Effect of protein intake on weight gain and plasma amino acid levels in uremic rats

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WANG, MARIAN, IRMA VYHMEISTER, JOEL D. KOPPLE, AND MARIAN E. SWENDSEID. Effect of protein intake on weight gain and plasma amino acid levels in uremic rats. Am. J. Physiol. 230(5): 1455-1459. 1976 — Chronically uremic rats weighing approximately 180–200 g and sham-operated controls of similar weight were pair-fed diets containing 5, 15, or 23% protein for 10–12 wk. At each level of protein intake, uremic animals gained less weight and had lower protein efficiency ratios than controls. In addition, certain plasma amino acid levels were altered in the uremic animals. These included tyrosine and the tyrosine/phenylalanine ratio, which were decreased, and citrulline, glycine, and the methylhistidines, which were increased. In both uremic and control rats, plasma concentrations of certain amino acids, primarily nonessential ones, varied inversely with protein intake; with the 5% protein diet, the ratio of essential to nonessential amino acids was significantly reduced. These observations indicate that both uremia and reduced protein intake may affect growth and amino acid metabolism in rats with chronic renal failure. The finding that uremic rats utilize protein less efficiently may indicate that marked reductions in protein intake may be particularly hazardous to the nutritional status of the uremic patient.

WEIGHT LOSS AND OTHER EVIDENCE of wasting are commonly observed in patients with chronic renal failure (4). In children, uremia is associated with impaired growth (9). Although there are almost certainly multiple causes for wasting and decreased growth in renal failure, it has been suggested that the reduced dietary intake of uremic subjects is a major factor (9, 14). A low intake of both energy (9) and protein (14) has been implicated as the cause of impaired growth and wasting. However, the effects of dietary intake on nutritional status in renal failure have often not been evaluated in a controlled prospective fashion. In addition, the effects of the uremic syndrome and dietary intake on growth and wasting have usually not been distinguished. The present study was therefore undertaken to compare the effects of both the uremic condition and varying protein intake on weight gains and plasma amino acid concentrations. Studies were conducted in chronically uremic and sham-operated control rats pair-fed isocaloric diets providing different amounts of protein. The results indicate that while severe protein restriction impairs growth and alters plasma amino acid concentrations in both uremic and control rats, renal failure per se is associated with impaired protein utilization and weight gain and altered amino acid levels.

MATERIALS AND METHODS

Female Sprague-Dawley rats weighing 180–200 g were made uremic by ligation of two-thirds to three-fourths of the primary and secondary divisions of the left renal artery, followed in 1 wk by contralateral nephrectomy (1). The remaining functional renal tissue was estimated to be approximately one-eighth of the original mass. The same number of control rats was subjected to a laparotomy.

After surgery, the rats were housed in individual, bottom-screened cages with controlled temperature (21°C) and lighting from 6:30 a.m. to 6:30 p.m. The uremic rats were assigned at random to diets providing 5, 15, or 23% protein as casein. The composition (g/100 g) of the 5% protein diet was as follows: vitamin-free casein, 6; glucose, 75.8; corn oil, 10; vitamin mix, 2; 2.2; mineral mix, 4; and cellulose, 2. The other diets containing casein were similar in composition except that a portion of the glucose was replaced by an equivalent weight of casein. All studies were conducted with the use of pair-feeding techniques. One week after nephrectomy, each uremic rat ingesting the 5 and 15% protein diets was paired with a sham-operated rat according to body weight. Each uremic rat given the casein diet containing 23% protein was pair-fed both with a sham-operated rat ingesting the same diet and with another uremic rat ingesting Purina chow (also containing 23% protein).

Body weights of all animals were measured twice weekly. Rats were placed in individual metabolic cages to collect 24-h urine specimens for the determination of

1 Percent of nitrogen in diet × 6.25.
2 Vitamin Diet Fortification Mixture, Nutritional Biochemical Corp., Cleveland. Composition per kilogram of diet (in g): retinyl acetate (200,000 IU/g), 0.099; cholecalciferol (400,000 IU/g), 0.0055; alpha tocopherol, 0.110; ascorbic acid, 0.996; inositol, 0.110; choline chloride, 1.656; menadione, 0.050; p-aminobenzoic acid, 0.110; niacin, 0.099; riboflavin, 0.022; pyridoxine hydrochloride, 0.022; thiamin hydrochloride, 0.022; calcium pantothenate, 0.066; (in mg) biotin, 0.44; folic acid, 1.99; vitamin B-12, 0.03.
3 Biological Test Salt Mixture "Hegsted," Nutritional Biochemical Corp., Cleveland. Composition per kilogram of diet (in g): CaCO3, 14.91; K2HP04, 16.78; CaHPO4, 3.73; MgSO4, 5.16; NaCl, 7.83; FeC2H302·5H2O, 1.32; KI, 0.040; MnSO4, 0.183; CuSO4, 0.011; ZnCl2, 0.011.
urea and creatinine. After the diets had been fed for 10–12 wk, uremic and control rats were killed. Animals were fasted overnight before killing to allow plasma levels to attain a steady state. The animals were anesthetized with ether, and then blood was withdrawn by cardiac puncture and centrifuged in heparinized tubes. A portion of the plasma was used for the determination of urea and creatinine, and the remainder was depro-teinized with sulfosalicylic acid (5 ml of 3% solution per ml of plasma). The protein-free filtrate was stored at −20°C until analyzed for amino acids with a Beckman 120 automatic amino acid analyzer. Urea was measured by the micro-method of Siest and Vigneron (23); creatine-nine was determined with the Technicon AutoAnalyzer. Data are expressed as mean values ± standard deviation. All the results were analyzed statistically by either the Student simple t test or paired-t test. The statistical significance of correlations was determined by examination of the correlation coefficient r.

RESULTS

Creatinine and urea clearances. Mean concentrations of creatinine and urea nitrogen in plasma and creatine-nine and urea clearances are shown in Table 1. With each level of dietary protein ingested, plasma urea ni-trogen and creatinine concentrations were significantly elevated in uremic as compared to pair-fed control ani-mals. Likewise, urea and creatinine clearances were significantly reduced in the uremic rats as compared to controls.

Weight gains. Figure 1 shows the mean weight gains of uremic and control rats during the 10- to 12-wk period when diets containing 5, 15, or 23% protein were adminis-tered. Data were evaluated for only those animals that had urea clearances below 0.3 ml/min and survived for the entire period as well as their pair-fed controls. With each diet, the uremic rats gained less weight than their pair-fed controls. From the onset of the study, weight gains of uremic rats ingesting 5 and 15% protein diets tended to be less than those of controls. The differ-

![Weight gain graph](image)

**FIG. 1.** Weight gains for control and uremic animals fed indicated diets varying in protein content for 10-12 wk periods.

At the end of the study, the total weight gain was no greater in either control or uremic rats receiving the 23% protein diet than in their respective counterparts fed the 15% protein diet. However, both uremic and control animals gained more weight with diets containing 15 or 23% protein than with an isocaloric diet containing 5% protein, although approximately the same amount of food per day was eaten. The protein efficiency ratios (PER) of the 5% protein diets, calculated as grams of weight gained per gram of protein consumed, were 0.79 ± 0.26 for control and 0.36 ± 0.29 for uremics (P < 0.05). PER's for the 15% protein diets were 0.52 ± 0.11 for controls and 0.31 ± 0.11 for uremics (P < 0.01). The ratios for the 23% protein diets were 0.24 ± 0.08 for controls and 0.13 ± 0.08 for uremic rats ingesting casein and 0.06 ± 0.08 for uremic rats ingesting chow (control vs. uremics: P < 0.05 and 0.001, respectively).

Plasma amino acid levels. The plasma amino acid levels of control and uremic rats pair-fed diets containing 23, 15, or 5% protein for 10–12 wk are shown in Table 2. The greatest number of differences between uremic

<table>
<thead>
<tr>
<th>Diet Treatment</th>
<th>Plasma Levels</th>
<th>Renal Clearances</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Creatinine</td>
<td>Urea nitrogen</td>
</tr>
<tr>
<td>23% protein</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Casein</td>
<td>0.9 ± 0.3</td>
<td>18.9 ± 3.1</td>
</tr>
<tr>
<td>Control</td>
<td>1.6 ± 0.7*</td>
<td>55.1 ± 20.1*</td>
</tr>
<tr>
<td>Uremic</td>
<td>1.4 ± 0.6*</td>
<td>38.0 ± 7.6*</td>
</tr>
<tr>
<td>Chow</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uremic</td>
<td>0.7 ± 0.1</td>
<td>13.4 ± 2.0</td>
</tr>
<tr>
<td>15% protein</td>
<td>2.3 ± 0.8*</td>
<td>34.0 ± 6.1*</td>
</tr>
<tr>
<td>Casein</td>
<td>0.6 ± 0.1</td>
<td>8.0 ± 3.0</td>
</tr>
<tr>
<td>Uremic</td>
<td>1.2 ± 0.14</td>
<td>13.8 ± 4.5*</td>
</tr>
</tbody>
</table>

All values are means ± SD; numbers in parentheses indicate numbers of rats. * Significantly different from control rats: P < 0.001, P < 0.05, and P < 0.01, respectively.

TABLE 1. Creatinine and urea nitrogen in plasma and creatinine and urea clearances of control and uremic rats pair-fed diets varying in protein content

![Amino acid levels graph](image)
and pair-fed control animals was found with casein diets containing 23% protein. When these diets were fed, plasma concentrations of lysine, tyrosine, serine, and ornithine were decreased and those of glycine and citrulline were elevated in uremic rats. The tyrosine-to-phenylalanine ratio was also decreased in uremic animals. Some of the alterations in plasma concentrations, such as elevated glycine and citrulline levels and reduced tyrosine to phenylalanine ratios, were observed with each diet ingested. In animals ingesting the 15% protein diet, the plasma levels of the methyl histidine levels were increased. At any level of protein fed, the ratio of essential to nonessential amino acids (E/N ratio) did not differ between control and uremic animals (Table 2). However, this ratio was significantly less in both uremic and control animals fed a 5% protein diet as compared to those ingesting greater amounts of protein. There were also other changes in plasma amino acids in both control and uremic animals that were inversely correlated with the dietary protein content. The amino acids that showed a significant inverse correlation with protein intake are listed in Table 3. With the exception of phenylalanine levels in uremic rats, this relationship was observed only with nonessential amino acids.

**DISCUSSION**

Although creatinine clearances for control rats did not vary with the protein content of the diet, the urea clearances were reduced with decreasing protein intake (Table 1). A reduction in urea clearance with low protein diets has also been observed in other mammals (1/7–19) and is believed to be due to an enhanced fractional reabsorption of urea in the distal nephron. In the present study, although uremic animals receiving 15 and 23% protein diets had low urea clearances, these clearances were further reduced in uremic animals ingesting low protein diets. Thus, at any level of protein intake, uremic animals always had significantly lower urea clearances than control rats.

The results of this study indicate that both the uremic condition and dietary protein restriction may retard growth and alter amino acid levels in the rat with chronic renal failure. Other studies have reported impaired growth in chronically uremic rats as compared to controls offered the same diets under ad libitum conditions (3, 26). However, the different growth rates observed in these studies may reflect lowered food intake by the uremic animals (3). We know of no other study in which uremic rats have been pair-fed isonitrogenous and isocaloric diets with controls. The finding that weight gain was significantly less in the uremic rats at each level of protein intake clearly indicates that uremic animals utilize dietary protein less efficiently than their nonuremic counterparts. This observation is confirmed by the significantly lower protein efficiency ratios calculated for uremic animals, which have not previously been reported. These findings suggest that growth and probably other anabolic processes appear to be affected by renal failure per se.

It is noteworthy that the lowered growth rate of the uremic rats apparently cannot be corrected by progressively increasing dietary protein. Although the 15% protein diet was associated with greater weight gain than the 5% diet in uremic animals, increasing protein intake to 23% was not associated with a further increase in weight gain (Fig. 1). Indeed, there is evidence that high protein diets may be deleterious in uremic rats. We (unpublished observation) have found that the survival rate of uremic rats receiving 23% protein diets is significantly less than those receiving 5% protein diets.

The mechanisms by which uremia affects the growth rate are unknown. There may be impairment of protein synthesis related to decreased availability of certain amino acids, to interference by uremic toxins, or to abnormal hormonal activity (13). Alternatively, protein degradation may be enhanced in uremia. Available data concerning protein synthesis in uremia are inconclusive. Shear (20) has found that in rats made acutely

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Control</th>
<th>15% Protein Diet</th>
<th>23% Protein Diet</th>
<th>5% Protein Diet</th>
</tr>
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<tbody>
<tr>
<td>His</td>
<td>3.6±0.6</td>
<td>3.7±1.1</td>
<td>4.5±1.3</td>
<td>3.9±0.2</td>
</tr>
<tr>
<td>Ile</td>
<td>7.4±1.7</td>
<td>7.8±2.0</td>
<td>6.9±2.1</td>
<td>7.6±1.5</td>
</tr>
<tr>
<td>Leu</td>
<td>15.2±1.7</td>
<td>11.3±2.7</td>
<td>10.7±2.1</td>
<td>11.3±2.7</td>
</tr>
<tr>
<td>Tyr</td>
<td>2.5±1.2</td>
<td>3.1±1.6</td>
<td>2.6±2.7</td>
<td>2.6±0.6</td>
</tr>
<tr>
<td>Phe</td>
<td>4.3±0.7</td>
<td>4.5±1.4</td>
<td>5.0±1.1</td>
<td>5.4±1.3</td>
</tr>
<tr>
<td>Thr</td>
<td>25.0±7.6</td>
<td>21.1±7.8</td>
<td>22.8±1.1</td>
<td>23.4±3.4</td>
</tr>
<tr>
<td>Val</td>
<td>14.9±3</td>
<td>13.2±4.7</td>
<td>13.4±3.6</td>
<td>14.6±2.6</td>
</tr>
<tr>
<td>Cys</td>
<td>5.0±2.3</td>
<td>4.2±2.3</td>
<td>1.9±0.5</td>
<td>1.4±0.5</td>
</tr>
<tr>
<td>Tyr</td>
<td>2.2±1.1</td>
<td>3.1±1.8</td>
<td>6.1±1.2</td>
<td>4.2±0.7</td>
</tr>
</tbody>
</table>

**TABLE 2. Plasma amino acid levels of control and uremic rats pair-fed diets varying in protein content**

Values are means ± SD expressed in μmol/100 ml of plasma. Dashed lines indicate that only trace amounts were present. *n = 5 for basic amino acids. **n = 4 for nonbasic amino acids. "Significantly different from control rats, P < 0.05, P < 0.01, P < 0.001, respectively. * Includes asparagine. ** Ratio of essential to nonessential amino acids excluding ornithine and citrulline which are not present in protein and Cys and Tyr.

**TABLE 3. Correlation coefficients between dietary protein content and indicated plasma amino acids in control and uremic rats**

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Control</th>
<th>Uremic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asp</td>
<td>-0.7370</td>
<td>-.7833</td>
</tr>
<tr>
<td>Ser</td>
<td>-0.8646</td>
<td>-0.7858</td>
</tr>
<tr>
<td>Gln</td>
<td>-0.6905</td>
<td>-0.3683</td>
</tr>
<tr>
<td>Cit</td>
<td>0.7493</td>
<td>0.3492</td>
</tr>
<tr>
<td>Gly</td>
<td>-0.8777</td>
<td>-0.7688</td>
</tr>
<tr>
<td>Ala</td>
<td>-0.7446</td>
<td>-0.6431</td>
</tr>
<tr>
<td>Phe</td>
<td>-0.3768</td>
<td>-0.5391</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Although creatinine clearances for control rats did not vary with the protein content of the diet, the urea clearances were reduced with decreasing protein intake (Table 1). A reduction in urea clearance with low protein diets has also been observed in other mammals (1/7–19) and is believed to be due to an enhanced fractional reabsorption of urea in the distal nephron. In the present study, although uremic animals receiving 15 and 23% protein diets had low urea clearances, these clearances were further reduced in uremic animals ingesting low protein diets. Thus, at any level of protein intake, uremic animals always had significantly lower urea clearances than control rats.

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uremic by ureteral ligation or bilateral nephrectomy and starved for 48 h, the in vivo incorporation of L-[14C]leucine into muscle protein was reduced, but L-[14C]leucine incorporation into liver and heart protein was increased. In chronically uremic rats, the uptake of L-[14C]leucine by liver ribosomes in vitro is enhanced (24). This finding suggests that ribosomal function is not impaired in uremia.

Chantler et al. (3) have attributed the decreased growth rate in uremic rats to increased caloric requirements. Male and female rats were made uremic by heminephrectomy at 22-24 days of age and were fed ad libitum a diet containing approximately 34% protein from a mixture of egg white and casein. They consumed fewer calories and gained less weight than did control rats, even when the uremic animals were compared to controls ingesting similar amounts of calories. In the current study, since all diets were isocaloric and pair-feeding techniques were used, it was not possible to assess the affect of caloric intake on growth in uremia.

The present study also indicates that renal failure as well as protein intake can affect plasma amino acid concentrations. When comparisons of amino acid concentrations were limited to uremic and pair-fed control rats ingesting the same protein diet, certain abnormalities were found. These included low plasma tyrosine levels and tyrosine/phenylalanine ratios and increased plasma citrulline and glycine concentrations.

Low plasma tyrosine levels and tyrosine/phenylalanine ratios have been reported by other investigators in both uremic animals and humans. In chronic uremia the defect appears to be due to impaired conversion of phenylalanine to tyrosine (7, 15, 27). The cause of this abnormality is not clear. We have previously found activity of phenylalanine hydroxylase, the enzyme mediating the conversion of phenylalanine to tyrosine, to be decreased in the diseased rat kidney (25). In addition, Young and Parsons (27) have suggested that activity of this enzyme in liver may be inhibited by uremic toxins. It has been suggested that as a result of decreased conversion to tyrosine, more phenylalanine may be diverted to other pathways of degradation and abnormal phenylalanine metabolites may accumulate (7, 10, 15). These abnormal metabolites may be toxic (16). It is of interest that in acutely uremic rats Shear has presented evidence that the low tyrosine levels and altered tyrosine/phenylalanine ratios are due primarily to increased activity of hepatic tyrosine aminotransferase and enhanced degradation of tyrosine (21).

There is evidence that elevated citrulline levels may be caused by impaired conversion of citrulline to arginine in the diseased kidney (2). In vitro activity of hepatic argininosuccinate synthetase and argininosuccinase, the enzymes catalyzing the conversion of citrulline to arginine, appears to be normal in chronically uremic rats (2). In contrast, activity of kidney argininosuccinase is markedly reduced in rats with renal failure (2).

The elevated methylhistidine levels are probably due to reduced excretion, as has been reported in uremic humans (6, 12). However, the possibility cannot be excluded that enhanced catabolism in uremic rats may have increased release of 3-methylhistidine from muscle and contributed to the elevated plasma methylhistidine levels (28).

Plasma glycine levels were also observed to be consistently increased in uremic rats. Felig and co-workers (5) have found that in human kidney there is a net uptake of glycine coupled with a release of arginine. It may be that in the uremic rat there is decreased utilization of glycine by the remaining renal tissue, together with decreased urinary excretion.

In addition to the effect of renal failure on plasma amino acid concentrations, certain amino acids also varied with protein intake in both uremic and control rats. These amino acids were generally nonessential, and they were inversely related to dietary protein content. The correlation between plasma amino acids and protein intake was in general similar in the uremic and control rats (Table 3). The rise in nonessential amino acids with decreased protein intake contributed to the significantly lower E/N ratio observed with the 5% protein diet (Table 2). This ratio is considered to be an index of protein nutritional status, and the lowered E/N ratio with the 5% protein diet, as well as the impaired weight gain, thus provide evidence for the nutritional inadequacy of this diet in both uremic and control rats.

The chronically uremic human subject manifests alterations in plasma concentrations of certain amino acids which are similar to those found in the uremic rats. These include lowered tyrosine/phenylalanine ratios, and increased levels of citrulline, glycine, and the methylhistidines (11). In addition, Gulyassy et al. (8) have found decreased total tryptophan and increased free tryptophan in plasma of chronically uremic patients. Siassi et al. (22) have made similar observations in chronically uremic rats. The findings of abnormal plasma amino acid levels and wasting or impaired growth in both chronically uremic humans and rats suggest that chronically uremic rats may serve as a valuable model for the study of nitrogen metabolism in patients with chronic renal failure. The observation that uremic rats utilize protein less efficiently may indicate that the marked reduction in protein intake which occurs commonly in uremic humans, secondary to either dietary prescription or illness, may contribute to tissue wasting in this condition.

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