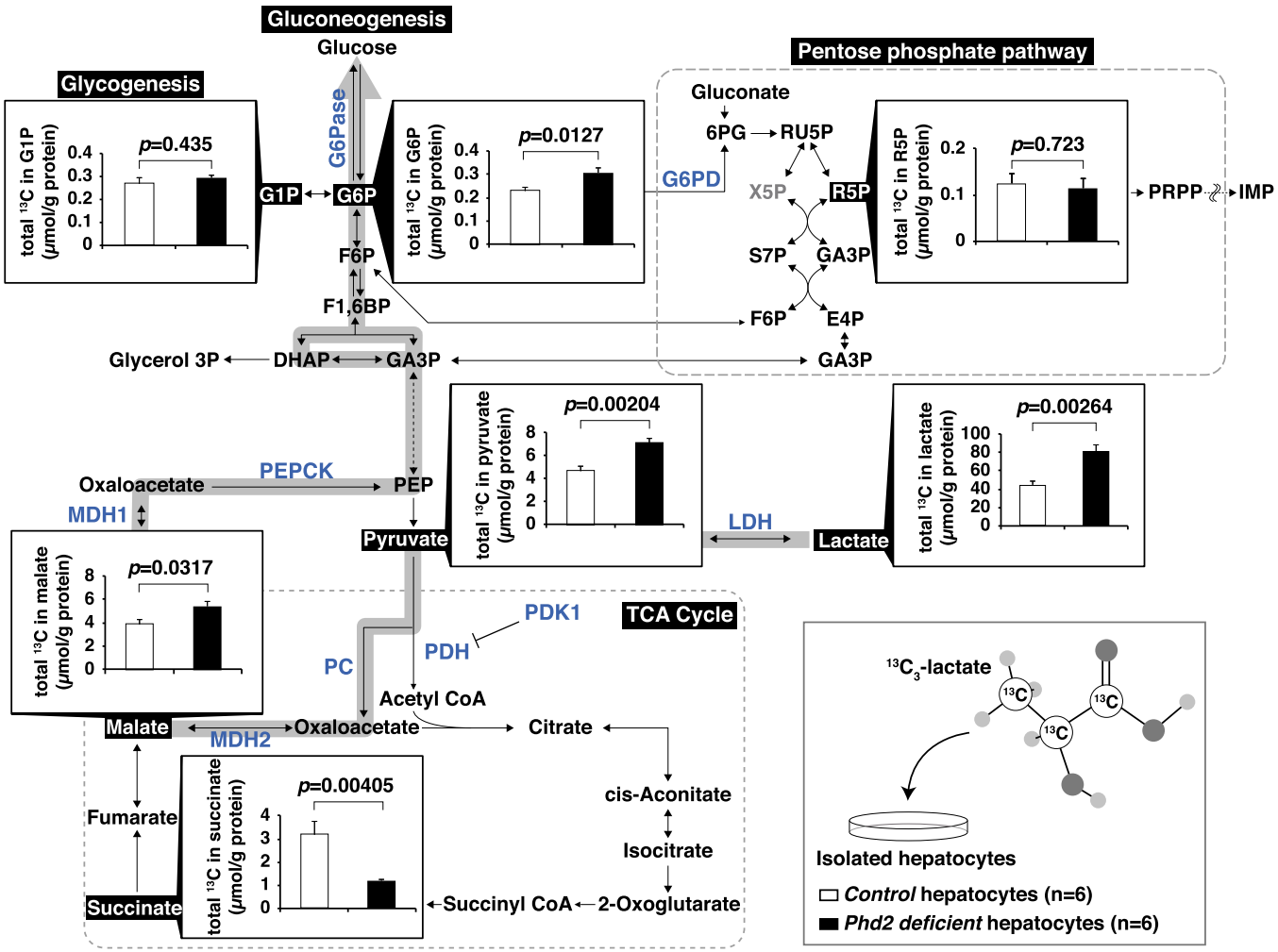

Fig. 3. Quantification of total ^{13}C atoms in $^{13}C_3$-lactate-derived metabolites. Cultured hepatocytes from *Control* and *Phd2-LKO* mice were labeled with $^{13}C_3$-lactate. Total ^{13}C atoms in indicated metabolites were quantified (μmol/g protein), using CE/MS. Data were not corrected for the natural abundances of $^{13}C_x$-glucose (*Materials and Methods*). Error bars, SEM.

observed that some HIF-responsive glycolytic mRNAs, such as *Slc2a2* and *Ldha* mRNA, were induced in *Phd2*-defective hepatocytes (Fig. 2*A*), but others, such as the critical *Hk2* (hexokinase 2), *Pfkl* (phosphofructokinase, liver), and *Pklr* (pyruvate kinase, liver, and red blood cell) mRNA, were not (Fig. S3). Moreover, the conversion of lactate to pyruvate consumes NAD and generates NADH, which inhibits glycolytic activity of GAPDH. Whether or not *Phd2* inactivation increases glycolysis in the liver while allowing the liver to consume lactate still remains to be determined. It is also possible that PHD2 regulates HIF-independent glucose metabolism.

Inactivation of *Phd2* in the Liver Improves Survival in Life-Threatening Lactic Acidosis. Increased blood lactate causes lactic acidosis, a critical medical condition characterized by blood lactate levels above 5 mM and blood pH levels below 7.35. To assess whether PHD2 loss in the liver can improve survival in the setting of lactic acidosis, *Phd2-LKO* and control mice were given intraperitoneal (i.p.) injection of lactate. Venous blood gas analysis 20 min after the lactate injection revealed a smaller decrease in pH in *Phd2-LKO* mice than in control mice (Fig. 4*A*). After administration of a lethal dose of lactate, *Phd2-LKO* mice also exhibited significantly higher survival rates than control mice (Fig. 4*B*). These results suggest that inactivating *Phd2* in the liver can reduce the mortality linked to lactic acidosis.

Lactic acidosis is often a complication of severe medical conditions, including endotoxin shock and ischemic diseases, and has also been observed in biguanide-treated diabetic patients with renal dysfunction (17). Lactic acidosis is frequently associated with multiple organ failure, which has an extremely poor survival rate (18). Because the outcome of multiple organ failure treatment is closely correlated with blood lactate levels (19–22), it is believed that reducing blood lactate levels would improve outcomes in this setting. To test whether pharmacological PHD2 inhibition could have a beneficial effect on such severe medical conditions, including endotoxin-induced shock, wild-type mice were treated with vehicle or the PHD inhibitor GSK360A by oral gavage immediately after the i.p. injection of a lethal dose of lipopolysaccharide (LPS). GSK360A treatment clearly attenuated the elevation of the blood lactate level (Fig. 4*C*) and was associated with a significantly better survival rate after the LPS injection (Fig. 4*D*). The presence of a GSK360A-induced hypoxic response was confirmed by the up-regulation of mRNAs that are direct targets of HIF (Fig. S4 and Table S1).

It is well established that the balance between peripheral lactate generation and its consumption in the liver determine the severity of lactic acidosis. Our study suggests that promoting lactate clearance by inhibiting hepatic PHD2 function, and thereby enhancing the hepatic arm of the Cori cycle, improves outcomes in patients with lactic acidosis and shock. In addition, there may be a beneficial immune response caused by enhanced

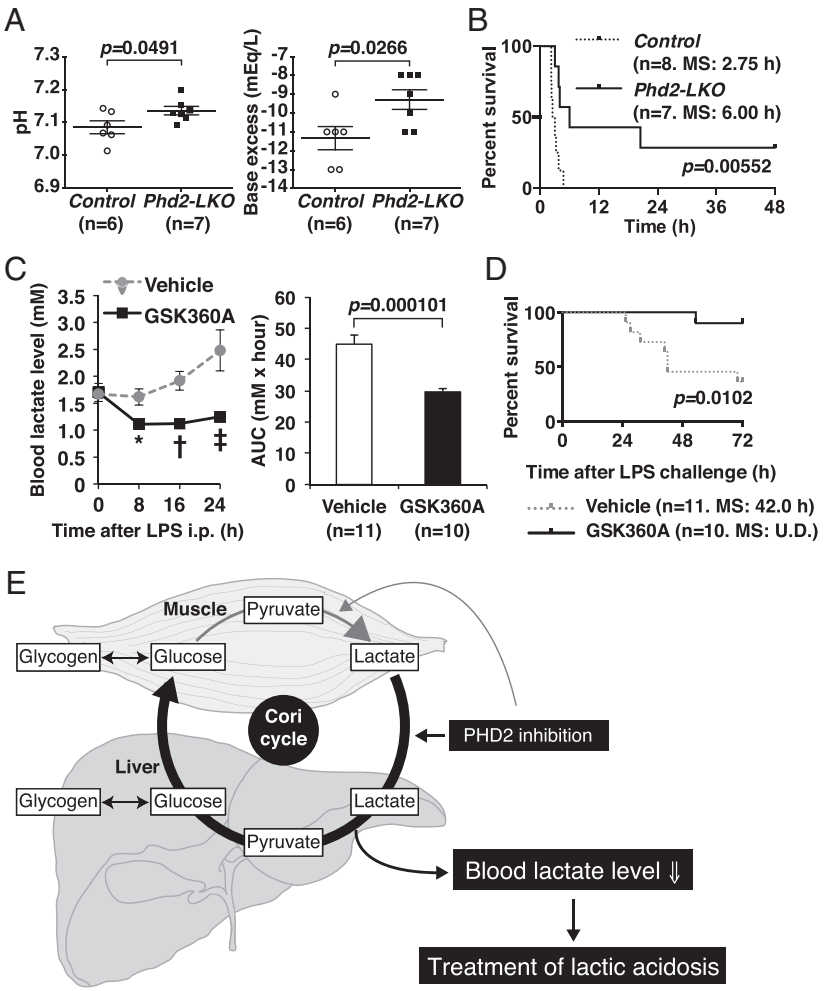

Fig. 4. Inactivation of *Phd2* in the liver causes resistance to lactic acidosis. (*A*) Venous blood gas analysis. pH (*Left*) and base excess (*Right*) were analyzed in the venous sample from the retro-orbital sinus 20 min after the injection of 0.5 mg/g body weight of lactate. Error bars, SEM. (*B*) Kaplan–Meier survival analysis after the injection of 0.5 mg/g body weight of lactate. MS indicates median survival. (*C*) The blood lactate analysis in indicated points after LPS injection in vehicle or PHD inhibitor GSK360A-treated wild-type mice (*Left*). AUC was also calculated (*Right*). Error bars, SEM. **P* = 0.00598; †*P* = 0.000641; ‡*P* = 0.00742 versus *Control*. (*D*) Survival analysis in LPS-induced endotoxin shock model. Wild-type mice were orally treated with vehicle or PHD inhibitor GSK360A immediately after the LPS injection. U.D., undefined. (*E*) Scheme of how the Cori cycle is activated in the liver upon PHD2-inactivation.

interleukin-10 (IL-10) production, which was reported previously (23). Conventional lactate reduction therapies include oxygen inhalation or the transfusion of red blood cells to improve molecular oxygen delivery, treatment with inotropes to increase cardiac output, and treatment with vasodilators to improve microvascular circulation (20, 21). None of these treatments enhances lactate clearance directly. Moreover, even though conventional oxygenation therapies down-regulate lactate production in peripheral tissues, they also appear to simultaneously suppress hepatic lactate consumption. Our study clearly demonstrates that inactivation of PHD2 in the liver triggers hypoxic responses that directly contribute to lactate clearance, resulting in better survival of lactic acidosis upon endotoxin shock by activating the Cori cycle (7) in the liver (Fig. 4*E*). Although chronic and systemic complete inactivation of PHD2 can cause detrimental adverse effects, such as cardiomyopathy, hepatic steatosis, and polycythemia (5, 6, 24), transient, acute inhibition of PHD2 might be safe and potentially beneficial in situations such as the treatment of myocardial infarction (25, 26), stroke (27), or organ transplantation (28). Moreover, as drugs that are given orally or via nasal tube are absorbed from the gut and delivered first to the liver via the portal vein, oral PHD inhibitors now under development might prove useful and safe for the treatment of lactic acidosis by preferentially targeting the PHD2 in the liver.

Materials and Methods

Mice. All experiments were approved by the Animal Care and Utilization Committee of Keio University School of Medicine (11050–1). Wild-type C57BL/6 mice were from CLEA Japan, Inc. All *Phd2 flox/flox* (*Phd2*$^{F/F}$) mice in this study were backcrossed to C57BL/6 strain at least five times, as previously reported (5). The transgenic mice expressing tamoxifen-inducible *Cre-recombinase* (*Cre-ER*) (004682 CAGG-Cre-ER; The Jackson Laboratory) and the albumin promoter-driven *Cre-recombinase* (*Alb-Cre*) were also described previously (6, 8, 9, 29). Further detailed breeding information is provided in the *SI Materials and Methods*. All the mice used in this study were 8–10-wk-old males except those used for urinary lactate measurement and venous blood gas analysis, and were maintained on a 12-h light/12-h dark cycle in a specific pathogen-free facility. Mice were fed ad libitum except in the in vivo $^{13}C_3$-lactate tracer study (fasted for 16 h). *Control* and *Phd2-SKO* mice were orally treated with 1 mg tamoxifen twice at 6 and 7 d before the blood lactate measurement (Fig. 1 *B* and *C*) and real-time RT-PCR (Fig. S1*A*), or were treated with 1 mg tamoxifen twice at 2 and 3 d before the urinary analysis (Fig. 1*D*). Oligonucleotide sequences for genotyping are shown in Table S2.

Treadmill Experiment. Forced exercise endurance in a treadmill experiment (Model MK-680S/CS; Muromachi Kikai) with an initial velocity of 6 m/min and an

increase in velocity of 2 m/min every 5 min, as described previously (30), were performed. Mice were forced to run at a 20° uphill incline to induce concentric exercise in the musculature, which results in the lactate accumulation because of the activation of anaerobic glycolysis. Exhaustion was defined as an inability to stay off shock grids (100 V) for at least 5 s. Before and 50 min after treadmill exercise, blood samples were obtained from the retro-orbital sinus (Fig. 1 *B* and *C*) or tail vein (Fig. 1 *F–H*) for analysis of lactate levels, using Lactate Pro Test Meter and Lactate Pro Test Strip (Cycle Classic Imports).

Lactate Tolerance Test. Lactate tolerance test was performed by the i.p. injection of 0.5 mg/g body weight of lactate (L-lactic acid. 252476; Sigma-Aldrich) dissolved in 0.9% NaCl. Whole-blood samples (5 μL each) were obtained from the tail veins at 0, 5, 10, 15, and 20 min after the injection. The blood lactate levels were measured using Lactate Pro Test Meter and Lactate Pro Test Strip. Kaplan–Meier survival analysis (up to 48 h) was also performed.

In Vivo Tracer Study. Mice were fasted for 16 h and were intraperitoneally injected with 0.5 mg/g body weight of $^{13}C_3$-lactate (L-lactic acid-$^{13}C_3$, 606065; Sigma-Aldrich). Twenty minutes after the injection, mice were anesthetized with isoflurane, and a blood sample was obtained from the retro-orbital sinus. Plasma compartment was purified with centrifuge twice at 15,000 × *g* for 5 min at 4 °C.

LC/MS-Based Quantification of Plasma Glucose. Plasma samples were further purified by methanol–chloroform, as previously reported (31). ^{13}C-labeled glucose ($^{13}C_1$–$^{13}C_6$), which is newly synthesized from administered $^{13}C_3$-lactate, was measured using the Nexera UHPLC (Ultra High Performance Liquid Chromatograph) system coupled with a LCMS-8030 triple quadrupole mass spectrometer (Shimadzu). Total ^{13}C atoms in glucose were quantified as a sum of 1 × $^{13}C_1$-glucose, 2 × $^{13}C_2$-glucose, 3 × $^{13}C_3$-glucose, 4 × $^{13}C_4$-glucose, 5 × $^{13}C_5$-glucose, and 6 × $^{13}C_6$-glucose. $^{12}C_6$-glucose indicates nonlabeled glucose ($^{13}C_0$-glucose). The data were not corrected for the presence of naturally occurring ^{13}C, which would be estimated to represent 6.6% of $^{12}C_6$-glucose in total $^{13}C_1$-glucose, 0.22% of $^{12}C_6$-glucose in total $^{13}C_2$-glucose, and so on.

CE/MS-Based Metabolome Analysis. Extracted metabolites from the hepatocytes were analyzed using Agilent CE Capillary Electrophoresis System equipped with an Agilent 1100 series MSD mass spectrometer, as previously reported (31, 32). Quantification of the metabolites was normalized with protein amount of the samples (μmol/g protein). Total ^{13}C atoms in indicated metabolites were calculated in a same manner as LC/MS-based quantification of ^{13}C-labeled glucose.

Endotoxin Shock Model. Eight-week-old wild-type C57BL/6 male mice were intraperitoneally injected with 40 mg/kg body weight of LPS (L2880, Sigma-Aldrich) and then treated with vehicle (1% methyl cellulose; 64625; Sigma-Aldrich) or 30 mg/kg body weight of PHD-inhibitor GSK360A (sc-490346; Santa Cruz Biotechnology) (33) by oral gavage immediately after the LPS injection. Whole-blood samples (5 μL each) were obtained from the tail veins, and the blood lactate levels were measured using Lactate Pro Test Meter and Lactate Pro Test Strip before and after the LPS injection.

Statistical Analysis. No power calculation was performed to determine sample size. Sample size for each group was chosen based on previous reports. No animals were excluded. Comparisons of normally distributed data between genotypes were analyzed with the use of unpaired Student *t* test. Area under the curve value was calculated for time course lactate measurement and analyzed by unpaired Student *t* test. Difference in survival rate was analyzed by Log-rank (Mantel–Cox) test, using GraphPad Prism software.

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