Role of Peroxisome Proliferator-Activated Receptor-α in the Mechanism Underlying Changes in Renal Pyruvate Dehydrogenase Kinase Isoform 4 Protein Expression in Starvation and after Refeeding

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The pyruvate dehydrogenase complex (PDC) occupies a strategic role in renal intermediary metabolism, via partitioning of pyruvate flux between oxidation and entry into the gluconeogenic pathway. Inactivation of PDC via activation of pyruvate dehydrogenase kinases (PDKs), which catalyze PDC phosphorylation, occurs secondary to increased fatty acid oxidation (FAO). In kidney, inactivation of PDC after prolonged starvation is mediated by up-regulation of the protein expression of two PDK isoforms, PDK2 and PDK4. The lipid-activated transcription factor, peroxisome proliferator-activated receptor-α (PPARα), plays a pivotal role in the cellular metabolic response to fatty acids and is abundant in kidney. In the present study we used PPARα null mice to examine the potential role of PPARα in regulating renal PDK protein expression. In wild-type mice, fasting (24 h) induced marked up-regulation of the protein expression of PDK4, together with modest up-regulation of PDK2 protein expression. In striking contrast, renal protein expression of PDK4 was only marginally induced by fasting in PPARα null mice. The present results define a critical role for PPARα in renal adaptation to fasting, and identify PDK4 as a downstream target of PPARα activation in the kidney. We propose that specific up-regulation of renal PDK4 protein expression in starvation, by maintaining PDC activity relatively low, facilitates pyruvate carboxylation to oxaloacetate and therefore entry of acetyl-CoA derived from FA β-oxidation into the TCA cycle, allowing adequate ATP production for brisk rates of gluconeogenesis. © 2001 Academic Press

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The liver and kidney are the only mammalian organs that possess sufficient gluconeogenic capacity and glucose 6-phosphatase activity to enable them to release glucose into the circulation as a result of gluconeogenesis. Until recently, the liver was considered to be the sole site of gluconeogenesis in normal postabsorptive individuals, and that kidney only functioned as an important source of glucose in acidotic conditions or after prolonged fasting. However, recent studies have demonstrated that the kidney releases significant amounts of glucose even in postabsorptive normal individuals (1). It has been calculated that in overnight-fasted normal humans, renal glucose release could account for 20% of overall endogenous glucose release, corresponding to approximately 40% of total body gluconeogenesis (reviewed in 2). These new data have led to the suggestion that the kidney could be as important a gluconeogenic organ as the liver in normal postabsorptive humans (2). Lactate, glutamine, alanine, and glycerol together account for greater than 90% of overall gluconeogenesis (3), but of these lactate has been reported to be the most important renal gluconeogenic substrate (2). Physiological increases in insulin concen-

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trations suppress renal glucose release (4, 5) primarily by intrarenal mechanisms, rather than by simply reducing substrate delivery (4, 6). This could arise by shunting precursors away from the gluconeogenic pathway and into the oxidative pathway, thereby compensating for decreased availability of nonesterified fatty acids (NEFA)[2] as an oxidative fuel during insulin infusion (2).

The pyruvate dehydrogenase complex (PDC), via partitioning of pyruvate flux between oxidation and entry into the gluconeogenic pathway, occupies a potentially strategic role in renal intermediary metabolism (7). Activation of PDC under conditions of an abundant glucose supply promotes glucose oxidation. Conversely, inactivation of PDC limits glucose oxidation. Mammalian PDC is a large assembly of six components, with nine distinct subunits. Four of these components execute the overall PDC reaction through a series of steps linked by cofactor-mediated active-site coupling: the pyruvate dehydrogenase component (E1; pyruvate dehydrogenase, PDH); the dihydrolipoyl acetyltransferase component (E2); the dihydrolipoyl dehydrogenase component (E3); and the E3-binding component (E3BP, previously termed protein X) (reviewed in 8–10). PDC activity is primarily controlled by a phosphorylation/dephosphorylation cycle catalyzed by a family of dedicated kinases (pyruvate dehydrogenase kinases, PDKs) (11) and two phosphatases (pyruvate dehydrogenase phosphate phosphatases, PDHP phosphatases) (12). These components of PDC respectively operate to decrease and increase the activity of the complex through phosphorylating and dephosphorylating the E1 component (reviewed in 7).

Increased renal PDK activity after prolonged (48 h) starvation is concomitant with up-regulation of renal protein expression of PDK2 and PDK4 (13, 14). By contrast, although heart and oxidative skeletal muscle contain both PDK2 and PDK4, starvation leads to targeted up-regulation only of PDK4 in these tissues (15, 16). In rat skeletal muscle, activation of peroxisome proliferator-activated receptor-α (PPARα) by the selective agonist WY14,643 leads to selective accumulation of PDK4 mRNA transcript and protein in the absence of any change in PDK2 protein expression (16). PPARα is expressed at high levels in the kidney (17–19). Furthermore, PPARα is critical to renal function, as demonstrated by findings that PPARα null mice subjected

to ischemia/reperfusion (I/R) injury demonstrate significantly enhanced renal cortical necrosis and worse kidney function compared with wild-type controls (20). Although it is well established that the regulation of FAO represents an important mechanism for a sustained balance of energy production/utilization in the kidney, it is not known whether it is involved in regulation of renal PDK protein expression, either directly or via PPARα.

In the present study, we analyzed the response of PDK2 and PDK4 protein expression to starvation and refeeding in relation to changes in renal PDK activity. Our aim was to determine whether suppression of PDK activity (and thus redirection of pyruvate to oxidation) might contribute to the attenuation of renal gluconeogenesis observed on refeeding when insulin concentrations are elevated. In addition, we investigated the possible role of PPARα in mediating these responses. In particular, we studied renal PDK isoform protein expression in fed, starved, and refed PPARα null mice to examine the potential role of PPARα in regulating renal PDK protein expression during nutritional transitions.

MATERIALS AND METHODS

Materials. General Laboratory reagents were from Roche Diagnostics (Lewes, East Sussex, UK) or from Sigma (Gillingham, Dorset, UK), with the following exceptions. Organic solvents were of analytical grade and obtained from BDH (Poole, Dorset, UK). Arylamine acetyltransferase was purified from pigeon liver acetone powder purchased from Europa Bioproducts (Ely, Cambridgeshire, UK). ECL reagents, hyperfilm, and secondary antibodies were purchased from Amersham Pharmacia Biotech (Little Chalfont, Buckinghamshire, UK). Anti-PDK2 antibodies were generated in the authors' laboratory in rabbits against recombinant PDK2 (21). Anti-PDK4 antibodies were generated in rabbits against individual recombinant PDK4 (11) and were generously provided by Professor Bob Harris. Protein A for Western blot analysis was obtained from ICN Pharmaceuticals (Basingstoke, Hants, UK). Bradford reagents were purchased from Bio-Rad (Hemel Hempstead, Hertfordshire, UK).

Animals. All studies were conducted in adherence to the regulation of the United Kingdom Animal Scientific Procedures Act (1986). PPARα null mice bred onto an Sv/129 genetic background were kindly provided by Dr. J. Peters and Dr. F. J. Gonzalez (National Institutes of Health, Bethesda, MD). Wild-type Sv/129 mice were used as controls. Mice were maintained on a reverse light/dark cycle (12-h dark phase 03:00–15:00, 12-h light phase 15:00–03:00) and were fed *ad libitum* (standard high-carbohydrate/low-fat rodent laboratory diet), starved for 24 h, or refed (6 h) *ad libitum* after starvation for 24 h. For the mouse studies, the starvation period of 24 h rather than 48 h was selected since a more prolonged period of starvation was considered to be inappropriate as the PPARα null mice develop severe hypoglycemia even after 24 h of starvation (22). Adult female albino Wistar rats (200–250 g) were purchased from Charles River (Margate, Kent, UK). Rats were maintained at a temperature of $22 \pm 2°C$ and subjected to a 12-h light/12-h dark cycle (12-h dark phase 20:00–08:00, 12-h light phase 08:00–20:00). Rats were allowed access *ad libitum* to water and were fed *ad libitum* (standard high-carbohydrate/low-fat rodent laboratory diet), starved for 48 h, or refed (6 h) *ad libitum* after starvation for 48 h.

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[2] Abbreviations used: ACS, acyl-CoA synthase; BSA, bovine serum albumin; CPT I, carnitine palmitoyltransferase I; DMSO, dimethyl sulfoxide; FAO, fatty acid oxidation; PDC, pyruvate dehydrogenase complex; PDH, pyruvate dehydrogenase; PDHa, active pyruvate dehydrogenase; PDK, pyruvate dehydrogenase kinase; PPARα, peroxisome proliferator-activated receptor-α; DR, direct repeat; RXR, retinoid X receptor; NEFA, nonesterified fatty acid; PMSF, phenylmethylsulfonyl fluoride; SDS, sodium dodecyl sulfate; TBS, Tris-buffered saline.

Tissue and blood sampling. Rats and mice were anesthetized by injection of sodium pentobarbital (60 mg/ml in 0.9% NaCl; 1 ml/kg body wt ip) and, once locomotor activity had ceased, kidneys were rapidly excised, freeze-clamped using aluminum clamps precooled in liquid nitrogen, and stored in liquid nitrogen until analysis. In the rat studies of PDK activity changes, the contralateral kidney was used for preparation of mitochondria. Blood was sampled and an aliquot was immediately added to ice-cold perchloric acid for metabolite analysis. A further aliquot was centrifuged for 5 min at $12,000g$ and plasma was stored at $-20°C$.

Enzyme assays. Active pyruvate dehydrogenase (PDHa) activity was assayed both in freeze-clamped tissue extracts and in isolated mitochondria, prepared as described in Ref. (21). PDHa was assayed spectrophotometrically by coupling to arylamine acetyltransferase (23, 24). Total PDC activity was assayed after complete activation through the action of endogenous PDC phosphate phosphatase as active PDC in mitochondria incubated for 15 min in the absence of respiratory substrate and in the presence of the uncoupler carbonyl cyanide *m*-chlorophenylhydrazone (25). PDK activities were determined in mitochondrial extracts at pH 7.0 by the rate of ATP-dependent inactivation of PDHa and computed as apparent first-order rate constants for ATP-dependent PDHa inactivation (21, 26). Phosphorylation of PDC occurs on three seryl residues (Ser264, Ser271, and Ser203) of the α-chain of the E1 (PDH) component (27). These seryl residues are designated as phosphorylation sites 1, 2, and 3, respectively. Analysis of phosphorylation and inactivation of purified PDC or mitochondrial PDC demonstrated that the relative initial rates of phosphorylation of the sites are: site 1 > site 2 > site 3 (reviewed in 28). With purified complex or complex *in vivo,* phosphorylation of site 1 accounts for >98.5% of inactivation during phosphorylation or in the steady state (27, 29, 30). Thus, ATP-dependent inactivation of PDHa essentially corresponds to phosphorylation of site 1 of the PDC complex, which accounts for the physiological function of PDK to inactivate PDC activity.

Immunoblotting. Tissue samples (approx. 100 mg) were homogenized using a Polytron tissue homogenizer (PT 10 probe; position 5, 15 s) in 1 ml ice-cold extraction buffer A (20 mM Tris, 137 mM NaCl, 2.7 mM KCl, 1 mM CaCl$_2$, 10% glycerol, 1% Igepal ([octylphenoxy]-polyethoxyethanol), 45 mM sodium orthovanadate, 0.2 mM PMSF, 10 μg/ml leupeptin, 1.5 mg/ml benzamidine, 50 μg/ml aprotinin, 50 μg/ml pepstatin A (in DMSO), pH 8.0. Homogenates were placed on ice for 20 min, centrifuged in an Eppendorf centrifuge (12,000 rpm for 20 min at 4°C) and the supernatants stored ($-20°C$) until analysis. Protein concentrations were determined using the Bradford method using bovine serum albumin (BSA) as standard. The assay was linear over the range of protein concentrations routinely used. Samples (20–50 μg of total protein) were subjected to SDS-PAGE using a 12% resolving gel with a 6% stacking gel. Following SDS-electrophoresis, resolved proteins were transferred electrophoretically to nitrocellulose membranes, and then blocked for 2 h at room temperature with Tris-buffered saline (TBS) supplemented with 0.05% Tween and 5% (w/v) nonfat powdered milk. The nitrocellulose blots were incubated overnight at 4°C with polyclonal antisera raised against specific recombinant PDK isoforms, washed with 0.05% Tween in TBS (3 × 5 min), and incubated with the horseradish peroxidase-linked secondary antibody IgG anti-rabbit (1:2000, in 1% (w/v) nonfat milk in TBST) for 2 h at room temperature. Bound antibody was visualized using ECL according to the manufacturer's instructions. The blots were then exposed to Hyperfilm and the signals quantified by scanning densitometry and analyzed with Molecular Analyst software (BioRad Ltd). The amounts of extracts loaded on to the gel were varied to establish that the relative densities of the bands corresponding to the PDK isoforms were linear with concentration. Immunoblots were performed under conditions in which autoradiographic detection was in the linear response range. For each representative immunoblot presented, the results are from a single gel exposed for a uniform duration, and each lane represents a preparation from a different animal.

Statistical analysis. Results are presented as the mean $\pm$ standard error (SE), with the numbers of rats in parentheses. Statistical analysis was performed by ANOVA followed by Fisher's post hoc tests for individual comparisons or Student's *t* test as appropriate (Statview, Abacus Concepts, Inc., Berkeley, CA). A *P* value of <0.05 was considered to be statistically significant.

RESULTS

Changes in renal PDK activity correspond with changes in renal PDK protein expression during starvation and refeeding after starvation. As demonstrated previously, starvation led to a significant increase in renal PDK activity, measured in isolated mitochondria, (fed, 0.41 ± 0.06 min^{-1}; 48-h starved, 0.72 ± 0.08 min^{-1}; $P < 0.05$) (13) together with marked suppression of renal PDHa activity *in vivo* (fed, $19.3 \pm 2.8\%$ of total PDH activity; 48-h starved, $11.3 \pm 2.5\%$ of total PDH activity; $P < 0.05$) (31), implying activation of PDK. Using Western blot analysis, we observed a modest (17%; $P < 0.05$) increase in the protein expression of PDK2 (Fig. 1), together with much more marked increase (3.1-fold; $P < 0.001$) in the amount of PDK4 protein, in the rat kidney after a 48-h starvation (Fig. 1). Thus, the amount of PDK4 protein relative to total PDK protein (PDK2 + PDK4) in kidney mitochondria is increased by starvation. The data presented in Fig. 1 demonstrate 2 bands for PDK2 and PDK4. Both bands were used for quantification. Multiple bands can also be observed in earlier reports of PDK protein expression (14), although no comment was made on this in the earlier report. At present, we do not have any explanation for the multiple bands, but they are consistently observed. As in previous studies (13, 31, 32), total PDC activities (measured in isolated mitochondria and expressed relative to the mitochondrial marker citrate synthase) were unchanged by starvation (results not shown). Furthermore, protein expression of PDC-E1α was unchanged after a 48-h starvation; results not shown. Refeeding (*ad libitum*) standard rodent diet for 6 h led to modest suppression of the renal protein expression of both PDK2 and PDK4 (Fig. 1). PDK4 protein expression after refeeding for 6 h remained significantly higher than levels observed in the fed state. The effect of refeeding to suppress renal PDK2 and PDK4 protein expression was concomitant with a 31% decrease ($P < 0.05$) in renal PDK activity (measured in isolated mitochondria) (48-h starved, 0.72 ± 0.05 min^{-1}; 6-h refed, 0.49 ± 0.06 min^{-1}). This moderate decrease in PDK activity was associated with a more marked (1.7-fold; $P < 0.001$) increase in renal PDHa activity *in vivo* (48-h starved, $11.3 \pm 3.5\%$ of total PDH activity; 6-h refed, $19.6 \pm 0.01\%$ of total PDH activity). It can be concluded that pattern of change of renal PDK activity in response to starvation and refeeding after starvation reflects
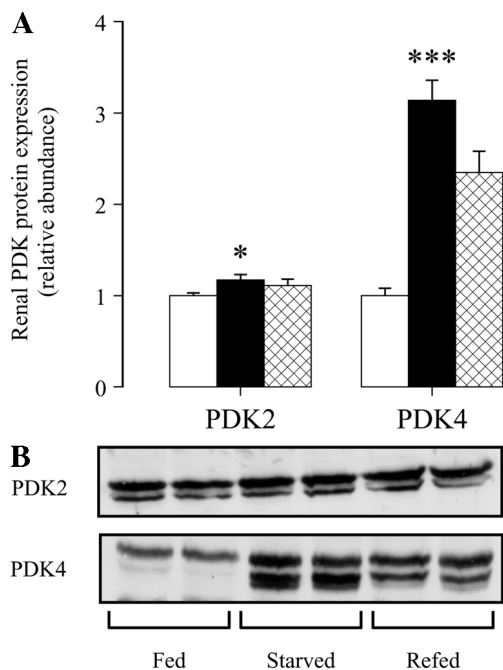
FIG. 1. Effects of 48-h starvation or 6-h refeeding after 48-h starvation on PDK2 and PDK4 protein expression in rat kidney. For starvation, food was removed for 48 h beginning at the end of the dark (feeding) phase of the cycle (08:00). For refeeding, food was provided *ad lib* for 6 h and animals were killed at 14:00. Rabbit polyclonal antisera raised against recombinant PDK2 and PDK4 were used to detect PDK2 and PDK4 protein using Western blot analysis. Each lane corresponds to 50 μg of kidney mitochondrial protein. Western blots were analyzed by scanning densitometry using Molecular Analyst 1.5 software. Quantification of Western analysis of renal PDK2 and PDK4 protein expression in fed rats (open bars), 48-h starved rats (closed bars), or 6-h refed rats (hatched bars) is shown in panel A. Data are means $\pm$ SE for 10 (fed), 11 (48-h starved), and 5 (refed) kidney preparations from individual rats in each experimental group. Typical immunoblots of PDK2 or PDK4 protein expression are shown for an individual kidney preparation from 3 $\times$ fed vs 3 $\times$ 48-h starved vs 3 $\times$ 6-h refed rats in panel B. Statistically significant effects of 48-h starvation are indicated by: *$P < 0.05$; ***$P < 0.001$. There were no statistically significant effects of refeeding.

changes in renal PDK protein expression, but that changes in metabolite effectors that acutely influence renal PDK activity predominantly mediate the initial phase of renal PDH reactivation after refeeding.

Effect of starvation on renal PDK protein expression in wild-type and PPARα null mice. Reiterating the response to starvation observed in the rat, we observed a modest (5%, $P < 0.05$) increase in the protein expression of PDK2 and a marked (4.6-fold; $P < 0.01$) increase in the amount of PDK4 protein, in the wild-type mouse kidney after a 24-h starvation (Fig. 2). The effect of starvation to increase renal PDK2 protein expression was somewhat exaggerated in the PPARα null mice (an increase of 20%; $P < 0.001$) (Fig. 2). In marked contrast, the effect of starvation to increase
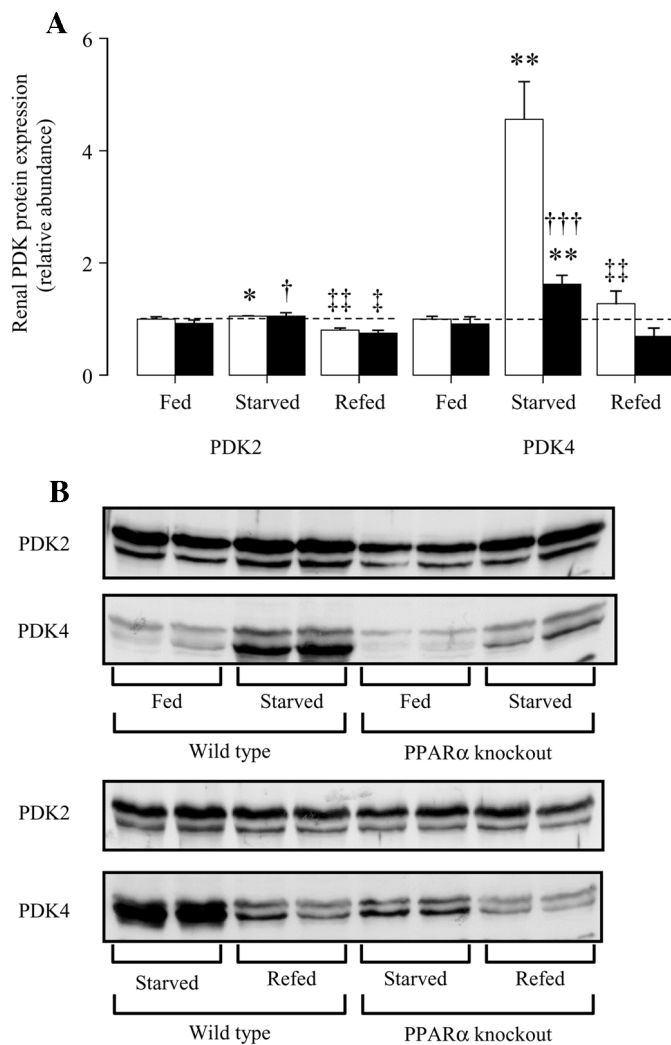

FIG. 2. Effects of 24-h starvation or 6-h refeeding after 24-h starvation on PDK2 and PDK4 protein expression in kidneys of wild-type mice and PPARα null mice. For starvation, food was removed for 24 h beginning at the midpoint of the dark phase of the cycle (09:00). For refeeding, food was provided *ad lib* for 6 h and animals were killed at 15:00. Mice fed *ad lib* were killed within 2 h of the end of the dark (feeding) phase. Rabbit polyclonal antisera raised against recombinant PDK2 and PDK4 were used to detect PDK2 and PDK4 protein using Western blot analysis. Each lane corresponds to 50 μg of kidney protein. Western blots were analyzed by scanning densitometry using Molecular Analyst 1.5 software. Quantification of Western analysis of renal PDK2 and PDK4 protein expression in fed, 24-h starved, or 6-h refed wild-type mice (open bars) or PPARα null mice (closed bars) is shown in panel A. Data are means $\pm$ SE for 12 (fed), 16 (24-h starved), and 4 (6-h refed) kidney preparations from individual mice in each experimental group. Representative immunoblots of PDK2 or PDK4 protein expression are shown for an individual kidney preparation from 2 $\times$ fed vs 2 $\times$ 24-h starved and 2 $\times$ 24-h starved vs 2 $\times$ 6-h refed mice in panel B. Statistically significant effects of 24-h starvation are indicated by: *$P < 0.05$; **$P < 0.01$. Statistically significant differences between wild-type mice and PPARα null mice are indicated by: †$P < 0.05$; †††$P < 0.001$. Statistically significant effects of 6-h refeeding are indicated by: ‡$P < 0.05$; ‡‡$P < 0.01$.

renal PDK4 protein expression was greatly attenuated (by 54%) in the PPARα null mice (a starvation-induced increase of 1.8-fold; $P < 0.05$) (Fig. 2). Protein expression of PDC-E1α after a 48-h starvation was similar in wild-type and PPARα null mice (results not shown), indicating that the differences between wild-type and PPARα null mice in terms of the response of PDK isoform protein expression to starvation did not reflect changes in total PDC protein. As a consequence of the selective response of renal PDK4 to starvation in the two groups, renal PDK4 protein expression in the PPARα null mice after a 24-h starvation was only 39% ($P < 0.001$) of corresponding control values. It can be concluded that increased renal PDK4 protein expression observed in wild-type mice after a 24-h starvation is markedly blunted in PPARα null mice.

Effect of refeeding after starvation on renal PDK protein expression in wild-type and PPARα null mice. Refeeding (*ad libitum*) of wild-type mice for 6 h led to suppression of the renal protein expression of both PDK2 (by 24%; $P < 0.01$) and PDK4 (by 59%; $P < 0.001$) (Fig. 2) to levels comparable to those found in the fed state. In contrast, refeeding (*ad libitum*) of PPARα null mice for 6 h led to modest suppression of renal PDK4 protein expression (18%; NS) and failed to affect renal PDK2 protein expression (Fig. 2).

DISCUSSION

Until recently, the liver was considered to be the sole site of gluconeogenesis in normal postabsorptive individuals. However, more recent studies indicate that renal glucose release could account for 20% of overall endogenous glucose release, corresponding to approximately 40% of total body gluconeogenesis, in overnight-fasted normal humans (reviewed in 2). Physiological increases in insulin concentrations suppress renal glucose release (4, 5). Lactate has been reported to be the most important renal gluconeogenic substrate (2) and thus the effect of insulin could be achieved through shunting precursors away from the gluconeogenic pathway and into the oxidative pathway via PDC. Increased renal PDK activity after prolonged (48 h) starvation is concomitant with up-regulation of renal protein expression of PDK2 and PDK4 (13, 14). Here we analyze changes in renal PDC activity elicited by starvation and refeeding after starvation, nutritional transitions known to be respectively associated with renal glucose output and suppression of renal glucose output, in relation to changes in PDK activity and PDK isoform protein expression. To establish the potential mechanism by which changes in PDK isoform protein expression might be achieved, we analyzed the potential role of PPARα by use of PPARα-deficient mice. PPARα not only is expressed at high levels in the kidney (17–19) but is critical to renal function (20).

Although it is well established that the regulation of FAO represents an important mechanism for a sustained balance of energy production/utilization in the kidney, it is not known whether it is involved in regulation of renal PDK protein expression, either directly or via PPARα. The present study provides insight into the potential importance of the response of PDK2 and PDK4 protein expression, and thus of renal PDK activity, to the partitioning of renal pyruvate between oxidation and gluconeogenesis and the role of PPARα in mediating the renal response to starvation.

The PPAR subfamily consists of three members, PPARα, -β, and -γ (33). Although the three PPARs bind to the same PPREs in their target genes, distinct functions for PPAR family members are suggested from their tissue-specific protein expression patterns. PPARα is abundant in kidney, liver, and heart (17, 18). PPAR activators are known to produce peroxisome proliferation in the kidney (19). Studies of gene expression of the different subtypes of PPARs in defined nephron segments have shown that PPARα is expressed predominantly in the renal cortex (17, 19), the major site of gluconeogenesis. Gene expression of acyl-CoA synthase (ACS), a known PPARα target gene, is significantly stimulated in renal cortex, but not medulla, in response to fenofibrate administration (19), consistent with PPARα distribution (19). It is established that PPARα plays a pivotal role in the overall metabolic response to fasting (22). Altered renal gluconeogenesis is increasingly recognized as contributing to fasting hyperglycemia in diabetes, and also assumes increasing importance for maintenance of glycemia after prolonged starvation (reviewed in 2, 34). Starvation causes relative hypoglycemia in mice lacking PPARα (22). Under normal circumstances, FAO suppresses PDC activity via the PDKs, which catalyze PDC inactivation by phosphorylation, an effect crucial to glucose conservation in starvation (13). In kidney, inactivation of PDC after prolonged starvation is mediated in part by up-regulation of the protein expression of two PDK isoforms, PDK2 and PDK4 (13, 14). In the present study, we used PPARα null mice to examine the potential role of PPARα in regulating renal PDK protein expression in response to starvation and refeeding after starvation. In wild-type mice, fasting (24 h) induced marked up-regulation of the protein expression of PDK4, together with modest up-regulation of PDK2 protein expression. In striking contrast, up-regulation of renal protein expression of PDK4 in response to fasting was greatly attenuated in PPARα null mice. The present results therefore demonstrate for the first time a critical role for PPARα in renal adaptation to fasting, and identify PDK4 as a downstream target of PPARα activation in the kidney.

The conversion of pyruvate to acetyl-CoA via PDC links glycolysis with the synthesis of malonyl-CoA. In

the fed state, malonyl-CoA potently inhibits mitochondrial long-chain fatty acid uptake at the level of carnitine palmitoyltransferase I (CPT I) (35). The kidney contains L (liver)-type CPT I (36), and measurements of free and acylated carnitine concentrations have suggested that flux through CPT I is minimal in kidney in the fed state (13). However, factors promoting PDC inactivation suppress malonyl-CoA formation and thereby favor acetyl-CoA production via the mitochondrial β-oxidation of long-chain fatty acids through relief of inhibition of CPT I. We propose that specific up-regulation of renal PDK4 protein expression in starvation via activation of PPARα, by maintaining PDC activity relatively low, functions to suppress malonyl-CoA formation from glucose and to promote pyruvate carboxylation to oxaloacetate. The latter is predicted not only to facilitate gluconeogenesis through sparing pyruvate, but also aid entry of acetyl-CoA derived from FA β-oxidation into the tricarboxylic acid cycle via citrate formation, and thereby to allow adequate ATP production to sustain brisk rates of gluconeogenesis. Thus, phosphorylation and inactivation of renal PDC activity elicited by increased PDK4 activity, secondary to activation of PPARα, may be an important mechanism to redirect available pyruvate toward gluconeogenesis and therefore represent an crucial component of the whole-body adaptation to maintain glycemia in response to starvation. Conversely, the failure to increase renal PDK4 protein expression in PPARα null mice may contribute to development of relative hypoglycemia in mice lacking PPARα (22) through impaired renal gluconeogenesis.

Studies with isolated mitochondria have shown that PDK activity can be modified by increases in NADH and acetyl-CoA concentrations (activation) and pyruvate (inhibition) (reviewed in 7). However, it has been shown using recombinant proteins that of the two PDK isoforms that are up-regulated in response to starvation in kidney, only one, PDK4, can be markedly activated by elevated $[NADH]/[NAD^+]$ in the absence of a concomitant elevation in acetyl-CoA (11). NADH and acetyl-CoA can be generated by increased rates of fatty acid β-oxidation, but since renal CPT I activity is suppressed by malonyl-CoA in the fed state (13), incoming fatty acids are largely reesterified rather than oxidized (37–39). It is therefore implied that the regulation of renal PDC via PDK is unlikely to be due to the increased mitochondrial $NADH/NAD^+$ concentration ratios elicited by long-chain FAO in the fed state, rendering PDHa activity relatively refractory to changes in mitochondrial $NADH/NAD^+$ ratios. However, in starvation and during the initial phase of refeeding after starvation, the potential exists for increased rates of FAO to impact on renal PDC activity by virtue of the effect of elevated mitochondrial $NADH/NAD^+$ concentration ratios on PDK4. Hence, renal PDK4 upregulation during starvation allows renal gluconeogenic flux to be coordinated by changes in FA supply and oxidation.

REFERENCES

1. Meyer, C., and Gerich, J. E. (2000) *Curr. Opin. Endocrinol. Diabetes* **7,** 19–24.
2. Gerich, J. E. Meyer, C., Woerle, H. J., and Stumvoll, M. (2001) *Diabetes Care* **24,** 382–391.
3. Gerich, J. E. (1993) *Baillieres Clin. Endocrinol. Metab.* **7,** 551–586.
4. Meyer, C., Dostou, J., Nadkarni, V., and Gerich, J. (1998) *Am. J. Physiol. Renal Physiol.* **275,** F915–F921.
5. Cersosimo, E., Garlick, P., and Ferretti, J. (1999) *Am. J. Physiol. Endocrinol. Metab.* **276,** E78–E84.
6. Cersosimo, E., Garlick, P., and Ferretti, J. (2000) *Metabolism* **49,** 676–683.
7. Sugden, M. C., and Holness, M. J. (1994) *FASEB J.* **8,** 54–61.
8. Patel, M. S., and Roche, T. E. (1990) *FASEB J.* **4,** 3224–3233.
9. Reed, L. J., and Hackert, M. L. (1990) *J. Biol. Chem.* **265,** 8971–8974.
10. Perham, R. N. (1991) *Biochemistry* **30,** 8501–8512.
11. Bowker-Kinley, M. M., Davis, W. I., Wu, P., Harris, R. A., and Popov, K. M. (1998) *Biochem. J.* **329,** 191–196.
12. Huang, B., Gudi, R., Wu, P., Harris, R. A., Hamilton, J., and Popov, K. M. (1998) *J. Biol. Chem.* **273,** 17680–17688.
13. Sugden, M. C., Holness, M. J., Donald, E., and Lall, H. (1999) *Metabolism* **48,** 707–715.
14. Wu, P., Blair, P. V., Sato, J., Jaskiewicz, J., Popov, K. M., and Harris, R. A. (2000) *Arch. Biochem. Biophys.* **381,** 1–7.
15. Sugden, M. C., Kraus, A., Harris, R. A., and Holness, M. J. (2000) *Biochem. J.* **346,** 651–657.
16. Wu, P., Inskeep, K., Bowker-Kinley, M. M., Popov, K. M., and Harris, R. A. (1999) *Diabetes* **48,** 1593–1599.
17. Braissant, O., Foufelle, F., Scotto, C., Dauca, M., and Wahli, W. (1996) *Endocrinology* **137,** 354–366.
18. Guan, Y., Zhang, Y., Davis, L., and Breyer, M. D. (1997) *Am. J. Physiol. Renal Physiol.* **273,** F1013–F1022.
19. Yang, T., Michele, D. E., Park, J., Smart, A. M., Lin, Z., Brosius, III, F. C., Schnermann, J. B., and Briggs, J. P. (1999) *Am. J. Physiol. Renal Physiol.* **277,** F966–F973.
20. Portilla, D., Dai, G., Peters, J. M., Gonzalez, F. J., Crew, M. D., and Proia, A. D. (2000) *Am. J. Physiol. Renal Physiol.* **278,** F667–F675.
21. Sugden, M. C., Fryer, L. G., Orfali, K. A., Priestman, D. A., Donald, E., and Holness, M. J. (1998) *Biochem. J.* **329,** 89–94.
22. Kersten, S., Seydoux, J., Peters, J. M., Gonzalez, F. J., Desvergne, B., and Wahli, W. (1999) *J. Clin. Invest.* **103,** 1489–1498.
23. Caterson, I. D., Fuller, S. J., and Randle, P. J. (1982) *Biochem. J.* **208,** 53–60.
24. Holness, M. J., Liu, Y. L., and Sugden, M. C. (1989) *Biochem. J.* **264,** 771–776.

25. Kerbey, A. L., Radcliffe, P. M., and Randle, P. J. (1977) *Biochem. J.* **164,** 509–519.
26. Kerbey, A. L., and Randle, P. J. (1982) *Biochem. J.* **206,** 103–111.
27. Sale, G. J., and Randle, P. J. (1981) *Eur. J. Biochem.* **120,** 535–540.
28. Randle, P. J. (1986) *Biochem. Soc. Trans.* **14,** 799–806.
29. Sale, G. J., and Randle, P. J. (1982) *Biochem. J.* **206,** 221–229.
30. Sale, G. J., and Randle, P. J. (1982) *Biochem. J.* **203,** 99–108.
31. Holness, M. J., Palmer, T. N., and Sugden, M. C. (1985) *Biochem. J.* **232,** 255–259.
32. Holness, M. J., and Sugden, M. C. (1987) *Biochem. J.* **241,** 421–425.
33. Kersten, S., Desvergne, B., and Wahli, W. (2000) *Nature* **405,** 421–424.
34. Stumvoll, M., Meyer, C., Mitrakou, A., Nadkarni, V., and Gerich, J. E. (1997) *Diabetologia* **40,** 749–757.
35. McGarry, J. D., and Brown, N. F. (1997) *Eur. J. Biochem.* **244,** 1–14.
36. Brown, N. F., Hill, J. K., Esser, V., Kirkland, J. L., Corkey, B. E., Foster, D. W., and McGarry, J. D. (1997) *Biochem. J.* **327,** 225–231.
37. Kleinzeller, A., and McAvoy, E. M. (1986) *Biochim. Biophys. Acta* **856,** 545–555.
38. Weidemann, M. J., and Krebs, H. A. (1969) *Biochem. J.* **112,** 149–166.
39. Wirthensohn, G., Gerl, M., and Guder, W. G. (1980) *Int. J. Biochem.* **12,** 157–161.