Inverse relationship between protein intake and plasma free amino acids in healthy men at physical exercise

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We have shown in adult humans given diets supplying the equivalent of 1 g protein·kg⁻¹·day⁻¹, but with varying intakes of individual indispensable amino acids and use of ¹³C-labeled amino acid tracers, that the rate of catabolism of leucine (15, 62), valine (38), phenylalanine (53), threonine (65), and tyrosine (3) is reflected by their concentrations in the circulating blood and presumably the concentration in the free amino acid pools (40, 49). Furthermore, there is a diurnal fluctuation in plasma amino acid concentrations (59) that is modulated by the pattern (27, 64) and composition of meal intake (16, 17, 21). There is also a meal-induced retention of amino acids and subsequent loss of amino acids (or body protein) throughout the 24-h day, the amplitude of which is also determined by the dietary protein level (43, 51).

Changes in plasma amino acid concentrations due to acute and more prolonged periods of physical exercise also have been described (13, 26, 33, 44, 46, 47, 50). However, essentially all the earlier studies have involved relatively short and infrequent periods of experimental observation during or after exercise, and the subjects studied had not usually been controlled strictly for their previous dietary protein intake level at energy balance. This makes it difficult to interpret and compare many of the earlier findings on plasma amino acid changes in relation to exercise and diet. Furthermore, there are few data available on the pattern of change in the concentration of specific amino acids in blood over a continuous 24-h period at a normal or high intake with or without short periods of moderate exercise. Hence, we thought it desirable to examine in some detail the pattern and degree of change in plasma amino acid concentrations over a continuous 24-h period in healthy subjects consuming diets supplying a normal and a high level of protein intake that is in the region of the recommended intakes proposed by some investigators (31, 32).

We hypothesized that the plasma amino acid concentrations, especially the nutritionally indispensable amino acids, during the fed period of the day would reflect the prevailing dietary protein level and that, for the range of protein intake studied, there would be fewer distinct diet-related differences in the concentrations of these amino acids in plasma during the fasting period of the day.

MATERIAL AND METHODS

Subjects

Eight healthy male volunteers [age, 29 ± 14 (mean ± SD) years; weight, 78 ± 7 kg; height, 187 ± 6 cm; body fat, 17 ± 6%] participated in the first study, and six healthy male volunteers [age, 29 ± 15 (mean ± SD) years; weight, 80 ± 12 kg, height, 186 ± 9 cm; body fat, 19 ± 5%] in the second study. One person participated in both studies, and his results reflected the group differences. The subjects were recruited...
from the population of students and employees at Uppsala University. They were physically fit but not competitive athletes, and all were in good health as determined by medical history and physical examination. None of them smoked or had excessive alcohol consumption. All subjects gave their written informed consent, and the study was approved by the Ethical Committee of the Faculty of Medicine at Uppsala University.

Diet. A standardized diet was consumed during the 7-day experimental period. The diet was based on two major components, 1) a milk drink as the principal protein source, flavored with banana or raspberry, and 2) specially prepared cookies as an energy source to balance energy expenditure. During the first experiment, the protein intake was 1 g·kg⁻¹·day⁻¹, and during the second period it was 2.5 g·kg⁻¹·day⁻¹. Milk protein from skim milk powder was the principal protein source in both diets, so that the dietary amino acid pattern was the same for both diets. The nonprotein fat-carbohydrate energy ratio also was kept at 40:60 for both diets. Energy intake was given to keep the subjects in energy balance. The macronutrient compositions of the two diets are shown in Table 1 together with a summary of their metabolic balances, and further details are given elsewhere (14, 22).

For the normal-protein intake (1 g·kg⁻¹·day⁻¹), the milk drink comprised the protein source, and the protein-free cookies were used as an additional energy source to balance energy expenditure. The cookies were baked with an essentially protein-free mix (low-protein and milk-free mix from Semper AB, Stockholm, Sweden), beet sugar, margarine, and sunflower oil, flavored with raisins or chocolate. The cookies contained 0.3% energy from protein and 46 and 54% from fat and carbohydrate, respectively. For the high-protein intake (2.5 g·kg⁻¹·day⁻¹) milk protein was also used as a protein source, but in this case it was also added to the cookies, which provided 40% of the total protein intake.

Energy expenditure. Basal metabolic rate (BMR) was calculated from the WHO/FAO/UNU equations (58). When calculating total energy expenditure, a physical activity level (PAL) factor of 1.55 (58) was used during days 1–5, the milk drink comprised the protein source, and the protein-free cookies were used as an additional energy source to balance energy expenditure. The specimens were cooled immediately in ice water, centrifuged (at 2,500 RPM), and the plasma was deproteinized within 30 min by means of 50 mg sulfosalicylic acid/ml plasma and centrifuged. The deproteinized specimens were kept frozen at −20°C until analyzed, which was usually done within 1–2 wk. Urine samples were collected during consecutive 3-h periods throughout the 24-h day (see below) and kept frozen at −20°C until used for analysis.

Experimental Design

The subjects were studied on an outpatient basis during days 1–5 at the Energy Metabolic Unit (UPPCAL) of the Department of Medical Sciences, Nutrition unit, Uppsala University. They were given the experimental diets for seven days and, at these times, glucose was performed on the cycle ergometer within the metabolic unit. During days 1–5, the food was given as three major meals (breakfast, lunch, and dinner) with two small meals between. During days 6 and 7, the food was equally distributed as 10 small hourly meals from 1200 until 2100. On day 7, venous blood samples were collected every 30 min (beginning at 0600 and continuing until 0600 the next day), except during the 90-min exercise period when samples were collected every 15 min. The specimens were cooled immediately in ice water, centrifuged (at 2,500 RPM), and the plasma was deproteinized within 30 min by means of 50 mg sulfosalicylic acid/ml plasma and centrifuged. The deproteinized specimens were kept frozen at −20°C until analyzed, which was usually done within 1–2 wk. Urine samples were collected during consecutive 3-h periods throughout the 24-h day (see below) and kept frozen at −20°C until used for analysis.

Methods

The amino acid levels in plasma and urine (within a coefficient of variance of 1–2% for most of the amino acids) were determined by means of an automatic amino acid analyzer (LKB 415Alpha Plus Amino Acid Analyser, Pharmacia-LKB Biochrom, Cambridge, UK) by use of the lithium buffer system according to the manual. Each analysis had a coefficient of variance of 1–2% for most of the amino acids (determined by a 50 μmol/l standard solution (Sigma Chemical, St. Louis, MO; product no. 9906)) was analyzed at regular intervals as well as whenever a new batch of solutions and reagents was used; the coefficient of variation was <1% over the course of these studies. Repeated analysis of a plasma reference showed a coefficient of variation between 1–2% for most amino acids. Cystine and proline were not determined reliably by our procedure, and so these amino acids are not reported here. Urine and plasma urea analysis was accomplished via an enzymatic procedure (Granatest 15-Plus from Diagnostica Merck, Darmstadt, Germany). Urine collection bottles contained 15 ml 6 N HCl as a preservative. Creatinine in plasma and urine was measured by routine methods (Boehringer Mannheim Diagnostica, Mannheim, Germany).

Glucagon levels in plasma were estimated with the Linco glucagon RIA kit (Linco Research, St. Louis, MO). Insulin in...
50 µl serum (S-insulin) was measured with a time-resolved noncompetitive sandwich fluoroimmunoassay (AutoDeffia 
 Insulin kit, Wallac Oy, Turku, Finland). Two different mouse monoclonal antibodies directed to different sites on the human insulin molecule were used. One was immobilized onto the walls of microtiter plates and the other in solution and labeled with europium chelate. The results were expressed in mU/l by use of the WHO 1st International Reference Preparation (66/304) of insulin for immunoassay as a reference. The cross-reactivity with proinsulin was 0.1%. The minimal detection limit was 0.3 mU/l. The within- and between-assay coefficients of variation were 1.5 and 2%, respectively.

Renal clearance. For the two diet groups, to determine amino acid clearance by the kidney, the blood sample taken at 0500 was used, and the urine output between 0300 and 0600 was measured and analyzed. Clearance was calculated as urine concentration × urine volume (milliliters per minute)/plasma concentration. The value was then expressed per standard 1.73 m² (determined from a nomogram based on height and weight).

Evaluation of the data. Seven specific time points were chosen for purposes of evaluation of the plasma AA data: 1) after overnight fasting (0830), 2) during physical exercise while fasting (1000), 3) postexercise while fasting (1200), 4) during feeding before exercise (1600), 5) during physical exercise while feeding (1730), 6) postexercise during feeding (1900), and 7) during sleep (0500). For those amino acids (methionine, phenylalanine, threonine, glutamine, and tyrosine) for which the 24-h data are depicted (see below) the mean values for the periods 0530–0830, 0900–1000, 1100–1200, 1300–1600, 1615–1730, 1800–2100, and 2200–0600 were used, and the urine output between 0300 and 0600 was measured and analyzed. Clearance was calculated as urine concentration × urine volume (milliliters per minute)/plasma concentration. The value was then expressed per standard 1.73 m² (determined from a nomogram based on height and weight).

RESULTS

Tables 2 and 3 summarize the plasma AA concentrations, as well as the insulin and glucagon levels, for the seven selected phases or times of day for subjects receiving the normal- and high-protein intakes, respect-

### Table 2. Plasma concentrations of free indispensable amino acids, glucose, insulin, and glucagon for 7 different periods on normal- and high-protein diets

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Response Code</th>
<th>Protein Intake</th>
<th>During Fasting</th>
<th>During Feeding</th>
<th>During Night Sleep</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before exercise</td>
<td>During exercise</td>
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<td>Before exercise</td>
<td>During exercise</td>
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<td></td>
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<tr>
<td>Histidine</td>
<td>5</td>
<td>N</td>
<td>93.8 ± 31.8</td>
<td>94.6 ± 20.7</td>
<td>90.0 ± 28.1</td>
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<tr>
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<tr>
<td>Isoleucine</td>
<td>2</td>
<td>H</td>
<td>56.7 ± 11.6</td>
<td>58.8 ± 12.6</td>
<td>60.7 ± 15.1</td>
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<tr>
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<tr>
<td>Leucine</td>
<td>2</td>
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<td>56.6 ± 24.3</td>
<td>49.8 ± 15.5</td>
<td>45.6 ± 17.6</td>
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<tr>
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<tr>
<td>Lysine</td>
<td>4</td>
<td>H</td>
<td>37.2 ± 7.7</td>
<td>33.0 ± 7.2</td>
<td>34.0 ± 7.4</td>
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<tr>
<td>Methionine</td>
<td>1</td>
<td>N</td>
<td>23.2 ± 7.5</td>
<td>24.9 ± 7.1</td>
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<td>59.9 ± 20.2</td>
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<tr>
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<tr>
<td>Threonine</td>
<td>3</td>
<td>H</td>
<td>174.8 ± 54.4</td>
<td>174.2 ± 51.4</td>
<td>151.7 ± 53.1</td>
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<tr>
<td>Tryptophan</td>
<td>5</td>
<td>N</td>
<td>95.7 ± 18.2</td>
<td>90.7 ± 15.0</td>
<td>82.3 ± 11.6</td>
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<tr>
<td>Tyrosine</td>
<td>1</td>
<td>N</td>
<td>56.1 ± 23.0</td>
<td>40.3 ± 20.9</td>
<td>35.8 ± 20.6</td>
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<tr>
<td>Valine</td>
<td>2</td>
<td>N</td>
<td>253.9 ± 92.6</td>
<td>233.4 ± 58.6</td>
<td>221.0 ± 66.2</td>
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<tr>
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<tr>
<td>Glucose</td>
<td>2</td>
<td>N</td>
<td>5.0 ± 0.3</td>
<td>4.7 ± 0.3</td>
<td>4.5 ± 0.4</td>
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</tr>
<tr>
<td>Insulin</td>
<td>2</td>
<td>N</td>
<td>5.3 ± 0.6</td>
<td>5.1 ± 0.5</td>
<td>4.8 ± 0.3</td>
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<tr>
<td>Glucagon</td>
<td>2</td>
<td>H</td>
<td>61.1 ± 2.4</td>
<td>26.0 ± 0.5</td>
<td>5.7 ± 3.4</td>
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<tr>
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</tr>
<tr>
<td>Insulin/glucagon ratio</td>
<td>2</td>
<td>H</td>
<td>0.12 ± 0.06</td>
<td>0.05 ± 0.02</td>
<td>0.08 ± 0.02</td>
</tr>
</tbody>
</table>

Values of plasma concentrations are means ± SD, expressed in µmol/l. Glucose values are mmol/l; insulin values are mU/l; glucagon values are ng/l. Response codes are based on statistical analyses as described in RESULTS. a = 7; b = 6; c = 5. N, normal-protein intake; values refer to means based on 8 individuals unless otherwise indicated. H, high-protein intake; values refer to means based on 6 individuals unless otherwise indicated.
Table 3. Plasma concentrations of free dispensable or conditionally indispensable amino acids for 7 different periods on normal- and high-protein diets

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Response Code</th>
<th>Protein Intake</th>
<th>During Fasting</th>
<th>During Feeding</th>
<th>During Night Sleep</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Before exercise</td>
<td>During exercise</td>
<td>After exercise</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>N 712.4 ± 154.1</td>
<td>N 402.3 ± 51.2</td>
<td>N 154.6 ± 74.2</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>5</td>
<td>N 74.0 ± 56.6*</td>
<td>N 403.1 ± 52.1</td>
<td>N 408.1 ± 39.3</td>
<td>N 397.2 ± 49.6</td>
</tr>
<tr>
<td>Glutamine</td>
<td>5</td>
<td>N 115.0 ± 24.7</td>
<td>N 211.9 ± 45.8</td>
<td>N 208.6 ± 37.4</td>
<td>N 192.1 ± 29.5</td>
</tr>
<tr>
<td>Ornithine</td>
<td>2</td>
<td>N 48.7 ± 15.2</td>
<td>N 44.1 ± 8.6</td>
<td>N 44.6 ± 8.0</td>
<td>N 55.6 ± 12.1</td>
</tr>
<tr>
<td>Serine</td>
<td>2</td>
<td>N 119.1 ± 46.7</td>
<td>N 34.3 ± 9.5</td>
<td>N 35.0 ± 8.7</td>
<td>N 59.3 ± 19.1</td>
</tr>
<tr>
<td>Taurine</td>
<td>2</td>
<td>N 71.3 ± 11.8</td>
<td>N 115.0 ± 24.7</td>
<td>N 128.2 ± 22.9</td>
<td>N 109.7 ± 21.1</td>
</tr>
</tbody>
</table>

Values for amino acid plasma concentrations are µmol/liter; means ± SD. Response codes are based on statistical analyses as described in RESULTS. N values refer to means based on 8 individuals, H values to means based on 6 individuals, unless otherwise indicated. a n = 7; b n = 6; c n = 5; d n = 4.

Phenylalanine (Fig. 2), together with leucine, isoleucine, valine, and serine (Tables 2 and 3) fell, again based on the statistical analysis, into response code 2. In contrast to methionine and tyrosine, phenylalanine concentration was lowered in the fast but not in the fed state at the high- vs. normal-protein intake.

Serine, isoleucine, and valine were all lower during the fast at the high-protein intake. The leucine concentration did not differ significantly during the fast between the two protein intakes, but together with valine and isoleucine, these concentrations were all higher with the high-protein level during the fed period, compared with values for the normal-protein diet.

Two amino acids, threonine (Fig. 3) and alanine (Tables 2 and 3), fell into response code 3. Concentrations of these amino acids were all lower at the high-protein intake regardless of phase (or exercise). The difference in plasma concentrations of threonine between the diets was about 102 µM.

Plasma glutamine and taurine concentrations are depicted in Figs. 4 and 5, respectively, which, together with arginine, citrulline, and lysine, fell into response code 4 (Tables 2 and 3). Glutamine plasma concentrations during the fast and fed periods were significantly (P < 0.001) higher on the normal vs. high-protein diets. Glutamine concentrations during the 24-h day for the normal-protein group approximated 700–750 µM or about 200–300 µmol·l−1 higher than those for the high-protein group. At the 1-g protein intake level, the glutamine concentrations did not differ significantly (P > 0.05) between the various phases of the day, but they were higher (P < 0.001) during the fed vs. fast phases when subjects consumed the high-protein diet.

Taurine concentrations were higher for the normal-protein intake during both the fast and fed periods.
Feeding increased plasma lysine, and the high-protein diet significantly reduced the concentration of lysine during the fast ($P = 0.0018$) but not the fed period. Arginine and citrulline concentrations were similarly lowered by the high-protein diet during the fast but not the fed phase of the day. Citrulline concentration decreased with feeding at normal- but not high-protein intake. In contrast, feeding increased the plasma arginine concentration at high- but not normal-protein intake.

The plasma concentrations of glutamic acid, histidine, ornithine, and tryptophan (response code 5; Tables 2 and 3) revealed significant effects of protein and phase and their interaction. This suggests that the differences between protein intake level depended on the phase of the day. In the case of ornithine, tryptophan, and histidine, the differences in concentrations between diets were significant during the fast and for glutamate during both the fast and the fed phases with the concentrations being lower in all cases at the high-protein intake.

Asparagine and glycine (Tables 2 and 3) are grouped in response code 6 because their plasma concentrations revealed no significant interactions, although all three main effects (phase, protein intake, and exercise) were significant. Glycine and asparagine concentrations were lower at the high-protein intake.

Glucagon concentrations (Table 2) were affected according to response code 2. Overall, they were significantly higher during the fed phase ($P < 0.0001$) at the high-protein intake, together with a tendency ($P < 0.09$) for this to be so during the fast. For insulin (Table 2), there was a small (~2 µU/l higher for the high-protein diet) but significant ($P < 0.02$) difference in the
plasma concentration between the diet groups before and after the exercise periods.

The insulin-to-glucagon ratio (Table 2) changes and differences between the diet groups followed response code 2; it was increased with feeding and to a greater extent at the normal-protein intake.

The impact of exercise per se on plasma amino acid concentrations cannot be determined precisely from this study, because it requires a nonexercise group for additional comparison and complete interpretation. However, the 90-min exercise periods took place during fasting, when plasma concentrations were expected to remain relatively stable in the absence of exercise. Similarly, the exercise occurred during the feeding with small meals, when the changes in plasma amino acid concentrations in response to feeding were also expected to be at a relatively steady level with this mode of feeding. Therefore, some comparisons between the exercise and nonexercise periods also might be made here. Thus it appears that exercise raised significantly ($P < 0.05$) the concentrations of most of the amino acids during the fed state, although there were no apparent marked effects when judged from these data. There was little evidence of any major effects of exercise during the fast period. Exercise increased the glucagon concentration in both the fast and the fed conditions, whereas exercise decreased insulin concentrations, particularly in the fed state.

Finally, we assessed whether or not changes in clearance of amino acids by the kidney might offer an explanation for the marked and constant differences in the 24-h or fasted-state plasma concentrations of the
amino acids discussed above. Our estimates of plasma amino acid clearance are summarized in Table 4. Here, it is worth noting that creatinine clearance was the same for the two diets, indicating that there were no profound differences in the glomerular filtration rate between the diet groups. In contrast, urea clearance was about half that for the high- vs. normal-protein diet. Second, if the clearance of amino acids for the normal-protein group, it can be seen that the clearance rate, which was not compensated for by an equivalent increase in the rate of entry of the amino acids into circulation from endogenous or exogenous sources. A quantitative evaluation of these data with respect to the observed plasma concentration changes will be presented in DISCUSSION. Finally, the concentration of the branched-chain amino acids (BCAA) in urine was below a reliable limit of detection; hence, clearances could not be established for these amino acids.

Thus the sustained lower concentrations of glutamine (Fig. 4), alanine, asparagine, glycine, and taurine (Fig. 5, Table 3) could be due in part to a more efficient renal clearance rate, which was not compensated for by an equivalent increase in the rate of entry of the amino acids into circulation from endogenous or exogenous sources. A quantitative evaluation of these data with respect to the observed plasma concentration changes will be presented in DISCUSSION. Finally, the concentration of the branched-chain amino acids (BCAA) in urine was below a reliable limit of detection; hence, clearances could not be established for these amino acids.

Table 4. Amino acid, urea, and creatinine clearance at normal- and high-protein intake

<table>
<thead>
<tr>
<th>Compound</th>
<th>P1, μM/l</th>
<th>U1, μM/l</th>
<th>C1, ml·min⁻¹·1.73 m⁻²</th>
<th>P2.5, μM/l</th>
<th>U2.5, μM/l</th>
<th>C2.5, ml·min⁻¹·1.73 m⁻²</th>
<th>Ratio, C2.5/C1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>432 ± 108</td>
<td>115 ± 50</td>
<td>0.39 ± 0.18</td>
<td>257 ± 43*</td>
<td>123 ± 47</td>
<td>0.84 ± 0.26</td>
<td>2.15</td>
</tr>
<tr>
<td>Arginine</td>
<td>78 ± 28</td>
<td>0</td>
<td>0</td>
<td>42 ± 6†</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Asparagine</td>
<td>107 ± 36</td>
<td>24 ± 13</td>
<td>1.07 ± 8.3</td>
<td>70 ± 11*</td>
<td>49 ± 22**</td>
<td>1.2 ± 0.7†</td>
<td>3.0</td>
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<tr>
<td>Aspartic acid</td>
<td>6.3 ± 2.9</td>
<td>37 ± 17</td>
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<td>30.1 ± 3.9*</td>
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<td>Citrulline</td>
<td>37 ± 8</td>
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<td>0</td>
<td>17 ± 3‡</td>
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<td>Glutamine</td>
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<td>89 ± 80</td>
<td>0.15 ± 0.09</td>
<td>402 ± 25‡</td>
<td>158 ± 76</td>
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<td>Glutamic acid</td>
<td>105.8 ± 92</td>
<td>95 ± 39p</td>
<td>2.6 ± 3.4†</td>
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<td>61 ± 24p</td>
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<td>Glycine</td>
<td>224 ± 41</td>
<td>314 ± 83</td>
<td>2.2 ± 1.1</td>
<td>121 ± 15†</td>
<td>327 ± 96</td>
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<tr>
<td>Histidine</td>
<td>91 ± 26</td>
<td>229 ± 97</td>
<td>3.8 ± 1.8</td>
<td>57 ± 12†</td>
<td>340 ± 116</td>
<td>11.0 ± 3.0†</td>
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<td>Isoleucine</td>
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<td>0</td>
<td>42 ± 13</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Leucine</td>
<td>126 ± 33</td>
<td>0</td>
<td>0</td>
<td>123 ± 23</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lysine</td>
<td>178 ± 39</td>
<td>34 ± 22</td>
<td>0.20 ± 0.16</td>
<td>130 ± 17*</td>
<td>75 ± 53</td>
<td>0.99 ± 0.78</td>
<td>3.5</td>
</tr>
<tr>
<td>Methionine</td>
<td>25 ± 7</td>
<td>0</td>
<td>0</td>
<td>22 ± 5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ornithine</td>
<td>52 ± 12</td>
<td>6 ± 3e</td>
<td>0.20 ± 0.08</td>
<td>37 ± 9*</td>
<td>4 ± 2e</td>
<td>0.16 ± 0.09</td>
<td>0.8</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>62 ± 20</td>
<td>24 ± 13</td>
<td>0.58 ± 0.28</td>
<td>45 ± 8</td>
<td>31 ± 15e</td>
<td>1.1 ± 0.24†</td>
<td>1.9</td>
</tr>
<tr>
<td>Serine</td>
<td>122 ± 29</td>
<td>116 ± 40</td>
<td>1.5 ± 0.7</td>
<td>76 ± 5f</td>
<td>150 ± 66</td>
<td>3.5 ± 1.2†</td>
<td>2.3</td>
</tr>
<tr>
<td>Taurine</td>
<td>64 ± 11</td>
<td>74 ± 41</td>
<td>1.9 ± 1.2</td>
<td>27 ± 6t</td>
<td>67 ± 61</td>
<td>3.9 ± 2.6</td>
<td>2.1</td>
</tr>
<tr>
<td>Threonine</td>
<td>190 ± 55</td>
<td>57 ± 20</td>
<td>0.48 ± 0.27</td>
<td>106 ± 18t</td>
<td>99 ± 48e</td>
<td>1.6 ± 0.74</td>
<td>3.3</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>39 ± 13</td>
<td>47 ± 12c</td>
<td>2.1 ± 1.2c</td>
<td>50 ± 15</td>
<td>44 ± 13'</td>
<td>1.8 ± 0.9</td>
<td>0.9</td>
</tr>
<tr>
<td>Valine</td>
<td>240 ± 77</td>
<td>0</td>
<td>222 ± 44</td>
<td>0</td>
<td>222 ± 44</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Urea, mg/ml</td>
<td>0.05 ± 0.02</td>
<td>3.6 ± 1.7</td>
<td>121 ± 49</td>
<td>0.13 ± 0.02</td>
<td>5.3 ± 1.8</td>
<td>70 ± 14*</td>
<td>0.6</td>
</tr>
<tr>
<td>Creatinine, µM/l</td>
<td>88 ± 7</td>
<td>7679 ± 2744</td>
<td>131 ± 100</td>
<td>84 ± 4</td>
<td>5396 ± 2434</td>
<td>133 ± 11</td>
<td>1.0</td>
</tr>
</tbody>
</table>

P1, U1, P2.5, and C1 and C2.5 are plasma (P) and urine (U) amino acid concentrations and renal clearance (C) for the 1- and 2.5-g protein intakes, respectively. U1 volume 1.9 ± 0.9 ml/min, U2.5 volume 2.4 ± 1.1 ml/min. Normal-protein, 1.0 g·kg body wt⁻¹·day⁻¹; high-protein, 2.5 g·kg body wt⁻¹·day⁻¹. *Values refer to the last urine sampling period (i.e., during the night) and the 0500 plasma value, mean ± SD. †P < 0.05, ‡P < 0.01, ††P < 0.001. n = 7, b n = 6, c n = 5, d n = 4, e n = 3, f n = 2.
DISCUSSION

The concentrations of the free amino acids in blood plasma are determined by various factors, including the level and pattern of amino acids supplied by the diet (40, 64), the tissue distribution of the metabolic pathways of amino acid metabolism, the status of the amino acid transport systems in the various organs (8, 55), and the pathophysiological condition of the organism, probably including the amount of muscle glycogen (57). Hence, changes in plasma amino acid concentrations and patterns are brought about by a complexity of factors that interact, making it difficult to identify fully the metabolic basis for alterations that are brought about, for example, by diet or physical exercise. Nevertheless, the present data offer new insights into how diet and exercise serve to collaborate in determining the concentrations of various amino acids in the circulation.

We had not anticipated the consistent and much lower concentrations of the dispensable (or conditionally indispensable) amino acids, as well as some of the indispensable amino acids, when the high- vs. normal-protein diet was consumed. Fernstrom et al. (21) had earlier shown that glycine and alanine concentrations were lower after subjects received 150 g egg protein/day than in those receiving 75 or 0 g protein daily. In this respect their findings are in agreement with ours. However, they report that threonine and serine plasma levels rose at all times of the day as more protein was added to the diet. In our study the concentration of threonine (Fig. 3) was lower throughout the entire day and serine significantly lower during the fast phase of the 24-h day when subjects received the high-protein diet. In addition, the concentrations of other indispensable amino acids (phenylalanine, lysine, isoleucine, valine, and tryptophan) were all reduced during the fast but not during the fed period of the day when the high-protein diet was consumed. On the other hand, leucine, methionine, and tyrosine concentrations were not reduced by feeding a high-protein diet.

The lower concentrations of the specific amino acids noted above were found to be related to an increased clearance of amino acids by the kidney when the high protein intake was given. Although the glomerular filtration rate (GFR), approximated from creatinine clearance, was not affected by the level of protein intake and remained within normal limits, the clearance rate was much higher at the high-protein intake for alanine, glutamine, asparagine, aspartic acid, lysine, phenylalanine, threonine, and taurine. This implies that the tubular reabsorption of these amino acids was reduced when the high-protein diet was consumed. Irrespective of the renal mechanism, however, an important issue is the extent to which changes in renal clearance might account for the differences in the amino acid levels between the two diets, and we now discuss this in reference to glutamine, alanine, taurine, and finally threonine and the BCAA.

Thus the rates of glutamine and alanine excretion in the urine for the normal- and high-protein groups amounted to 0.1–0.24 µmol·kg⁻¹·h⁻¹, based on the urine analysis conducted. There might have been differences in these rates at specific periods of the day, but because the plasma differences for these two amino acids existed throughout the entire 24-h period, this would not detract significantly from the following discussion; thus these hourly losses via the kidney can be compared with the rates of glutamine and alanine entry into the blood circulation, which are ~350 and 250 µmol·kg⁻¹·h⁻¹, respectively (9, 11, 42, 60). Although these rates can be affected by diet (36, 61) and hormonal status (12), for example, this does not minimize the argument that their urinary excretion rates amount to a small fraction (0.1%) of their plasma entry rates for the normal- and high-protein groups. On this basis, therefore, it does not appear that the changes observed in kidney clearance are responsible for the altered plasma AA concentrations. Under conditions of an adequate but not excessive protein intake, the rates of de novo glutamine and alanine (12) synthesis are about 198 and 100 µmol·kg⁻¹·h⁻¹, respectively, or ~40–60% of the plasma entry rates. These rates are also high in relation to the absolute urinary losses and the differences observed here between the two diet groups. Hence, it appears that the altered output of these amino acids via the kidney would not bring about the plasma concentration changes observed; rather, the rates of entry of glutamine and alanine into plasma were probably reduced with high-protein feeding, and this might also have been associated with increased clearance of the amino acids by the splanchnic bed. Alanine and glutamine account for ~60–80% of the amino acids released from skeletal muscles in postabsorptive hours, with glutamine being dominant (18–20). The glutamine released from muscle is metabolized to an important extent in the intestinal tissues (1) where the nitrogen is then released as alanine and ammonia (29). Similarly, dietary glutamate is almost entirely metabolized within the intestinal tissues (4, 52). Also, it might be estimated, as noted above, that about half the alanine and glutamine release from skeletal muscle is due to de novo synthesis, and the other half is due to direct release of the amino acids from protein. It seems possible, therefore, that one or both of these sources of glutamine and alanine may be reduced with the feeding of a high-protein diet. Matthews and Campbell (36) found that plasma glutamate and glutamine fluxes varied inversely with the level of dietary protein intake, and these investigators attributed the changes to de novo production of the amino acids. At the 0.1, 0.8, and 2.2 g protein·kg⁻¹·day⁻¹ intakes, the mean plasma glutamine concentrations reported by these investigators were 616, 492, and 411 µM, respectively. Similarly, we have reported an inverse relationship between the dietary protein intake level and plasma alanine flux (61). In addition, an increased protein intake appears to restrain body protein breakdown (22, 37). This being the case, the direct release of alanine and glutamate from proteins would also be attenuated. Clearly, additional studies are required on the impact of high-protein diets on the
kinetics of glutamine and alanine metabolism and the interrelationships between the skeletal muscle and splanchnic region.

Taurine (2-aminoethanesulfonic acid) also showed much lower concentrations with the high- vs. normal-protein diet; it is readily excreted in the urine and metabolized within the liver, where it is exported as a bile acid conjugate (25, 28). There does not seem to be any oxidation of the amino acid by body tissues. We do not know what the dietary taurine intake was in our experiment, but it is present in relatively low concentrations in cow’s milk, which served as the source of protein in our experiment. Hence, the lower concentration in the high-protein group would not appear to be due to distinct differences in taurine intake. Furthermore, the dietary losses of taurine via the urine amounted to about 200 μmoles/day (<3 μmol·kg⁻¹·day⁻¹) in both diet groups, and the turnover of the bulk of taurine in the body is relatively slow (25). Although additional taurine losses via the urine occur in the form of inorganic sulphate, formed by the gut microflora, these are not substantial. From these facts, and assuming that in adults the body taurine pool is ~100–150 mmoles (25), it is apparent that there would be little change in the turnover of taurine due to protein intake. Therefore, we interpret our findings to mean that the dietary protein level altered either the entry of taurine into the plasma or its reuptake by body tissues, unless losses of taurine in the bile were substantially increased, which seems unlikely. Our interpretation is consistent with the conclusion drawn by Huxtable (28) that transport, rather than biosynthesis and metabolism, was of greater importance in the regulation of body taurine homeostasis.

Finally, the nutritionally indispensable amino acids showed a diverse pattern of response to the high-protein diet: plasma threonine levels were consistently lower throughout the 24-h period in the high-protein group, whereas the concentrations of isoleucine, valine, tryptophan, and phenylalanine were lower in the fast period only. Threonine is deaminated in the liver via threonine-serine dehydratase (SDH, EC 4.2.1.16), and its activity increases with total protein intake in growing rats (2, 30). Therefore, it is possible that the splanchnic clearance and catabolism of dietary and endogenous threonine was higher when subjects received the high-protein diet. Coupled, perhaps, with reduced rates of tissue (muscle) protein turnover (22), as noted above, this would help to explain the plasma pattern of threonine observed here. Andersen et al. (2) reported that with higher liver SDH activity, when rats were adapted to high (75% casein)-protein diets, the plasma concentrations declined and reached substantially below those for rats given lower-protein diets (5 and 2.5% casein). Furthermore, the threonine levels noted here for the high-protein group are reminiscent of those we have reported for limiting, and possibly inadequate, dietary threonine intakes (56). BCAA are catabolized to an important extent in the muscle (24), and dietary changes in the supply of protein or of BCAA have been shown to result in adaptive changes in the activity of branched-chain α-ketoacid dehydrogenase (EC 1.2.4.4) in liver and muscle of experimental animals (24). Thus the capacity for BCAA catabolism appears to be responsive to dietary change, and this might be why lowered concentrations of isoleucine and valine were observed in the fast state when the high-protein diet was consumed.

Previously (22), we reported a higher loss of leucine via sweat for subjects receiving the high-protein intake. The difference in daily loss between the two diet groups was about 10 mg/day or approximately <0.02% of the plasma leucine flux. Therefore, it would seem unlikely on this basis that increased sweat losses of amino acids at the higher intake would contribute significantly to the plasma amino acid responses observed.

Although we tend to favor the idea that the reduced plasma concentrations for glutamine and alanine, and perhaps of isoleucine and valine, are due to lower outputs from peripheral tissues (muscle), it is also possible that their rates of clearance, as well as those for glycine, serine, and threonine, may have also been increased due to an induction of amino acid degrading enzymes, especially in the splanchnic region. This would result, in association with lower production and/or release from proteins, in a depletion of tissue free amino acids, as well as in the size of the plasma pool, during the fasting state. Thus the pool would then be maintained at a smaller size than that for the case of a normal protein intake, where not only the intake but the rate of degradation is probably lower. Evidence in support of this is to be found in the rat feeding experiments carried out by Harper et al. some years ago (2, 30, 48) and more recently by Moundras et al. (39). Whether an increased uptake of amino acids by the splanchnic region would also have been promoted by the high glucagon and lowered insulin-to-glucagon ratio that we see for the high-protein group is not known. However, an increased splanchnic catabolism of amino acids has been proposed as an in vivo action of glucagon (10), although this might not apply to all amino acids, such as leucine (35). However, glucagon appears to be an important regulator of hepatic glutamine uptake, which increases independently of the fasted state (23, 54).

Finally, the apparent changes in plasma amino acids in response to the physical exercise deserve a brief comment. For both diets, the plasma amino acid levels did not appear to respond to exercise in a quantitatively profound way to the two 90-min exercise periods. Generally, their concentrations increased with exercise, and this was most evident during the fed phase. Whether alterations in plasma volume contributed to these changes cannot be determined. Furthermore, in contrast to the findings by Paul et al. (46, 47), we did not observe marked reductions in the BCAA or tryptophan or increases in glutamine and alanine during exercise while the subjects remained in the fast state. It is possible that the difference in findings between the experiments are related to the fact that our subjects had undergone the exercise program for six days before the measurements, whereas the subjects studied by
Paul et al. (46, 47) had not been preconditioned to the bike-ride exercise, which in their case was set at 60% of peak oxygen uptake (\( V_{\text{O2peak}} \)), compared with 46% in the present study. On the other hand, Blomstrand et al. (6), observed little decrease in the BCAA in endurance subjects given a mixed diet and exercised at 75% \( V_{\text{O2max}} \) (6), observed little decrease in the BCAA in endurance exercise, at least, accelerates amino acid transport (5), and it is possible that the 24-h plasma amino acid patterns observed here were modulated by the exercise periods that each subject experienced during the six days before and on day 7 of the 24-h metabolic study.

In conclusion, we have observed profound diet-induced differences, apparently affected to only a relatively minor extent by exercise, in plasma concentrations of the various amino acids, especially the nutritionally dispensable or conditionally indispensable amino acids. The plasma concentration of glutamine during the high-protein intake period was low, and similar to that in stressed and catabolic patients (41, 45). Taurine levels were also reduced by 50% when the high-protein diet was given. This amino acid may have a protective effect in cardiovascular disease (28). For these reasons alone it is important to further explore the mechanism of action of a high-protein diet on plasma amino acid concentrations and their control.

We thank Prof. A. Harper and Dr. Steven F. Abcouwer for their helpful ideas regarding the interpretation of our findings.

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