Kidney Protein Dynamics and Ammoniagenesis in Humans with Chronic Metabolic Acidosis

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Abstract. To evaluate the effects of chronic metabolic acidosis on protein dynamics and amino acid oxidation in the human kidney, a combination of organ isotopic (13C-leucine) and mass-balance techniques in 11 subjects with normal renal function undergoing venous catheterization was used. Five of 11 studies were performed in the presence of metabolic acidosis. In subjects with normal acid-base balance, kidney protein degradation was 35% to 130% higher than protein synthesis, so net protein leucine balance was markedly negative. In acidic subjects, kidney protein degradation was no different from protein synthesis and was significantly lower (P < 0.05) than in controls. Kidney leucine oxidation was similar in both groups. Urinary ammonia excretion and total ammonia production were 186% and 110% higher, respectively, and more of the ammonia that was produced was shifted into urine (82% versus 65% in acidemic subjects versus controls). In all studies, protein degradation and net protein balance across the kidney were inversely related to urinary ammonia excretion and to the partition of ammonia into urine, but not to total ammonia production, arterial pH, [HCO3–], urinary flow, the uptake of glutamine by the kidney, or the ammonia released into the renal veins. The data show that response of the human kidney to metabolic acidosis includes both changes in amino acid uptake and suppression of protein degradation. The latter effect, which is likely induced by the increase in ammonia excretion and partition into the urine, is potentially responsible for kidney hypertrophy.

Both in vitro and in vivo studies in animals have shown that metabolic acidosis causes several biochemical and morphologic changes in tubule cells, which are ultimately associated with kidney hypertrophy (1,2). Protein synthesis and degradation, the determinants of protein metabolism, are key factors in the hypertrophy process. However, information regarding the signals and mechanisms by which metabolic acidosis might cause kidney hypertrophy is still incomplete, and whether these effects take place in the human kidney is unknown. A further complicating element is that the effects of acidosis on protein turnover, amino acid metabolism and/or transport, and gluconeogenesis. It has been shown that in tubule epithelial cells, the associated increase in ammonia production, rather than the acidosis per se, is responsible for favoring tubular hypertrophy (10–12). This effect is related to the inhibition of protein degradation, owing to changes in lysosomal pH and cathepsin activity (12). In addition, other mechanisms may be responsible for tubular hypertrophy. Ammonium chloride, used to induce acidosis, may decrease amino acid oxidation (11), another effect that could account for the increase in tubular protein content. Furthermore, chronic acidosis activates early genes, which are associated with growth and could therefore promote protein synthesis (6). In a previous in vivo study in the rat, kidney hypertrophy was shown to be associated with both a decrease in protein degradation and an increase in protein synthesis (13).

We previously evaluated protein turnover across the human kidney in studies on the basis of the organ mass balance associated with leucine isotope kinetics (14,15). Kidney protein turnover, as compared with muscle and splanchnic turnover, is characterized by the highest rates of protein synthesis and amino acid oxidation. These effects are mainly the expression of tubular epithelial cell metabolism, because glomeruli make up only 5% of the kidney weight (16). In terms of mass...
balance, the high kidney tubule protein turnover is likely to confer sensitivity to the signals and mediators that control protein balance, leading to great increases in kidney protein content in the presence of small changes in the rate of protein degradation (17). To explore how metabolic acidosis might influence protein metabolism in the human kidney, we studied protein dynamics and amino acid oxidation across the kidney in subjects in whom metabolic acidosis was induced by administering acidifying agents for 6 d (chronic metabolic acidosis) and in subjects with normal acid-base balance (controls). To test the hypothesis that the kidney is a major site for gluconeogenesis in metabolic acidosis, we also measured the exchange of individual amino acids, lactate, and glucose across the kidney. Furthermore, we evaluated the individual role of some of the factors that may affect protein turnover. These included the production of ammonia and its distribution within the urine and the renal veins, as well as the acid-base parameters in plasma and urine.

Materials and Methods

Subjects

Eleven amino acid kinetic studies across the kidney were carried out on 11 subjects in the postabsorptive state. Standard blood chemistry values, GFR, acid-base and electrolyte measurements, and oral glucose tolerance tests were all normal. All subjects were within 10% of their ideal body weight. Their usual diet provided 30 to 35 kcal/kg and 0.9 to 1.1 g protein/kg body weight. Medication was discontinued 4 d before the study. None of the subjects had signs of gastrointestinal or hepatic disease, congestive heart failure, diabetes mellitus, or other endocrine disorders. Nine subjects (five men, four women) had arterial hypertension. Their mean BP was between 121 and 130 mmHg. A renal vein catheterization for renin activity was performed for diagnostic purposes. The final diagnosis was essential hypertension. Two men had cardiac valvular disease, and a right-sided cardiac catheterization was indicated for the hemodynamic evaluation. Five of the 11 studies (three men and two women, aged 45 ± 3 yr) were performed during the presence of metabolic acidosis induced by a combination of HCl (1.2 mEq/kg/d), CaCl2 (0.6 mEq/kg/d), and NH4Cl (1.1 mEq/kg/d), provided orally at 8-h intervals for 6 d. The last dose of acidifying was calculated as 

\[
\text{Ra} = \frac{\text{[DPM-}^{14}\text{C}_{\text{art}} - \text{DPM-}^{14}\text{C}_{\text{ven}}]}{\text{[SA-}^{14}\text{C}_{\text{art}}]} \times \text{flow}
\]

where [DPM-14Cart] and [DPM-14Cven] are isotope concentrations (in DPM/ml) of 14C-leucine in arterial and venous blood, respectively; [SA-14Cart] is the specific activity (in DPM/nmol) of leucine in the arterial blood; and flow is renal blood flow (in ml/min × 1.73 m² of body surface).

Kidney leucine oxidation (\text{Leu Ox}) was calculated by

\[
\text{Leu Ox} = \frac{\text{[DPM-}^{14}\text{C Bic}_{\text{ven}}]}{\text{[SA-}^{14}\text{C Leu}_{\text{ven}}]} \times \text{flow}
\]

Analytical Measurements

The methods we used for the whole blood assay of amino acids, α-ketoisocapric acid (kic), 14C-bicarbonate levels, and for isotope concentrations and specific activities are reported extensively elsewhere (14,15,18–22). The GFR was measured by iothalamate infusion and clearance (21,22). Here, the term “ammonia” refers to the sum of ammonium ions and free ammonia base.

Calculations

All of the kinetic calculations based on whole blood data were performed under steady-state conditions (16,17,23). Kidney leucine kinetics, i.e. the rates of leucine-carbon appearance (Ra), disappearance (Rd), oxidation, disposal into protein synthesis (PS), and net balance (NB), were calculated by a noncompartmental arteriovenous approach, as described elsewhere (16). Because there is no certainty regarding the use of leucine or of kic in estimating kidney protein turnover, the data were calculated by using leucine alone (leucine primary pool kinetics) and including kic in the calculations as well. The latter approach is defined as leucine-carbon (Leu-C) kinetics. Rates of disappearance (Rd) were calculated as follows:

\[
\text{Rd} = \frac{\text{[DPM-}^{14}\text{C}_{\text{art}} - \text{DPM-}^{14}\text{C}_{\text{ven}}]}{\text{[SA-}^{14}\text{C}_{\text{art}}]} \times \text{flow}
\]

where [DPM-14Cart] and [DPM-14Cven] are isotope concentrations (in DPM/ml) of 14C-leucine in arterial and venous blood, respectively; [SA-14Cart] is the specific activity (in DPM/nmol) of leucine in the arterial blood; and flow is renal blood flow (in ml/min × 1.73 m² of body surface).

Kidney leucine oxidation (Leu Ox) was calculated by

\[
\text{Leu Ox} = \frac{\text{[DPM-}^{14}\text{C Bic}_{\text{ven}}]}{\text{[SA-}^{14}\text{C Leu}_{\text{ven}}]} \times \text{flow}
\]

where [DPM-14Cart] and [DPM-14Cven] are isotope concentrations (in DPM/ml) of 14C-leucine in arterial and venous blood, respectively.
When kic was included in the calculations, Leu-C oxidation was calculated by Equation 4, but SA-14C Leu-en was replaced by the SA-14C of kic in the vein (SA-14C-kic-en), as follows:

\[
\text{Leu-C Ox} = \frac{[\text{DPM-}^{14}\text{C Bic-en}] - [\text{DPM-}^{14}\text{C Bic-en}]}{\text{SA-}^{14}\text{C kic-en}} \times \text{flow}
\]

The rate of nonoxidative leucine disposal into protein synthesis (PS) was calculated by subtracting leucine oxidation from leucine Rd:

\[
\text{Leu PS} = [\text{Leu Rd} - \text{Leu-Ox}]
\]

Similarly, the rate of nonoxidative leucine-carbon disposal into protein synthesis (Leu-C PS) was calculated by subtracting Leu-C oxidation from Leu-C Rd:

\[
\text{Leu-C PS} = [\text{Leu-C Rd} - \text{Leu-C Ox}]
\]

Net leucine balance (Equation 2) simply measures the net leucine exchange across the kidney, without taking into consideration the actual amount of leucine entering protein. The latter (defined as net protein balance of either leucine or leucine-carbon, i.e. NPB-Leu or NPB-Leu-C, respectively) must be calculated by subtracting Leu PS (or Leu-C PS), from Ra, as follows:

\[
\text{NPB-Leu} = [\text{Leu Ps} - \text{Leu Ra}]
\]

and

\[
\text{NPB-Leu-C} = [\text{Leu-C PS} - \text{Leu-C Ra}]
\]

Major model assumptions are the following: (1) it is assumed that there is a single, well mixed intracellular leucine pool, out of and into which entries and exits take place; (2) no interstitial leucine and kic compartment is taken into consideration; (3) no release of tracer recycled from protein breakdown into the intracellular compartment is assumed to occur within the duration of the study; and (4) the metabolic behavior of the 14C tracer is the same as the behavior of the unlabeled material (14,15).

Whole body amino acid kinetics was calculated by conventional calculations (14). A fixed whole body fixation factor for 14C-CO2 of 20% was used, but no fixation correction for 14C-bicarbonate exchange across the kidney was used. Renal arterial blood flow was calculated from the clearance and extraction of p-aminohippuric acid by the Wolf equation (24). Renal arterial blood flow was calculated by dividing plasma flow over \((1 - \text{hematocrit})\). Total ammonia production was obtained by summing urinary ammonia and ammonia added to renal veins. The rate of uptake or release of metabolites by the organs was calculated by the arteriovenous difference times blood flow.

**Statistical Analyses**

Data are expressed as \(\mu\text{mol/min} \times 1.73 \text{ m}^2\) (±SEM). Statistical analysis was performed by ANOVA for repeated measures to compare arterial with venous data as well as two sets of organ kinetic parameters. One-way ANOVA was used to compare data from different groups. Linear regression and correlation were used to evaluate the relationship between two variables. Statistical analysis was performed by the StatView statistical package (Abacus, Berkeley, CA). \(P < 0.05\) was considered statistically significant.

**Results**

**Substrate and Isotope Concentrations, Specific Activities (Sa), and Blood Flow**

There was no statistically significant difference in arterial blood leucine concentrations between metabolic acidosis subjects and controls; the kic concentrations in the former group were approximately 40% lower. In control subjects, leucine concentrations in the renal vein were slightly, although significantly, greater than in the artery. This indicates leucine release in the renal vein. There were no differences in arterial or renal venous leucine concentrations in acidic subjects. Kic concentrations in the renal vein were lower than in the artery both in acidic and control subjects, thus implying kic uptake by the kidney in both conditions. Both 14C-leucine and 14C-kic concentrations were significantly lower in the renal vein than in arterial blood in acidic subjects and in controls (Table 1). 14C-bicarbonate concentrations in the renal vein were 12% and 16% higher than concentrations in the artery in controls and in acidic subjects, respectively, thus indicating similar

<table>
<thead>
<tr>
<th></th>
<th>Chronic Metabolic Acidosis</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Artery</td>
<td>Renal Vein</td>
</tr>
<tr>
<td><strong>Substrate concentration</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>leucine</td>
<td>153 ± 17</td>
<td>152 ± 15</td>
</tr>
<tr>
<td>Kic</td>
<td>17 ± 2a</td>
<td>14 ± 2a</td>
</tr>
<tr>
<td><strong>Isotope concentration</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14C-Leucine</td>
<td>478 ± 49</td>
<td>447 ± 44b</td>
</tr>
<tr>
<td>14C-Kic</td>
<td>43 ± 7</td>
<td>38 ± 6a</td>
</tr>
<tr>
<td>14C-Bicarbonate</td>
<td>130 ± 20</td>
<td>152 ± 28b</td>
</tr>
<tr>
<td><strong>Specifc activity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14C-Leu Sa</td>
<td>3.14 ± 0.23</td>
<td>2.95 ± 0.1</td>
</tr>
<tr>
<td>14C-Kic-Sa</td>
<td>2.49 ± 0.24</td>
<td>2.65 ± 0.25</td>
</tr>
</tbody>
</table>

\(^a P < 0.05\) and \(^b P < 0.025\), vein versus artery. \(^c P < 0.05\), chronic metabolic acidosis versus controls.
Co2 production from 14C-leucine oxidation. In control subjects, 14C-leucine Sa in the renal vein was significantly lower as compared with the artery (by 12%), indicating amino acid release. The same was true for 14C-kic. In acidic subjects, 14C-leucine Sa in the renal vein was slightly lower than in the artery (P = 0.06, NS), and 14C-kic Sa was similar to the artery, indicating a blunted release of unlabeled leucine and kic in the renal vein.

**Blood Flow, GFR, Acid-Base Parameters, and Ammoniagenesis**

Blood flow to the kidney was 780 ± 75 ml/min/1.73 m2 in acidic subjects and 934 ± 98 ml/min/1.73 m2 in control subjects, respectively (P = NS) (Table 2). GFR was 102 ± 17 ml/min/1.73 m2 in acidic subjects and 138 ± 11 ml/min/1.73 m2 in control subjects, respectively (P = NS). Arterial pH and bicarbonate, as well as urinary pH, were significantly lower in acidic subjects than in controls. Urinary ammonia excretion and total ammonia production were 186% and 110% higher, respectively, in acidic subjects versus controls. The ratio of urinary ammonia to total ammonia production (an expression of the distribution of produced ammonia between urine and renal venous blood), was higher in acidic subjects than in controls.

**Leucine Kinetics across the Kidney**

As expected, the data expressed as leucine-carbon yielded somewhat greater rates of kidney protein kinetics than those calculated by the leucine primary pool (leucine carbon pool includes both leucine and kic turnover). In the control subjects, the rate of leucine appearance across the kidney was about 35% to 130% higher than protein synthesis (by using both the leucine-carbon and the primary pool data, P < 0.01 and 0.05, respectively). Thus, net protein leucine balance was markedly negative when either calculation was used (Table 3).

In the acidic subjects, the rate of leucine appearance across the kidney was significantly lower (P < 0.05) than in control subjects when both the leucine-carbon and the primary pool data were used, and the rate was no different from the rate of protein synthesis. Thus, when either calculation is used, net protein leucine balance was not different from zero. Leucine oxidation was similar in both groups. Protein synthesis in the acidic group tended to be lower, although not significantly so, than in controls.

Despite a decrease in the kidney protein turnover rate, we found that the rate of whole body leucine appearance, an index of whole body proteolysis, tended to be higher (approximately 10%) in acidic versus control subjects (3.1 ± 0.44 μmol/min/kg versus 2.8 ± 0.12 μmol/min/kg, respectively), although the difference was not statistically significant.

**Amino Acid Exchange across the Kidney**

There were no differences in arterial amino acid levels between the two groups. In controls, the kidney took up glutamine, citrulline, and kic from the circulation, whereas it released threonine, serine, alanine, cysteine, tyrosine, and arginine in addition to leucine (Table 4). Increased uptake and reduced release of several amino acids by the kidney was observed in acidic subjects. Glutamine extraction was more than two times higher than in subjects with normal acid-base balance. Furthermore, an uptake of glycine was also observed. The release of glutamate, serine, tyrosine, and leucine into the renal vein was lower than in the control group.
Table 4. Arterial amino acid levels and amino acid exchange across the kidney in subjects with metabolic acidosis and controls\(^a\)

<table>
<thead>
<tr>
<th></th>
<th>Arterial Level ((\mu)mol/L)</th>
<th>Rate ((\mu)mol/min (\cdot) 1.73 m(^2))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls</td>
<td>Acids</td>
</tr>
<tr>
<td>Threonine</td>
<td>92 ± 9</td>
<td>102 ± 17</td>
</tr>
<tr>
<td>Serine</td>
<td>98 ± 4</td>
<td>84 ± 5</td>
</tr>
<tr>
<td>Glutamine</td>
<td>480 ± 20</td>
<td>448 ± 7</td>
</tr>
<tr>
<td>Glutamate</td>
<td>143 ± 27</td>
<td>132 ± 10</td>
</tr>
<tr>
<td>Glycine</td>
<td>226 ± 14</td>
<td>238 ± 2</td>
</tr>
<tr>
<td>Alanine</td>
<td>237 ± 11</td>
<td>267 ± 26</td>
</tr>
<tr>
<td>Citrulline</td>
<td>27 ± 3</td>
<td>43 ± 10</td>
</tr>
<tr>
<td>Valine</td>
<td>154 ± 9</td>
<td>170 ± 17</td>
</tr>
<tr>
<td>Cysteine</td>
<td>33 ± 5</td>
<td>38 ± 4</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>31 ± 4</td>
<td>35 ± 3</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>41 ± 3</td>
<td>43 ± 4</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>36 ± 2</td>
<td>37 ± 2</td>
</tr>
<tr>
<td>Ornithine</td>
<td>66 ± 6</td>
<td>72 ± 3</td>
</tr>
<tr>
<td>Lysine</td>
<td>134 ± 8</td>
<td>146 ± 9</td>
</tr>
<tr>
<td>Histidine</td>
<td>85 ± 8</td>
<td>70 ± 4</td>
</tr>
<tr>
<td>Arginine</td>
<td>59 ± 5</td>
<td>67 ± 2</td>
</tr>
</tbody>
</table>

\(a\) Values are given as mean ± SEM. \(^{b}\) \(P < 0.05\) and \(^{c}\) \(P < 0.025\), artery versus vein; \(^{b}\) \(P < 0.05\), chronic metabolic acidosis versus controls.

**Glucose and Lactate Exchange across the Kidney**

Blood glucose and lactate levels were similar in both groups. No significant arteriovenous differences in glucose were detectable in controls; the kidney took up lactate from arterial blood (lactate fractional extraction approximately 12%). A net release of glucose into the renal veins was detected in acidic subjects. In addition, no significant arteriovenous differences for lactate were observed (Table 5).

**Determinants of Kidney Protein Turnover**

Both net protein balance and protein degradation across the kidney were inversely related to urinary ammonia and to the urinary \(\text{NH}_3^+\)/total \(\text{NH}_4^+\) production ratio (Figures 1 and 2 are representative of calculations made by the use of leucine primary pool). The use of leucine carbon pool yielded similar results, with protein degradation versus urinary ammonia and the urinary \(\text{NH}_4^+\)/total \(\text{NH}_4^+\) production ratio \(r = -0.53, P < 0.05\) and \(r = 0.62, P < 0.03\), respectively. Protein degradation rates, but not the net protein balance, were directly related to urinary pH. No relationship was observed between net protein balance or degradation and total ammonia production, arterial pH or bicarbonate, urinary flow, the uptake of glutamine, or the ammonia released into the renal veins (Table 6).

**Determinants of Kidney Glucose Exchange**

Rates of glucose exchange across the kidney were closely related to arterial bicarbonate levels. Glucose exchange across the kidney was also related to arterial and urinary pH, as well as to urinary and total ammonia production. When taking into consideration the possible substrate or substrates for renal gluconeogenesis, close relationships were observed between glucose exchange and glutamine uptake, as well as net protein balance across the kidney, thus supporting the possibility that besides glutamine, other amino acids deriving from net protein breakdown are used as sources of glucose (Table 7).

**Discussion**

In the postabsorptive state, the human kidney is characterized by high rates of protein turnover, with protein degradation rates largely overcoming protein synthesis (14,15). Despite the small kidney mass, leucine appearance from kidney protein degradation is as high as 10% of whole body protein degradation. This is likely an expression of the breakdown of both structural and filtered proteins (17). The study presented here shows that in subjects with metabolic acidosis induced by 6-d administration of acidifying agents, the appearance of leucine from intrarenal protein degradation is markedly reduced and that the rates of kidney protein synthesis are no different from rates of protein degradation. As a consequence of these findings, no appreciable release of leucine from protein breakdown into the renal veins is observed in acidemia. According to our data the main effect that chronic metabolic acidosis has on human kidney protein turnover is that of downregulating protein degradation.

In addition, our data show that in chronic metabolic acidosis the human kidney takes up higher amounts of glutamine and glycine from arterial blood, and returns lower amounts of several other amino acids, some of which may serve as ammonia precursors, to the systemic circulation. In keeping with the stimulation of gluconeogenesis from amino acids observed in tubular cells, a net glucose release into the renal veins has been found. Taken together, our data show that the response of

Table 5. Whole blood glucose and lactate exchange across the kidney in subjects with metabolic acidosis and in controls

<table>
<thead>
<tr>
<th></th>
<th>Chronic Metabolic Acidosis</th>
<th>Controls</th>
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</thead>
<tbody>
<tr>
<td>Glucose ((\mu)mol/L)</td>
<td>3.91 ± 0.15</td>
<td>4.60 ± 0.35</td>
</tr>
<tr>
<td>Lactate ((\mu)mol/L)</td>
<td>674 ± 121</td>
<td>651 ± 80</td>
</tr>
</tbody>
</table>

\(^{a}\) \(P < 0.05\), vein versus artery.
the human kidney to chronic metabolic acidosis is a coordinated one that includes both changes in kidney glutamine and amino acid uptake (which supply substrates for ammoniagenesis) and suppression of kidney protein degradation. The latter effect is potentially responsible for tubular hypertrophy, which is observed in experimental chronic metabolic acidosis. The effects on protein metabolism in the kidney are opposite to the ones have been described in skeletal muscle and splanchnic organs, indicating that metabolic acidosis affects protein metabolism in individual organs differently.

The marked stimulation of ammoniagenesis and the increased partition into the urine of ammonia that was produced in subjects with metabolic acidosis studied here are in agreement with previous findings (24–26). NH₄⁺ transport through the epithelial cell membranes is believed to occur by substitution of NH₄⁺ for H⁺ or K⁺ on diverse cation transporters (27) or by NH₄⁺ diffusion coupled to active H⁺ secretion (28).

NH₄⁺ could pass freely across lipid bilayers through concentration gradients or could be transported by specific carriers (29). The distribution of NH₄⁺ into the urine augments the effects of the increase in ammoniagenesis on acid-base balance in as much as only ammonia excreted in the urine corresponds to a bicarbonate gain. Data from the study presented here, obtained both from subjects with metabolic acidosis and from subjects with normal acid-base balance, show that changes in the urinary excretion of ammonia and the partition of produced ammonia into urine are related to kidney protein balance and protein degradation.

Our data therefore suggest that the ammonia that is produced exerts a physiologic, inhibitory action on kidney protein degradation. That ammonia acts to suppress proteolysis by a direct action to raise lysosomal pH has been previously shown by incubating tubule cells with 1 to 20 mmol NH₄Cl (10–12). It is noteworthy that in our study, the kidney protein degradation rates were not related to total ammonia production, nor to the ammonia released into the renal veins. Neither protein degradation, nor kidney protein balance were related to various
metabolic parameters involved in the renal response to acidosis, such as arterial pH or bicarbonate, or glutamine uptake by the kidney. Although the small sample size and the possible interrelations among the variables we studied (protein synthesis versus degradation, urinary pH, and ammonia concentration) may be a limit of our study, our findings suggest that the processes that cause the concentration of ammonia to increase at the apical membrane, or the process of urine acidification itself, may mediate lysosomal cathepsin inhibition and hinder protein degradation. Regarding the signals sent to suppress kidney protein degradation, previous in vitro studies have shown that neither the suppression of cathepsin activity nor the induction of tubule cell hypertrophy is mediated by the Na/H + antiport activation or by a decrease in intracellular pH (11). Hence, it is likely that rather than expressing a direct effect, the correlation we observed between urinary pH and protein degradation actually expresses the effects of other related variables, such as the ammonia shift in urine. This is also indicated by the finding that net kidney protein balance was not related to urinary pH.

Branched-chain ketoacid dehydrogenase is the rate-limiting enzyme in branched-chain amino acid oxidation and catabolism. In our study, leucine oxidation rates across the kidney tended to be lower in acidemic subjects, indicating that amino acid oxidation is not increased by metabolic acidosis in the kidney, in agreement with studies showing no change in branched-chain ketoacid dehydrogenase activity in the kidneys of acidic rats (30,31).

In rats and humans, glutamine is the major precursor of the ammonia produced during acidosis (25,26,32). Data presented here show that glutamine uptake by the kidney in acidic subjects was 120% higher than what was observed in subjects with normal acid-base balance. These data are in keeping with studies in rat kidney showing that adaptation to metabolic acidosis is sustained by the induction of multiple enzymes and various transport systems, such as glutaminase and phosphoenolpyruvate carboxykinase (2,33). Despite the increase in the utilization of glutamine by the kidney that we observed in our study in acidic subjects, blood glutamine levels were found to be only slightly reduced. Thus, an increased production of glutamine by peripheral tissues and/or a decreased utilization by splanchnic organs likely occurred.

In normal acid-base conditions the glutamine N extraction by the kidney is greater than the amount of ammonia that is produced, thus indicating that N supplied by glutamine extraction is more than sufficient to account for ammonia production (18,25,26). In the acidic subjects we studied, the ratio of ammonia production to glutamine nitrogen extracted by the kidney was close to unity. This implies a complete efficiency in the use of both amino and amide group of glutamine or that some ammonia may derive from other amino acids.

The kidneys of acidic subjects displayed a reduction in the release of several amino acids, mainly leucine, serine, glutamate and tyrosine, and the appearance of an uptake for glycine. These observations partly differ from what was observed several years ago by Owen and Robinson (31), who showed significant changes in amino acid balance across the kidney with regards to glutamine and glutamate alone in subjects with NH4 +/Cl−-induced metabolic acidosis. Besides the differences caused by the acidifying protocols, it is possible that these variations may be due to the bicarbonate level that was reached in our study (17 mmol/L), which was lower than in the previous one (22 mmol/L) (31).

It is noteworthy that an uptake of glycine by the human kidney can be observed 24 h after the onset of metabolic acidosis (26), thus showing that pathways for glycine transport and or metabolism are precociously stimulated by acidemia. Although the reduced release of glutamate is consistent with its

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**Table 6. Regression analysis of the determinants of protein degradation and net protein balance across the kidney in subjects with normal acid-base balance and in those with chronic metabolic acidosis**

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>[HCO3]</th>
<th>pH</th>
<th>Total NH4⁺ Production</th>
<th>Urinary NH4⁺ Excretion</th>
<th>Urinary NH4⁺/Total NH4⁺ Production</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>P</td>
<td>R</td>
<td>R</td>
<td>P</td>
<td>R</td>
</tr>
<tr>
<td>Protein degradation</td>
<td>0.09</td>
<td>NS</td>
<td>0.427</td>
<td>NS</td>
<td>0.666</td>
<td>0.015</td>
</tr>
<tr>
<td>Net protein balance</td>
<td>0.423</td>
<td>NS</td>
<td>0.512</td>
<td>NS</td>
<td>0.110</td>
<td>NS</td>
</tr>
</tbody>
</table>

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**Table 7. Regression analysis of determinants of glucose release from the kidney in subjects with chronic metabolic acidosis and in those with normal acid-base balance**

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>[HCO3]</th>
<th>pH</th>
<th>Total NH4⁺ Production</th>
<th>NH4⁺ Excretion</th>
<th>NH4⁺/Total NH4⁺ Production</th>
<th>Glutamine Uptake</th>
<th>Net Protein Balance</th>
</tr>
</thead>
<tbody>
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<td>R</td>
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<tr>
<td>Glucose release</td>
<td>−0.55</td>
<td>0.05</td>
<td>0.83</td>
<td>0.001</td>
<td>−0.63</td>
<td>0.02</td>
<td>0.56</td>
<td>0.035</td>
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use as an ammoniagenic source, the decrease in kidney amino acid output (such as tyrosine and serine) is in agreement with the decrease in intrarenal protein breakdown that is observed in acidemic subjects. Tyrosine, a semiessential amino acid whose renal production is blunted in acidosis, partly derives from phenylalanine hydroxylation and partly from intrarenal protein breakdown (34,35). Serine is likely produced from glycine by the intrarenal breakdown of cysteinyl-glycine, which is released by glutathione hydrolysis from peripheral and splanchnic tissues and filtered by glomeruli (36). Furthermore, the addition of less serine to the systemic circulation could follow an increase in the intrarenal use of glycine as an ammoniagenic source.

As a new finding in humans, and in agreement with several experimental observations in rats (1,2,9), we found that during metabolic acidosis the human kidney adds glucose to the systemic circulation. The kidney has a substantial capacity for gluconeogenesis, and glutamine is a major substrate for this process. The human kidney has been shown to significantly contribute to maintaining blood glucose levels. Several years ago, Aber et al. (44) observed a net release of glucose into the renal veins, which was negatively correlated to arterial pH, in patients with respiratory acidosis. Similar findings were observed by Cahill (45) in morbidly obese subjects during prolonged fasting. More recently, net kidney gluconeogenesis has been shown to occur during hypoglycemia (40) and in patients with diabetes mellitus (46).

In our study, the correlation between kidney glucose balance and arterial bicarbonate levels is in keeping with the observations that in kidney epithelial cells, the induction of phosphoenolpyruvate carboxykinase and glutaminase during acidosis is initiated in direct response to a decrease in extracellular pH and/or bicarbonate (47). Our data, in association with the previously mentioned findings, strongly support the concept that the gluconeogenetic process in the kidney is coupled with ammoniagenesis and maintaining acid-base balance. One could estimate from published data (42) and from the measurements made in our laboratory that in chronic metabolic acidosis the renal release of glucose accounts for approximately 20% of the glucose released into the liver veins under normal conditions. This percentage is similar to that which is observed during hypoglycemia (40) and diabetes (46). A shift in kidney gluconeogenetic substrates is likely to occur during acidosis because the uptake of lactate observed in subjects with normal acid-base balance is no longer evident in acidic subjects. On the basis of carbon composition (glutamine has five carbons, glucose six) and on a complete efficiency of the gluconeogenic pathway, it is estimated that 1.2 mol of glutamine are needed to contribute to each mole of de novo synthesized glucose (43). Therefore, the net uptake of glutamine observed in acidic subjects could account for as much as 60% of the glucose released by the kidney. However, a more definitive conclusion regarding the fate of glutamine carbons cannot be drawn because no glutamine tracer was used.

It should be mentioned that our study was performed only in the postabsorptive state, a condition in which the concentrations of amino acids depends on the balance between release from proteins and utilization by tissues. We did not study the fed state, a condition during which the N balance across the kidney becomes positive. Moreover, we studied protein turnover after the recent onset of acidosis, and it is possible that more marked changes in kidney protein turnover rates are observed after several days or weeks, when hypertrophy may take place.

Acidemia leads to several cardiovascular effects, including arteriolar vasodilation and a decrease in cardiac output and renal plasma flow (47). The subjects with metabolic acidosis that we studied displayed a tendency toward decreased kidney blood flow, which although not statistically different from controls, could become significant with a large number of patients and, by mathematical reasons, could account for part of the decrease in the calculated rates of protein turnover. It is of note that a similar finding (renal blood flow values tendentially lower in acidemic subjects than in controls) was also observed by Owen and Robinson (31). However, the subjects with metabolic acidosis studied here displayed a decreased venous concentration and the release of unlabeled leucine from proteolysis. Our results are therefore preeminently supported by a decrease in the leucine arteriovenous concentration difference and the release of unlabeled leucine and kic in the renal vein.

In humans the kidney accounts for approximately 10% of whole body protein turnover rates (14). Despite a significant decrease in the kidney protein degradation, rates of whole body protein turnover tended to be higher in subjects with metabolic acidosis as compared with controls. One could therefore hypothesize that extrarenal protein turnover accounts for a greater percentage of whole body protein turnover or that it is accelerated. This hypothesis is supported by previous observations that muscle protein turnover is accelerated by metabolic acidosis (3).

Studies have provided evidence that early mesangial/tubular hypertrophy is a predecessor of the subsequent development of glomerulosclerosis and interstitial fibrosis. These conditions are characterized by irreversible morphologic changes to the kidney’s architecture, which ultimately lead to end-stage renal disease. Data from the study presented here further suggest the relevance of ammonia as a physiologic inhibitor of kidney protein breakdown in humans, and in the pathogenesis of tubular hypertrophy and chronic kidney damage.

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