Substrate Interactions in the Short- and Long-Term Regulation of Renal Glucose Oxidation

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The present study evaluated the substrate competition between fatty acids (FA) and glucose in the kidney in vivo in relation to the operation of the "glucose-FA" and "reverse glucose-FA" cycles. In fed rats, neither inhibition of adipocyte lipolysis by 5-methylpyrazole-3-carboxylic acid (MPCA) nor inhibition of mitochondrial long-chain FA oxidation by 2-tetradecylglycidate (TDG) influenced the renal ratio of free/acylated carnitine or the percentage of total renal pyruvate dehydrogenase complex (PDHC) in the active (dephosphorylated) form (PDHa). The additional provision of glucose, a precursor for the synthesis of malonyl-coenzyme A (coA), did not influence renal PDHa activity or the renal ratio of free to acylated carnitine, implying that FA oxidation is maximally suppressed in the fed state. A reverse glucose-FA cycle may therefore be important in suppressing renal FA oxidation in the fed state. After 48 hours of starvation, MPCA and TDG decreased short- and long-chain acylcarnitine concentrations (40% to 50%, $P < .01$) and elevated the renal ratio of free/acylated carnitine (2.5-fold, $P < .001$, and 3.3-fold, $P < .001$, respectively), indicating that FA oxidation is increased after starvation. Despite suppression of renal FA oxidation, renal PDHa activity in 48-hour starved rats was only partially restored by treatment with MPCA or TDG. The additional administration of glucose did not remedy this. The failure to reverse completely the effects of prolonged starvation in suppressing PDHC activity by acute inhibition of FA oxidation suggests additional regulatory mechanisms that dampen the PDHC response to acute changes in substrate supply. Estimations of PDH kinase (PDK) activity in renal mitochondria showed a significant 1.7-fold stable increase ($P < .01$) after 48 hours of starvation. Analysis of PDK pyruvate sensitivity in renal mitochondria incubated with respiratory substrate (5 mmol/L 2-oxoglutarate/0.5 mmol/L L-malate) showed that the pyruvate concentration required for 50% activation was substantially decreased by starvation. Enzyme-linked immunosorbent assay (ELISA) analysis over a range of PDHC activities demonstrated that increased PDK activity was concomitant with a significant (at least $P < .01$) 1.8-fold increase in the protein expression of the ubiquitously expressed PDK isoform, PDK2. We hypothesize that changes in protein expression and activity of individual PDK isoforms may dictate the renal response to incoming FA (esterification *v* oxidation) through modulation of the relationship between glycolytic flux and PDHC activity, and thus the provision of precursor for malonyl-coA production.

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THERE IS renewed interest in the kidney as a quantitatively important site of glucose disposal and production. The kidney accounts for about 20% of overall glucose disposal in the postabsorptive state.[1,2] Its fractional extraction of glucose is approximately 3%, and by extrapolation, the kidney could account for up to 30% of glucose disposal postprandially, when glucose concentrations are elevated.[1-3] The kidney is also a quantitatively important site of fatty acid (FA) uptake: in postabsorptive man, the kidney accounts for about 10% of overall FA disposal.[4] A reciprocal relationship between renal glucose and FA uptake, previously demonstrated in fasted rats,[5] was recently demonstrated in man.[4] Substrate competition between FA and glucose (the "glucose-FA cycle") is established[6-8]; however, muscle has hitherto been considered the quantitatively major site of operation of this cycle. The new data suggest that the renal contribution to glucose "sparing" via the glucose-FA cycle may be quantitatively important for whole-body glucose homeostasis.

Regulation of the rate-limiting step for glucose oxidation, the pyruvate dehydrogenase complex (PDHC), underlies the operation of the glucose-FA cycle.[7] Increased FA supply/oxidation reduces glucose oxidation by inhibiting PDHC (reviewed in Sugden and Holness[7]). This spares pyruvate for gluconeogenesis. Conversely, in some tissues, increased glucose supply reduces FA oxidation (the "reverse glucose-FA cycle") through an increase in malonyl-coenzyme A (coA), which inhibits the transport of long-chain FA into the mitochondria via carnitine palmitoyltransferase I (CPT I).[7] The conversion of pyruvate to acetyl-coA via PDHC links glycolysis with the citric acid cycle and the synthesis of malonyl-coA. The operation of the reverse glucose-FA cycle has been under intensive investigation in the liver.[9-12] More recently, it has emerged that an analogous substrate interaction may also be important for restricting FA oxidation in the skeletal muscle,[13-16] heart,[17-21] and pancreatic β cell.[22-24] The kidney contains the L (liver) type of CPT I,[25,26] and a substantial portion of FA taken up by the kidney is incorporated into triacylglycerols,[27-29] findings implying that the fate of incoming FA is regulated in the kidney and thus that a reverse glucose-FA cycle may operate in this tissue.

PDHC activity is regulated by interconversion between phosphorylated (inactive) and nonphosphorylated (active) forms.[7] Short-term effects of increased FA oxidation to suppress PDHC activity are mediated by increased mitochondrial acetyl-coA/coA and NADH/NAD^+ concentration ratios, which activate PDH kinase (PDK).[30] Conversely, changes in glycolytic flux may lead to changes in mitochondrial concentrations of pyruvate, an inhibitor of PDK activity.[31-33] In the liver—like the kidney, an important gluconeogenic tissue—extended starva-

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tion evokes an additional stable enhancement of PDK activity[34-37] together with a tissue-specific increase in the protein expression of PDK isoform 2 (PDK2),[34] one of four PDK isoforms identified thus far in mammalian tissues.[38,39] Increased hepatic PDK2 expression can account for approximately 40% of the PDK activity increase observed in the liver after prolonged starvation.[34] Increased PDK2 expression assists in the conservation of the three-carbon compounds required for hepatic glucose synthesis, as well as the suppression of hepatic FA synthesis.

The aim of this study was to elucidate the factors governing renal glucose oxidation in relation to possible reciprocal regulatory interactions between the oxidation of lipid and carbohydrate. We examined the acute substrate-led regulation of renal PDHC activity (phosphorylation) status in vivo by varying either the FA supply (through pharmacologic inhibition of lipolysis) or the rate of mitochondrial long-chain FA oxidation (through inhibition of CPT I). The impact of these acute manipulations on renal active PDHC (PDHa) activity was evaluated in relation to the free and acylated carnitine concentrations, indices of renal FA oxidation. Potential effects of increased glycolytic flux to pyruvate were assessed indirectly by providing glucose in conjunction with suppression of the FA supply. We examined the impact of prolonged starvation on renal PDK2 protein expression to determine the relationship, if any, between PDK activity and altered PDK protein expression. Finally, we assessed whether changes in PDK activity are associated with altered regulatory characteristics that might be indicative of PDK isoform switching.

MATERIALS AND METHODS

Materials

2-Tetradecylglycidate ([TDG] McN-3802) was a generous gift from McNeil Pharmaceuticals (Spring House, PA). 5-Methylpyrazole-3-carboxylic acid (MPCA) was generously provided by Upjohn (Crawley, West Sussex, UK). Other biochemicals and chemicals were from Boehringer or Sigma Chemical (Poole, Dorset, UK). Female Wistar rats were purchased from Charles River (Margate, Kent, UK).

Animals and Pharmacologic Interventions

Female Wistar rats (180 to 220 g) were maintained on a 12-hour light/dark cycle and were either fed ad libitum or starved for 48 hours (grid-bottom cages). Experiments were performed at the start of the light phase, when fed rats were in the postprandial state. To inhibit adipose tissue lipolysis, MPCA or an equivalent amount of saline was administered by intraperitoneal injection (0.66 mg/100 g body weight) 2 hours before sampling.[40,41] To inhibit CPT I, TDG was administered intragastrically as a suspension (2.5 mg/100 g body weight) in 0.5% (wt/vol) carboxymethylcellulose 2 hours before sampling.[42] Administration of 0.5% (wt/vol) carboxymethylcellulose did not affect the parameters under investigation. Glucose (2 mmol/100 g body weight) or water (1 mL/100 g body weight) was administered intragastrically 2 hours before sampling.[40] In further experiments, dichloroacetate ([DCA] 0.25 mL of a 5% wt/wt solution in 0.15 mol/L NaCl, pH 7.4) or 0.15 mol/L NaCl was injected intraperitoneally at 0.5-hour intervals until the rats were sampled at 2 hours.[43]

Measurement of Free and Acylated Carnitines

The kidney was removed and immediately freeze-clamped while the rats were anesthetized with pentobarbital (60 mg/kg body weight). This procedure minimizes changes in carnitine profiles due to ischemia. The frozen kidney was ground to a powder at −40°C and assayed for free and acylated carnitine in $HClO_4$ extracts.[44] Free (nonesterified) carnitine was taken as the carnitine found when extracts were examined without prior exposure to alkali. $HClO_4$-insoluble carnitine was assumed to be long-chain esters (chain length > 10 carbon atoms), and the $HClO_4$-soluble fraction was assumed to contain short-chain derivatives and free carnitine.[45]

Enzyme-Linked Immunosorbent Assays

Antibodies to purified recombinant PDK2 (Zeneca Pharmaceuticals, Macclesfield, UK) were raised in New Zealand white rabbits.[34] Serum was screened for antibodies by Western blotting using preimmune serum as a control. The antiserum was specific to PDK2 with negligible cross-reaction to PDK1. Enzyme-linked immunosorbent assays (ELISAs) were performed using clarified extracts of kidney mitochondria as described previously for liver.[34]

PDH and PDK Assays

PDHa (in freeze-clamped extracts of whole kidney and in freshly isolated renal mitochondria) and total PDHC (in freshly isolated renal mitochondria) were assayed spectrophotometrically by coupling to arylamine acetyltransferase.[41,46] Total PDHC activity (expressed relative to the mitochondrial marker citrate synthase) was unchanged by 48 hours of starvation (ad libitum, 132 ± 9, n = 12; 48-hour starved, 128 ± 17, n = 6, NS). PDK activity was determined (pH 7.0) in extracts of renal mitochondria by the rate of adenosine triphosphate (ATP)-dependent inactivation of PDHa and computed as apparent first-order rate constants for ATP-dependent PDHa inactivation.[47]

Metabolite Analysis

Arterial blood samples were obtained at death. Plasma was assayed spectrophotometrically for nonesterified FA using a kit (Wako C-test kit; Alpha Laboratories, Eastleigh, UK). Glucose and lactate concentrations were determined in KOH-neutralized $HClO_4$ extracts of whole blood by enzymatic spectrophotometric assays.[48,49]

Statistical Analysis

Experimental data are expressed as the mean ± SE. The statistical significance of differences between groups was assessed by Student's unpaired *t* test. Curve-fitting was performed using *Fig P* software (Biosoft, Cambridge, UK).

RESULTS

Effects of Pharmacologic Modulation of FA Supply or Oxidation on Plasma FA and Blood Metabolite Concentrations

We examined the effects of limiting the supply of FA for β-oxidation without any accompanying increase in carbohydrate supply by two methods. First, adipose tissue lipolysis was inhibited by MPCA. This intervention elicited a 44% ($P < .01$) decline in plasma FA concentrations in the fed state (where FA concentrations were already very low), and there was almost complete (91%, $P < .01$) suppression of FA concentrations after 2 hours of MPCA treatment in the 48-hour starved group. Secondly, mitochondrial long-chain FA oxidation was inhibited by administration of TDG, an inhibitor of CPT I. In fed rats, this manipulation was associated with a 1.7-fold increase ($P < .001$) in plasma FA concentrations. This finding suggests that suppression of mitochondrial FA oxidation limits the rate of FA removal from the bloodstream and that there is normally significant tissue disposal of FA in the fed state. In 48-hour starved rats,

Table 1. Effects of Modulation of FA Supply or Oxidation on Circulating Metabolite Concentrations and Renal Ratios of Free to Acylated Carnitine in Fed or 48-Hour Starved Rats

Parameter	Fed			48-Hour Starved		
	Control	TDG	MPCA	Control	TDG	MPCA
Metabolites (mmol/L)						
Plasma NEFA	0.18 ± 0.01	0.31 ± 0.02*	0.10 ± 0.02†	0.44 ± 0.03‡	1.76 ± 0.12*‡	0.04 ± 0.01*§
Blood glucose	7.59 ± 0.21	6.97 ± 0.30	8.01 ± 0.27	5.67 ± 0.08‡	2.25 ± 0.19*‡	3.01 ± 0.18*‡
Blood lactate	2.27 ± 0.18	1.90 ± 0.16	1.97 ± 0.31	0.93 ± 0.08‡	0.92 ± 0.12‡	1.13 ± 0.09§
Renal ratio of free to acylated carnitine	1.64 ± 0.12	1.71 ± 0.04	1.91 ± 0.11	0.66 ± 0.06‡	1.62 ± 0.04*	2.18 ± 0.16*

NOTE. Results are the mean ± SE.
Statistically significant effects of TDG or MPCA: *$P < .001$ and †$P < .01$.
Statistically significant effects of starvation: ‡$P < .001$ and §$P < .05$.

TDG administration led to a greater (fourfold, $P < .001$) increase in plasma NEFA concentrations than observed in the fed state, presumably reflecting the higher rates of FA release and utilization in starvation. Blood glucose concentrations were higher in the fed state and decreased in response to administration of TDG (60%, $P < .001$) and MPCA (47%, $P < .001$) in starved rats but not in fed rats. Blood lactate concentrations were significantly higher ($P < .05$) in the fed state irrespective of whether FA supply or oxidation was suppressed (Table 1).

Effects of Modulation of FA Supply or Oxidation on Renal Free and Acylated Carnitine Concentrations

Measurements of free and acylated carnitine concentrations in freeze-clamped kidney extracts (Fig 1A) indicated the specific effects of pharmacologic modulation of FA supply and mitochondrial β-oxidation rates on renal metabolism. Starvation (48 hours) led to a 1.6-fold increase ($P < .01$) in short-chain (S) and long-chain (L) acylcarnitine concentrations, whereas the free carnitine (F) concentration decreased to 63% of the fed value ($P < .001$). In fed rats, MPCA led to a significant (73%, $P < .001$) decline in renal long-chain acylcarnitine concentrations from 13.3 ± 1.2 (n = 6) to 3.6 ± 0.9 (n = 6) nmol/g wet weight, with a trend (16%, NS) for an increased renal ratio of free/acylated carnitine (F/S + L) (Table 1). TDG was without significant effect on free or acylated carnitine concentrations in fed rats. In 48-hour starved rats, MPCA and TDG significantly decreased short- and long-chain acylcarnitine concentrations (by ~35% to 50%, $P < .01$) and elevated F/S + L ratios 2.5-fold ($P < .001$) and 3.3-fold ($P < .001$), respectively (Table 1). In TDG- and MPCA-treated rats, renal concentrations of free and acylated carnitine were essentially similar irrespective of whether the rats were fed or starved. In MPCA-treated rats, concentrations of long-chain acylcarnitine were 3.7-fold higher ($P < .001$) when the rats were starved, but were nevertheless 38% lower ($P < .01$) than the levels found in untreated starved rats.

Effects of Modulation of FA Supply or Oxidation on Renal PDHa Activity

Starvation (48 hours) led to a significant decline (63%, $P < .001$) in the percentage of total PDHC existing in the active (dephosphorylated) form (PDHa), from 25% ± 1% (n = 6) to 9% ± 0.3% (n = 6), in the absence of any significant change in total PDHC activity (the sum of active and inactive forms). The effects of acute modulation of FA supply or oxidation on renal PDHa activity are shown in Fig 1B. TDG and MPCA did not influence renal PDHa activity in fed rats, but led to partial reactivation of PDHC in 48-hour starved rats. After treatment with TDG, PDHa activity was restored to only approximately 50% of the fed level. Since steady-state free and acylated carnitine concentrations did not differ significantly between fed and starved rats treated with TDG, the failure to restore PDHa activity is unlikely attributable to an increased rate of FA oxidation in treated starved rats. Similarly, despite essentially complete suppression of plasma FA levels by MPCA in starved

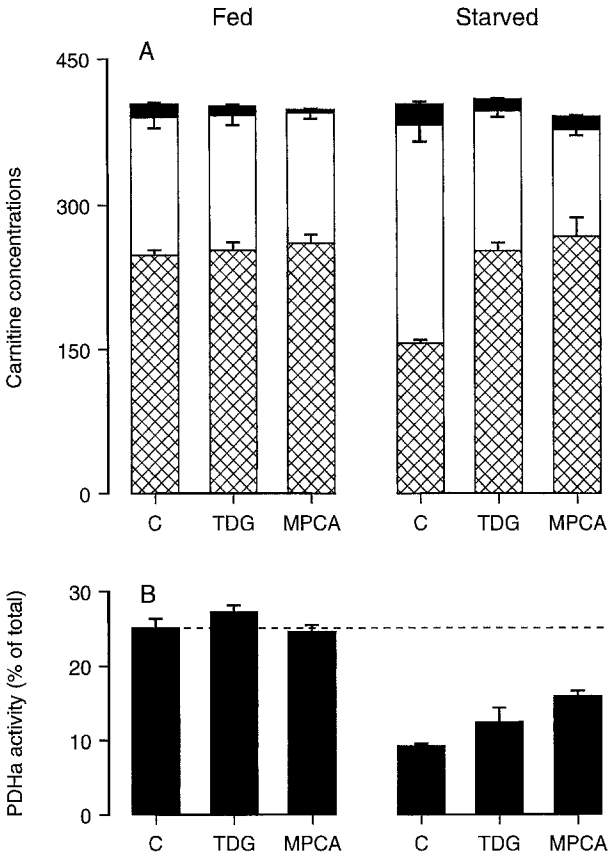

Fig 1. Effects of TDG and MPCA on free, short-chain, and long-chain carnitine concentrations and PDHa activity in kidneys from fed or 48-hour starved rats. Free (▨), short-chain (□), and long-chain (■) carnitine concentrations and PDHa activity were measured in freeze-clamped kidney extracts. The total height of the bar corresponds to the total carnitine concentration in each group. Results are the mean ± SE for ≥6 rats. Statistical significance of the effects of starvation, TDG, and MPCA is reported in the text.

rats (Table 1), PDHa activity at the end of the 2-hour treatment period was restored to only approximately 64% of the fed level.

Effects of Increased Glucose Supply on Plasma and Blood Analytes

In 48-hour starved rats, inhibition of FA oxidation by restricting adipose tissue lipolysis or mitochondrial entry of long-chain acyl-coA was, as expected, associated with reduced glycemia (Table 1). To investigate whether the failure to elicit more complete renal PDH reactivation on inhibition of lipolysis or β-oxidation was a consequence of this, an oral glucose load was administered in combination with injection of MPCA. Plasma FA and blood glucose and lactate concentrations 2 hours after administration of glucose in fed and 48-hour starved rats are shown in Table 2. In fed rats, the glucose load was cleared within 2 hours of administration (results not shown), and therefore, glycemia at 2 hours was unaffected. However, blood lactate concentrations 2 hours after glucose administration were increased 37% ($P < .01$). In addition, plasma NEFA was suppressed to values comparable to those found in fed rats treated with MPCA (compare data in Tables 1 and 2). At 2 hours after glucose administration to starved rats, blood glucose and lactate concentrations were increased (32% and 238%, respectively, $P < .001$), whereas plasma FA concentrations were decreased to values not significantly different from those found in the fed state. Administration of glucose in combination with MPCA normalized blood glucose in starved rats to the values found in fed rats (Tables 1 and 2).

Effects of Increased Glucose Supply on Renal Free and Acylated Carnitine Concentrations

Free and acylated carnitine concentrations in the kidney of fed and 48-hour starved rats 2 hours after glucose administration are shown in Fig 2A. Whereas glucose produced relatively few and minor changes in renal concentrations of free and acylated carnitine in fed rats, as indicated by the absence of any significant change in the ratio of free to acylated carnitine, an index of FA oxidation (1.72 ± 0.14, n = 6, for fed + glucose *v* 1.64 ± 0.12, n = 6, for fed, NS), glucose administration to starved rats increased the ratio of free to acylated carnitine (from 0.66 ± 0.07, n = 6, to 1.37 ± 0.16, n = 6, $P < .01$) by virtue of increases in free carnitine and decreases in both short-chain and long-chain acylcarnitine (compare values in Figs 1A and 2A). Concentrations of free and long-chain

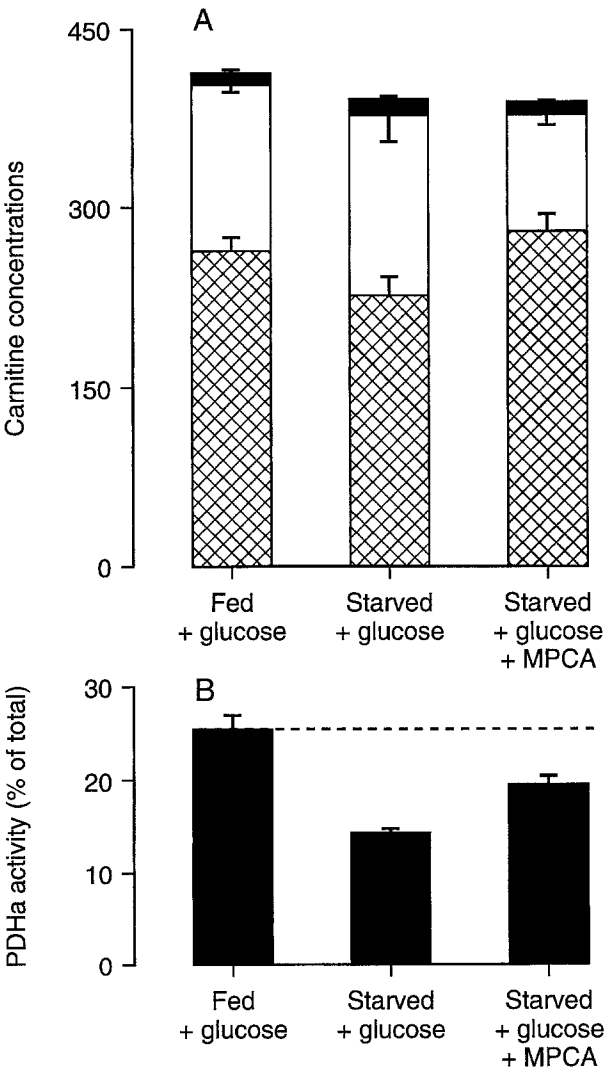

Fig 2. Effects of glucose in the absence or presence of MPCA on free, short-chain, and long-chain carnitine concentrations and PDHa activity in kidneys from fed or 48-hour starved rats. Free (▨), short-chain (□), and long-chain (■) carnitine concentrations and PDHa activity were measured in freeze-clamped kidney extracts. The total height of the bar corresponds to the total carnitine concentration in each group. Results are the mean ± SE for ≥6 rats. Statistical significance of the effects of starvation, TDG, and MPCA is reported in the text.

Table 2. Blood and Plasma Metabolite Concentrations and Renal Ratios of Free to Acylated Carnitine in Fed or 48-Hour Starved Rats After Oral Administration of Glucose Alone or Together With MPCA

Parameter	Fed	48-Hour Starved	
	Glucose	Glucose	Glucose + MPCA
Metabolites (mmol/L)			
Plasma NEFA	0.09 ± 0.01	0.20 ± 0.05	0.01 ± 0.01‡
Blood glucose	7.62 ± 0.15	7.48 ± 0.01	7.88 ± 0.22
Blood lactate	3.12 ± 0.16	2.22 ± 0.12*	1.91 ± 0.24
Renal ratio of free to acylated carnitine	1.72 ± 0.14	1.37 ± 0.16	2.57 ± 0.09†

NOTE. Results are the mean ± SE.
Statistically significant effects of starvation: *$P < .01$.
Statistically significant effects of MPCA: †$P < .001$ and ‡$P < .01$.

acylcarnitines 2 hours after glucose administration did not differ significantly between fed rats that were administered glucose and starved rats that were administered MPCA in combination with glucose. The concentrations of short-chain acylcarnitine were lower (35%, $P < .05$) in starved rats treated with glucose plus MPCA compared with rats treated with glucose alone. This suggests that an increase in glucose supply alone is unable to suppress FA oxidation completely when rats are starved.

Effects of Increased Glucose Supply on Renal PDHa Activity

The effects of an increased glucose supply on renal PDHa activity are shown in Fig 2B. Administration of glucose had no significant effect on renal PDHa activity in fed rats, but

significantly increased renal PDHa activity in starved rats (1.6-fold, $P < .001$; compare Figs 1B and 2B). However, renal PDHa activity in starved rats treated with glucose remained approximately 43% lower ($P < .001$) than the corresponding values for fed rats (14% ± 0.4% PDHa *v* 25% ± 2% PDHa). Renal PDHa activity in starved rats treated with both glucose and MPCA was higher than that found when only MPCA was administered (20% ± 1% PDHa *v* 16% ± 1% PDHa, $P < .05$), but remained approximately 23% lower than that found in the fed state.

Effect of 48-Hour Starvation on PDK Activity and PDK2 Immunoreactive Protein in Isolated Mitochondria

The results of these studies are consistent with the concept that, as in the liver, prolonged starvation evokes a long-term mechanism(s) for restricting PDHC activation in response to subsequent suppression of the exogenous supply of FA and/or an increased supply of glucose and lactate. Estimations of PDK activity in freshly isolated renal mitochondria showed that 48 hours' starvation led to a significant 1.7-fold stable increase in PDK activity (from 0.30 ± 0.03 to 0.52 ± 0.05 min^{-1}, $P < .01$). By ELISAs using rabbit anti–rat recombinant PDK2 serum, we examined whether this reflected any change in the protein expression of PDK2, which we have demonstrated previously to be an important component of the response to starvation in liver.[34] Mitochondria were prepared from the kidneys of control and 48-hour starved rats in parallel (with four separate mitochondrial preparations for each group), and extracts were compared directly in quadruplicate through side-by-side comparison in the same well block and over the same range of PDHC activity (Fig 3). Plateaus were reached at about 0.25 m-unit PDHC per well with mitochondrial extracts from both groups. The amount of immunoreactive PDK2 in renal mitochondrial extracts increased significantly after 48 hours' starvation. The mean ratio of activity for extracts (starved:fed) at individual points in the ELISAs was 1.76 ± 0.05 (n = 16). While the relative contribution of PDK2 to total PDK activity is not known, the increase in PDK2 protein could account for a substantial proportion of the overall increase in renal PDK activity.

Effects of Pyruvate on Active and Inactive Forms of PDHC in Kidney Mitochondria From Fed and 48-Hour Starved Rats

The mean concentration of active PDHC measured in freshly prepared renal mitochondria from fed rats incubated for 5 minutes at 30°C in the presence of respiratory substrate (5 mmol/L 2-oxoglutarate/0.5 mmol/L L-malate) was 19% ± 3% (n = 9) of the total complex, with a 42% decline ($P < .05$) to 11% ± 2% (n = 9) of the total complex when mitochondria were prepared from 48-hour starved rats. Thus, the relative activity of active PDHC measured in mitochondria incubated with respiratory substrate broadly reiterates that found in freeze-clamped tissue, and in both freeze-clamped tissue and isolated mitochondria, starvation for 48 hours results in a substantial reduction in the proportion of active PDHC. The addition of pyruvate to mitochondria incubated with respiratory substrate (to generate ATP) leads to PDHC activation through suppression of PDK activity.[33,50,51] Since the individual PDK isoforms identified to date differ with respect to regulation by pyruvate,[38] a change in pyruvate sensitivity might imply a switch in PDK isoform expression. We therefore examined whether prolonged starvation was associated with any change in PDK sensitivity. The effects of addition of pyruvate (0.1 to 100 mmol/L) on the concentration of active PDH complex in kidney mitochondria prepared from fed rats and incubated with 5 mmol/L 2-oxoglutarate/0.5 mmol/L L-malate are shown in Fig 4. As observed in previous studies with cardiac mitochondria,[33]

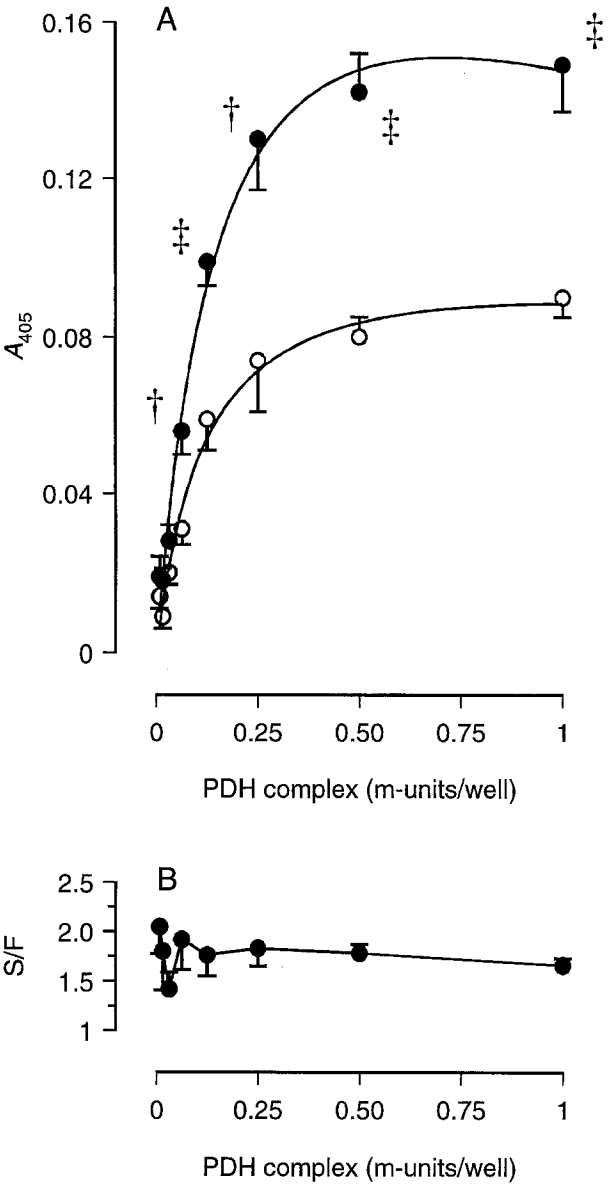

Fig 3. ELISA analysis of PDK2 protein expression in extracts of rat kidney mitochondria from fed or 48-hour starved rats. Kidney mitochondria were extracted by freezing and thawing (3 times) in mitochondrial extraction buffer. The extracts were clarified by centrifugation, and ELISA analysis was performed as described previously for liver.[32] Results are the mean ± SE for 4 ELISAs (4 wells per assay) from 4 mitochondrial preparations each from control rats (○) and 48-hour starved rats (●) in A. The ratios of starved/fed (S/F, mean ± SE) for individual points in the ELISAs, which provide quantitative estimates of the increase in PDK2 protein expression induced by starvation, are shown in B. Statistically significant effects of starvation: †$P < .05$, ‡$P < .01$.

pyruvate addition inhibited PDK in freshly prepared kidney mitochondria with activation of PDHC. The pyruvate concentration producing 50% active PDHC in mitochondria from kidneys of fed rats was approximately 15 μmol/L. When a comparison was made with mitochondria prepared from 48-hour starved rats, a decreased responsiveness of PDHC to activation by pyruvate was clearly evident, although differences between fed and starved groups achieved significance only at the highest (nonphysiologic) pyruvate concentration (Fig 4). Even at 100 mmol/L pyruvate (the highest concentration tested), only about 25% of total PDHC was present in the active form in mitochondria prepared from 48-hour starved rats. As a consequence, the percent of PDHa was 67% lower ($P < .05$) than observed in mitochondria prepared from fed rats.

The pyruvate analog DCA activates PDHC through inhibition of PDK.[52] In studies in vivo, DCA administration increased renal PDHa activity in starved rats but not in fed rats. The effect of DCA in 48-hour starved rats was insufficient to normalize differences in renal PDHa activity between fed and starved rats (Table 3). As a consequence, renal PDHa activity after DCA treatment remained approximately 30% ($P < .05$) lower than that found in fed rats after DCA treatment.

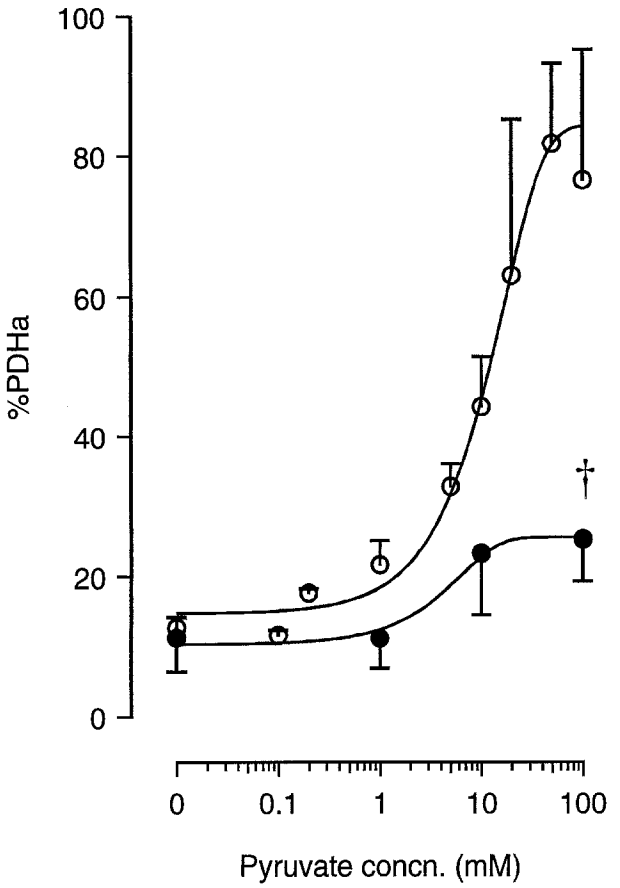

Fig 4. Effect of increasing pyruvate concentrations on steady-state PDHC activity in renal mitochondria. Mitochondria were prepared from fed (○) or 48-hour starved (●) rats. Mitochondria (0.5 to 1 mg protein) were incubated for 5 minutes at 30°C in 0.5 mL KCl medium containing 5 mmol/L 2-oxoglutarate/0.5 mmol/L L-malate with the concentrations of pyruvate shown. Each point is the mean of duplicate observations for ≥3 mitochondrial preparations from each group.

Table 3. Effect of DCA on Renal PDHa Activity In Vivo in Fed or 48-Hour Starved Rats

Treatment	PDHa Activity (% of total)	
	Fed	48-Hour Starved
Control	25.1 ± 1.2	9.2 ± 0.3*
DCA	26.4 ± 2.2	18.7 ± 1.3†‡

NOTE. Results are the mean ± SE.
Statistically significant effects of starvation: *$P < .001$ and †$P < .05$.
Statistically significant effects of DCA treatment: ‡$P < .001$.

DISCUSSION

The present study analyzed the relationship between acute alterations in FA oxidation rates in vivo (assessed by changes in free and acylated carnitine concentrations) and changes in renal PDHa activities in extracts of whole kidney. Pharmacologic inhibition of lipolysis or long-chain FA oxidation did not markedly alter renal free and acylated carnitine concentrations in fed rats, suggesting that renal FA oxidation is already low in the fed state. The provision of glucose, a potential precursor for malonyl-coA synthesis, did not increase the renal ratio of free to acylated carnitine in fed rats. The kidney contains the L (liver)-type isoform of CPT I.[25,26] Hepatic FA oxidation is suppressed in the fed state.[7] PDHa activity was not increased by glucose or DCA administration in fed rats, although PDK was demonstrated in separate experiments to be sensitive to changes in pyruvate concentrations. Taken together, the data suggest that both CPT and PDK activities in kidney are maximally suppressed in the fed state, when the glucose supply is abundant.

A potential problem with attributing the operation of the reverse glucose-FA cycle in the kidney stems from the distribution of glycolytic and oxidative enzymes along the nephron.[53] The proximal convoluted tubules have the highest capacity to oxidize FA but the lowest rate of glycolysis. Conversely, the distal segment of the nephron has the highest capacity for glycolysis but the lowest rate of FA oxidation. The kidney contains components of the insulin signaling machinery including insulin receptor substrate-4 (IRS-4)[54] and a new regulatory component of phosphatidylinositol-3-kinase (p55 PIK), which has been implicated in insulin receptor signaling,[55] but the insulin-regulated glucose transporter GLUT4 is located solely in the thick ascending limbs, whereas GLUT2 and GLUT5 (other members of the glucose transporter family, which have differing characteristics of regulation and are not considered to be regulated by insulin[56]) are exclusively located in the proximal tubules.[57] Gluconeogenesis primarily occurs in the proximal tubules, where FA represent the major metabolic fuel. A potential limitation of the design used in the present experiments is therefore that the kidney was studied as a single tissue. Nevertheless, since glycolysis and FA oxidation both occur throughout the nephron,[58] it is reasonable to suggest that the reverse glucose-FA cycle is of regulatory importance for suppression of renal FA oxidation in the fed state. Stimulation of renal glucose uptake by insulin has been observed in vivo without a change in glycemia.[1] It was proposed that the insulin-mediated increase in renal glucose uptake in this study most likely occurs in the distal nephron, and may reflect an enhancement in both glycogen synthesis and glucose oxidation.[1] Substrate-led regulation may be of greater significance in the proximal tubule, which does not contain the insulin-

regulatable glucose transporter (GLUT4). Thus, increased glucose uptake by the proximal tubule when glucose is abundant would be predicted to limit fat oxidation as a consequence of increased malonyl-coA production. In turn, the restraint on fat oxidation would result in a decrease in gluconeogenesis. Within the context of the operation of the conventional glucose-FA cycle in starvation, a previous study has reported greater inhibition of glucose uptake by palmitate in medullary—rather than cortical—rat kidney slices.[53] The present studies demonstrate partial reversal of the effects of prolonged starvation to suppress renal PDHC activity by acute inhibition of FA oxidation. However, the failure to observe complete reversal of the effects of prolonged starvation to suppress PDHa activity by acute inhibition of FA oxidation, together with the inability of DCA treatment to normalize PDHa activity between the fed and starved groups, suggest the existence of additional regulatory mechanisms that dampen the response to changes in regulatory metabolites.

The percentage of active PDHC existing in a particular tissue is determined by the relative PDK and PDHC phosphatase activities prevailing in the mitochondria. The present studies demonstrate an enhancement of renal PDK activity after prolonged starvation that is stable to mitochondrial preparation and therefore distinct from (and independent of) acute effects of acetyl-coA, NADH, and pyruvate. PDK activity in rat tissues may correspond to up to four distinct PDK isoforms.[38] Increased protein expression of PDK2 comprises a major component of long-term enhancement of hepatic PDK activity in response to prolonged starvation.[34] The present study clearly demonstrates for the first time that a similar mechanism operates in the kidney. Previous studies using the same antibody to PDK2 failed to detect any increase in PDK2 expression in the heart after prolonged starvation.[31] Increased protein expression of PDK2 may therefore be characteristic of tissues in which the regulation of PDHC is important for directing (eg, lipogenesis) or facilitating (eg, gluconeogenesis) biosynthesis, rather than those in which PDHC subserves a predominantly bioenergetic function such as the heart.

Studies with individual recombinant PDK isoenzymes have shown that as well as differing in specific activity, each isoenzyme is characterized by unique regulatory properties with respect to suppression by pyruvate (assessed using the pyruvate analog DCA) and activation by acetyl-coA and NADH.[38] PDK2 (a low–specific activity isoform) is relatively sensitive to pyruvate inhibition, whereas PDK3 and PDK4 are not. NADH alone stimulates PDK2 only weakly, and the stimulation is greatly enhanced by further addition of acetyl-coA, whereas PDK4 is activated twofold by NADH alone with no further effect on addition of acetyl-coA. In addition to PDK2, mRNA for PDK3 (a high–specific activity, pyruvate-insensitive isoform) is expressed in kidney.[38] There is also modest mRNA expression of PDK4.[38] The present studies indicate that starvation renders renal PDK somewhat refractory to suppression by pyruvate and DCA. If increased protein expression of PDK2 were solely responsible for the increase in PDK activity, it would be predicted that the characteristics of regulation by pyruvate would be retained. The activity state of renal PDH in starved rats was also found to be relatively refractory to inhibition of FA oxidation: although pharmacologic inhibition of CPT I activity in 48-hour starved rats resulted in concentrations of free acid-soluble (short-chain) and acid-insoluble (long-chain) acylcarnitine that did not differ significantly from those found in fed rats—implying almost complete suppression of acetyl-coA production from FA—the effects on renal PDHa activity, although significant, were modest. Taken together, the altered characteristics of regulation of renal PDK activity observed after starvation (refractory with respect to suppression of FA oxidation and to an increased pyruvate supply) are compatible with increased expression of a relatively pyruvate-insensitive isoform (eg, PDK3), although direct quantitative assessment of changes in PDK3 expression are presently precluded by the lack of availability of a sufficiently specific antibody. The relative insensitivity of renal PDK to suppression by pyruvate would be predicted to facilitate the sparing of lactate and alanine for gluconeogenesis during starvation. In addition, changes in PDK isoform expression—as well as influencing the cellular capacity for the oxidation of glucose and of potential gluconeogenic precursors (lactate, alanine, pyruvate, etc.)—may influence the renal response to incoming FA (esterification *v* oxidation) through modulation of the relationship between changes in glycolytic flux and malonyl-coA production.

In summary, the present study demonstrates that renal glucose oxidation via PDHC is regulated acutely by changes in substrate supply. In the fed state, regulation of PDHC may be important for the operation of the reverse glucose-FA cycle, whereby an abundant supply of glucose suppresses FA oxidation. In contrast, there is a switch to a predominant effect of FA over glucose oxidation after prolonged starvation. The study also demonstrates that the level and pattern of PDK isoform expression may be an important factor in determining the activity status of renal PDHC, in modulating the response to changes in the concentration of metabolite effectors, and in dictating whether the glucose or FA supply has the predominant regulatory influence over the selection of oxidative substrate.

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