Daily delivery of dietary nitrogen to the periphery is stable in rats adapted to increased protein intake

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The objective of this work was to assess the effects of long-term adaptation to a diet with an HP content (50% dry matter) on the anabolic and catabolic capacities of different tissues closely involved in the adaptation of AA and nitrogen metabolism, i.e., gut, liver, kidney, and muscle, to an increase in protein intake. For this purpose, rats were adapted for 15 days to either an HP diet (i.e., 50% protein) or an adequate-protein (AP) diet (i.e., 14% protein). Protein and non-protein nitrogen were measured in the different tissues and the plasma. The postprandial distribution and the plasma. The postprandial distribution and macronutrient content vary between meals. As a consequence, the body must be able to respond acutely to both daily transitions between the fasted and fed states and changes in the protein content of meals. A long-term adaptation to the habitual level of protein intake also occurs. It is established that one of the principal mechanisms of adaptation to an increase of this habitual protein intake is the stimulation of splanchnic AA oxidation. The activity of different gut and liver enzymes and transport systems increases markedly after an adaptation period, thereby augmenting the capacity for AA catabolism (7, 12, 20, 26, 29, 34, 35, 38).

However, although the modulatory role of liver catabolic pathways is well established, less is known about the cooperative catabolic and anabolic capacities of different splanchnic and peripheral tissues to cope with an excess of dietary AA. The current view is that the splanchnic area has a large but limited buffering capacity to respond to a high-protein (HP) intake (8, 20, 27). Above the limit, metabolic adaptive adjustments may be altered to an accommodation status that can have deleterious effects. The regulation of both splanchnic anabolism and peripheral AA and nitrogen metabolism under these conditions is still not fully understood. There seems to be a small increase of body protein synthesis (6, 14, 28, 32), which may in the fed state contribute to absorb the surplus dietary AA, even if the mean daily protein turnover rate does not appear to change (15). It may be possible to induce the liver protein synthesis rate through an increase in dietary protein intake (19). The results for the gut mucosa are more controversial, depending on the species and the physiological state (3, 9). Muscle protein synthesis does not seem to be enhanced by HP intake (2) but, in contrast, may be surprisingly reduced (40). Such observations likely depend on both the type of muscle studied and on the composition of the diet in energy nutrients (46).

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MATERIALS AND METHODS

Animals and Diets

Male Wistar rats (n = 112; Harlan), initially weighing 196 ± 1 g, were housed in single stainless steel wire cages in a room lit for 12 h daily (2030–0830) and maintained at a temperature of ~23°C. The animals were divided into the following three groups: two groups of rats (total n = 76) were adapted for 15 days to a control diet providing an adequate intake of protein (AP group, 14% of total milk protein), whereas the third group of 36 rats was fed for 15 days on an HP diet (HP group, 50% of total milk protein). The composition of the diets is given in Table 1. Rats were fed according to a schedule of three meals a day to adapt them to prompt consumption of the diet (0830–0845: 3 g of food; 1330–1430 and 1830–1930: free access to food), as previously described (27). On the morning of day 16 (0830–0845), 36 AP rats, i.e., rats adapted to an adequate protein diet, ingested an experimental 3-g meal containing 14% of 15N-labeled milk protein (0.42 g protein). This group was taken as the control (AP-14). The other 40 AP rats that were adapted to an adequate protein diet ingested a 3-g meal containing 50% of 15N-labeled milk protein (1.50 g of protein). This group is referred to as AP-50. The 36 rats of the HP group ingested a 3-g meal containing 50% of 15N-labeled milk protein (1.50 g of protein). This group was named HP-50. For more clarity, Fig. 1 summarizes the experimental schedule. The composition of the experimental meals is described in Table 1. The study was carried out in accordance with the recommendations of the French Committee for Animal Care. As previously described (27), the 15N-labeled milk was produced at the Institut National Agronomique experimental farm in Grignon with the help of Dr. P. Schmidely (Department of Animal Sciences, INAP-G, Grignon, France), and the isotopic enrichment of isolated milk protein was 0.4535 atom percent excess.

Table 1. Composition of diëts and experimental meals

<table>
<thead>
<tr>
<th></th>
<th>AP</th>
<th>HP</th>
<th>Experimental 15N-Labeled Meals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diëts</td>
<td>g/kg</td>
<td>% of energy</td>
<td>g/kg</td>
</tr>
<tr>
<td>Total milk protein</td>
<td>140</td>
<td>14.7</td>
<td>500</td>
</tr>
<tr>
<td>Sucrose</td>
<td>100</td>
<td>10.5</td>
<td>50</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>622.4</td>
<td>65.4</td>
<td>313.3</td>
</tr>
<tr>
<td>Vitamin mixture AIN-93M</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Salt mixture AIN-93M</td>
<td>35</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Soy oil</td>
<td>40</td>
<td>9.4</td>
<td>40</td>
</tr>
<tr>
<td>Cellulose</td>
<td>50</td>
<td></td>
<td>50</td>
</tr>
<tr>
<td>Trypto-choline</td>
<td>23</td>
<td></td>
<td>23</td>
</tr>
<tr>
<td>Energy</td>
<td>15.9 kJ/g</td>
<td></td>
<td>15.9 kJ/g</td>
</tr>
</tbody>
</table>

Experimental Protocol

The rats were killed at 0, 1, 2, 3, 4, and 5 h after the 15N-labeled meal. The average number of animals at each time point was six. They were injected with 13.6 mg/100 g body wt of pentobarbital sodium for anesthesia and 5,000 IU heparin (Laboratoires Leo, Saint-Quentin en Yvelines, France) to permit blood sampling.

Sampling Procedures

Blood was removed from the peritoneal cavity after rupture of both the abdominal aorta and vena cava. Plasma was stored at ~80°C until analysis. Urine was collected from the bladder and from an absorbent paper, which had been placed under the cage from the beginning of the experiment. Stomach, gut and cecum digesta, liver, small intestine and colon mucosa, one kidney, and one gastrocnemius muscle were sampled. The small intestine was divided into three equal parts that were everted and scraped as well as the colon to collect the mucosa. All samples were weighed and stored at ~80°C until analysis.

Analytical Methods

Urea was measured in blood and urine using the urease/glutamate dehydrogenase reaction (HYCEL kit, Le Rheu, France). Urinary urea was extracted by cation exchange chromatography on Dowex resin (Dowex AG50X8; Bio-Rad, Ivry sur Seine, France) as previously described (18) and was stored at 4°C until isotopic determination. Plasma was deproteinized with 1 mol/l HCl and then neutralized with 0.1 mol/l NaH2PO4. Plasma urea was then extracted using the same procedure.

For AA analysis, plasma was first deproteinized with sulfosalicylic acid (50 mg/ml). The supernatant was dried and resuspended in a lithium citrate buffer (pH 2.2) for analysis. Plasma AA concentrations were determined using HPLC with a postcolumn ninhydrin derivatization system (Amino System 3000; Bio-Tek Instruments, St. Quentin en Yvelines, France).

Tissue samples were crushed in 4 vol of 0.9% NaCl. TCA (10% final concentration) was used to precipitate the protein. After centrifugation, the supernatant (containing free AA and small peptides) was frozen and dried, and the pellet...
(containing protein) was washed one time in 0.9% NaCl and then frozen and lyophilized. The soluble fraction was designated as the nonprotein fraction, and the insoluble fraction was designated as the protein fraction.

Isotopic Determinations

Before the isotopic determination of $^{15}$N enrichment, resins were eluted with 2.5 mol/l KHSO$_4$. An isotopic ratio mass spectrometer (Optima; Fisons Instruments, Manchester, UK) coupled to an elemental analyzer (NA 1500 series 2; Fisons Instruments) was used to measure $^{15}$N enrichment in urinary and blood urea as well as in protein and nonprotein fractions of tissues and gastrointestinal digesta, as previously described (27). Total nitrogen in the protein and nonprotein fractions of tissues (i.e., gut and colon mucosa, liver, muscle, kidney) was measured using the elemental nitrogen analyzer.

Calculations and Statistics

All results were expressed in quantities per 100 g of body weight, since the rats in the three groups did not have exactly the same weight at the end of the adaptation period. Body muscle weight was evaluated assuming that it represented 45% of total body mass, as previously described (27). The gastrocnemius was taken to be representative of the whole muscular mass.

Dietary nitrogen in samples. The dietary nitrogen present in samples (N$_{diet}$, mmol) was calculated as follows

$$N_{diet} = N_{tot} \times (APE_s/APE_m)$$

where N$_{tot}$ is the amount of total nitrogen in the sample, and APE$_s$ and APE$_m$ are the $^{15}$N enrichment excess of the sample and the meal, respectively.

The dietary nitrogen present in the urea body pool (N$_{diet-urea}$, mmol) was calculated according to the formula

$$N_{diet-urea} = C_{urea} \times 0.67/0.92 \times BW \times (APE_{urea}/APE_m)$$

where BW is the body weight, C$_{urea}$ is the concentration of urea in the plasma, and APE$_{urea}$ is the $^{15}$N enrichment excess of the plasma urea sample. Sixty-seven and ninety-two percent were the mean percentage of body water in the rat and the mean percentage of water in plasma, respectively (36). The total transfer of dietary nitrogen to urea was calculated as the sum of the dietary nitrogen excreted in urinary urea and the dietary nitrogen present in the body urea pool.

Intestinal flux of dietary nitrogen absorption. The intestinal flux of dietary nitrogen absorption ($Q_A$) was calculated from the amounts of dietary nitrogen that disappeared from the stomach and intestinal lumen within 1 h

$$Q_A (\% \text{ of ingested N/h}) = \frac{(N_{dietS_t} - N_{dietS_{t+1}} + N_{dietI_t}) - (N_{dietC_{t+1}} - N_{dietC_t} - N_{dietI_{t+1}})}{100/N_{ingest}}$$

where N$_{dietS_t}$, N$_{dietC_t}$, and N$_{dietI_t}$ are the amount of dietary nitrogen recovered at time point t from the stomach, small intestine, and cecum, respectively, and N$_{ingest}$ is the amount of ingested nitrogen.

Protein fractional synthesis rate in gut mucosa. Protein fractional synthesis rates in the gut mucosa (FSR, %/day) were calculated assuming that the luminal AA were the precursor pool for protein synthesis (39), according to the formula

$$FSR = \frac{(APE_{mucosa}/APE_m) \times (24/t)}{100}$$

where APE$_{mucosa}$ is the $^{15}$N enrichment excess of the mucosa sample, and t is the incorporation time expressed in hours.

Daily incorporation of dietary nitrogen. The daily incorporation of dietary nitrogen into different body pools was extrapolated from experimental data for the AP-14 and HP-50 groups, assuming that the morning meal was representative of the other meals during the day, using the formula

$$N_{incorp} = N_{diet} \times N_{daily}/N_{ingest}$$

where N$_{incorp}$ is the amount of dietary nitrogen daily transferred to the body pool (tissue or deamination), N$_{daily}$ is the amount of nitrogen consumed daily (480 and 1,600 mg for AP and HP rats, respectively), and N$_{ingest}$ is the nitrogen content of the experimental meal (64 and 240 mg for AP-14 and HP-50 rats, respectively).

The results are expressed as means ± SE. Differences between groups were tested using ANOVA (Proc GLM, SAS version 6.11). P < 0.05 was considered to be significant.
RESULTS

Daily Food Intake, Weight Gain, and Biochemical Characteristics in the Fasted State

Growth and food intake were measured every day for 15 days in rats fed AP or HP diets (Fig. 2). During the first 2 days of the adaptation period, rats in the HP group lost weight (−6.9 ± 0.6 g/day). Thereafter, the weight gain was 5.5 ± 0.2 g/day for the rats in the AP group and 5.2 ± 0.1 g/day for those in the HP group and did not differ significantly. The 15-day cumulative weight gain in rats consuming the 50% protein diet was significantly lower than that seen among rats consuming the 14% protein diet (54.9 ± 2.2 vs. 67.6 ± 2.2, P < 0.001). This was because of diminished food intake during the first 3 days. The food efficiency ratio calculated over the 15-day adaptation period (cumulative weight gain/cumulative food intake) was significantly higher among rats in the AP group than among those in the HP group (0.020 ± 0.0004 and 0.017 ± 0.001 g/kJ, respectively, P < 0.0001).

After the 15-day adaptation period, the weights, protein, and nonprotein nitrogen contents of different tissues (Table 2), as well as fasted plasma urea and AA levels (Table 3), were measured in rats adapted to AP or HP diets. The contribution of proximal and median intestinal mucosa, colon mucosa, liver, and kidneys to body mass was significantly heavier (P < 0.05) in animals given the HP diet for 2 wk compared with those given the AP diet. Similar differences were obtained when the total mass of each organ was taken into account (data not shown). For instance, the liver weight was increased from 9.3 ± 0.2 g in the AP group to 11.2 ± 0.2 g in the HP group. The total amount of protein nitrogen was significantly higher in the proximal and median intestinal mucosa in the HP group. The total nonprotein nitrogen content was higher in the liver (±93 mmol/100 g body wt) and to a greater extent in the muscle of animals in the HP group. The differences between groups were similar whether or not nitrogen was reported to the body weight. Fifteen days of adaptation to the HP diet resulted in striking changes to certain biochemical plasma characteristics. In the fasted state, plasma urea concentrations were more than double among HP rats compared with AP rats, whereas plasma protein concentrations did not differ significantly. Plasma AA concentrations measured in the fasted state were also markedly influenced by the level of protein in the adaptation diet. All AA, except the branched-chain type (BCAA) and Arg, were depressed, and particularly the essential AA His, Met, and Thr and the nonessential AA Gly, Ser, and Gln

Table 2. Organ weights and total protein and nonprotein nitrogen of tissues collected from rats adapted for 2 wk to either a 14% protein or 50% protein diet

<table>
<thead>
<tr>
<th>Adaptation Protein Level</th>
<th>Organ Weight, g/100 g body wt</th>
<th>Total Protein Nitrogen, mmol/100 g body wt</th>
<th>Total Nonprotein Nitrogen, mmol/100 g body wt</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AP</td>
<td>HP</td>
<td>AP</td>
</tr>
<tr>
<td>Liver*</td>
<td>3.52 ± 0.05</td>
<td>3.91 ± 0.04$\ddagger$</td>
<td>6.50 ± 0.14</td>
</tr>
<tr>
<td>Proximal intestine mucosa*</td>
<td>0.43 ± 0.01</td>
<td>1.04 ± 0.13$\ddagger$</td>
<td>0.56 ± 0.01</td>
</tr>
<tr>
<td>Median intestine mucosa*</td>
<td>0.40 ± 0.01</td>
<td>0.52 ± 0.02$\ddagger$</td>
<td>0.39 ± 0.01</td>
</tr>
<tr>
<td>Distal intestine mucosa*</td>
<td>0.32 ± 0.01</td>
<td>0.33 ± 0.01$\ddagger$</td>
<td>0.34 ± 0.01</td>
</tr>
<tr>
<td>Colon mucosa*</td>
<td>0.20 ± 0.01</td>
<td>0.14 ± 0.01$\ddagger$</td>
<td>0.19 ± 0.01</td>
</tr>
<tr>
<td>Body muscles†</td>
<td>45.0</td>
<td>45.0</td>
<td>78.51 ± 2.94†</td>
</tr>
<tr>
<td>Kidneys$\ddagger$</td>
<td>0.32 ± 0.01</td>
<td>0.40 ± 0.01$\ddagger$</td>
<td>0.65 ± 0.02</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 76 rats in the AP group and 36 rats in the HP group. *Total protein nitrogen (mmol) = protein N (mmol/g fresh organ) × organ weight (g). †Total protein (or nonprotein) nitrogen = protein (or nonprotein) N (mmol/g fresh muscle) × body muscle weight (g, body wt × 0.45). $\ddagger$P < 0.05, AP vs. HP.
The most spectacular reduction was found for Met, the concentration of which was nine times lower in the HP than in the AP group (4.2 ± 0.9 vs. 36.1 ± 4.8 μmol/l, respectively). In contrast, concentrations of BCAA were increased by 70% in HP rats.

Nitrogen Kinetics and Distribution in the Tissues After the Ingestion of a 15N-Labeled Protein Meal

After adaptation for 15 days to an AP or an HP diet, the rats received a low (0.42 g) or a high (1.50 g) 15N-labeled protein meal. The digestion kinetics and the postprandial incorporation of dietary nitrogen in the protein and nonprotein fractions of gut mucosa, liver, plasma protein, kidney, and muscles were measured during the first 5 h after ingestion of the meal.

Digestive kinetics. The digestive kinetics responded differently to an acute or chronic increase in protein intake. The gastric emptying rate, expressed as a percentage of nitrogen ingested, was moderately affected by adaptation to the HP diet (AP-50 vs. HP-50 groups; Fig. 3A). When compared with the AP-50 group, a slight delay in emptying rate was observed during the first 2 h in the AP-14 group, but, after 3 h, an inversion was seen, with slower gastric emptying of protein in the AP-50 group. More dietary nitrogen was recovered in the intestinal lumen of rats in the HP-50 group than in the AP-50 group (Fig. 3B). As a consequence, the protein content of the meal (14 or 50%) had an effect on the hourly flux of dietary nitrogen absorption by the small intestine (Fig. 3C). In the control group (AP-14), the maximum flux was delayed compared with that seen in the AP-50 and HP-50 groups (2 vs. 1 h). Moreover, the maximal flux of dietary nitrogen absorption was reduced by the adaptation period, since the peak value was lowered from 52 to 42% of ingested nitrogen in the AP-50 and HP-50 groups, respectively.

Incorporation of dietary nitrogen. The incorporation of dietary nitrogen in the protein and nonprotein frac-
tions of some tissues is shown in Fig. 4. In the gut mucosa, incorporation did not differ between AP-50 and HP-50 rats (Fig. 4A). It increased significantly during the postprandial period and finally reached 93 ± 8 μmol/100 g body wt in the HP-50 group and 104 ± 11 μmol/100 g body wt in the AP-50 group. There was almost no difference in the amount of dietary nitrogen recovered in the nonprotein fraction in the two groups, except at 1 and 5 h where values were slightly but significantly higher in the HP-50 group. No significant differences were found in the colon in either the protein or the nonprotein fractions (data not shown). Protein FSR (%/day) for the mucosa were calculated in the proximal, median, and distal small intestine and in the colon (Table 4). The 15-day adaptation period had no effect (AP-50 vs. HP-50 data), and FSR was highest in the proximal segment and lowest in the colon.

In the liver, the incorporation of dietary nitrogen in both the protein and nonprotein fractions (Fig. 4B) was higher in HP-50 rats during the first 3 h (at 2 h, for instance: protein nitrogen, 179 ± 37 vs. 107 ± 12 μmol/100 g body wt in HP-50 and AP-50 groups, respectively; and nonprotein nitrogen: 46 ± 4 vs. 28 ± 4 μmol/100 g body wt in HP-50 and AP-50 groups, respectively, \( P < 0.05 \)). In muscle, marked differences were observed in the dietary nitrogen kinetics of both protein and nonprotein fractions (Fig. 4C). During the first 4 h after the meal, more dietary nitrogen was recovered in the nonprotein fraction of HP-50 rats than in that of AP-50 rats (at 2 h: 374 ± 37 vs. 187 ± 35 μmol/100 g body wt in HP-50 and AP-50 groups, respectively, \( P, 0.05 \)). In contrast, more dietary nitrogen was incorporated in the muscle protein of AP-50 animals. However, because of the high variability of results in the AP-50 group, the differences were only significant at 3 h (868 ± 152 vs. 306 ± 136 μmol/100 g body wt in AP-50 and HP-50 groups, respectively, \( P < 0.05 \)).

Finally, Table 5 shows the overall distribution of dietary nitrogen in the different samples collected 5 h after the meals in the three groups of rats. Nonnegligible amounts of dietary nitrogen still remained in the gastrointestinal tract (stomach and gut), particularly in the AP-50 group (9.6% of ingested nitrogen). In the other groups, nonabsorbed nitrogen represented 3.5 and 5.0% of ingested nitrogen. More dietary nitrogen was incorporated in plasma proteins when rats were given HP food for the first time (AP-50 group) than after a 15-day adaptation period. Five times more dietary nitrogen was seen in the kidney protein fraction of rats in the HP-50 group.

**Plasma AA Kinetics and Dietary and Total Nitrogen Transfer to Urea**

The maximum variations in plasma AA concentrations, observed after the 50% protein meal, were attenuated after HP adaptation, since the postprandial concentration of total AA increased by 31% in AP-50 rats and by 18% in HP-50 rats (data not shown). Transfers of dietary nitrogen to body and urinary urea differed considerably in rats receiving the HP diet for the first time (AP-50) and after a 15-day adaptation period.

Table 4. *FSR (%/day) of gut mucosa, calculated 1 h after meals in rats adapted or not to an HP or AP diet and ingesting a 50% 15N-labeled protein meal*

<table>
<thead>
<tr>
<th>Gut Mucosa</th>
<th>Proximal intestine</th>
<th>Median intestine</th>
<th>Distal intestine</th>
<th>Colon</th>
</tr>
</thead>
<tbody>
<tr>
<td>AP-50</td>
<td>66.1 ± 4.2</td>
<td>51.9 ± 4.7</td>
<td>23.1 ± 1.9</td>
<td>15.9 ± 6.0</td>
</tr>
<tr>
<td>HP-50</td>
<td>58.6 ± 4.6</td>
<td>40.8 ± 5.2</td>
<td>19.6 ± 2.8</td>
<td>12.9 ± 1.5</td>
</tr>
</tbody>
</table>

Values are means ± SE; *n* = 36 animals in the HP group and 40 animals in the AP group. Values did not differ statistically between groups (\( P < 0.05 \)).
In the AP-50 group, the transfer of dietary nitrogen to body urea regularly increased to reach a maximum of $0.571 \pm 0.108 \text{ mmol/100 g body wt}$ at $5 \text{ h}$, whereas a peak of $0.647 \pm 0.127 \text{ mmol/100 g body wt}$ was observed among HP-50 rats (Fig. 5A). Dietary nitrogen excretion in urinary urea (Fig. 5B) was greatly enhanced by adaptation to the HP diet; for instance, $5 \text{ h}$ after the meals the amount of dietary nitrogen recovered in urinary urea reached $1.679 \pm 0.266 \text{ mmol/100 g body wt}$ in HP-50 rats and only $0.723 \pm 0.121 \text{ mmol/100 g body wt}$ in rats belonging to the AP-50 group ($P < 0.001$). This amount reached only $0.089 \pm 0.017 \text{ mmol/100 g body wt}$ in the AP-14 group.

The kinetics also differed, since a plateau was achieved in the HP-50 group after $3 \text{ h}$, whereas a sustained rise was observed in the AP-50 group.

Cumulative total and dietary nitrogen transfer to urinary plus body urea, i.e., total deamination, was evaluated in the three groups during the postprandial period (Fig. 6). The production of total urea over the entire study period was greatly enhanced in the HP-50 group compared with the AP-50 group ($6.02 \pm 0.99 \text{ vs. } 3.36 \pm 0.36 \text{ mmol N/100 g body wt}$ in the HP-50 and AP-50 groups, respectively, $5 \text{ h}$ after the meal). In these two groups, dietary nitrogen represented an important part of the total nitrogen transferred to urea (between 25 and 40% in the HP-50 group and 19 and 42% in the AP-50 group), although it never represented $>23\%$ in the AP-14 group. Finally, $5 \text{ h}$ after the meal, the total deamination, calculated as the sum of dietary nitrogen transferred to urinary and body urea, was $1.7$ times higher in the HP-50 group than in the AP-50 group ($2.19 \pm 0.48 \text{ vs. } 1.29 \pm 0.22 \text{ mmol/100 g body wt}$, respectively, $P < 0.001$) and only reached $0.13 \pm 0.03 \text{ mmol/100 g body wt}$ in the AP-14 group.

**DISCUSSION**

The purpose of this work was to evaluate the adaptation of nitrogen and AA metabolism to an HP diet (50% protein) in rats. Our findings pointed out that the...
The principal adaptive regulation pathways responding to an increase of the habitual protein content of the diet were seen in the splanchnic tissues and subsequently in the kidneys, with dramatic stimulation of both AA deamination and urea production. The liver is known to play a central role in postprandial dietary AA metabolism, since it modulates the inflow of AA from the intestine and controls AA supply to peripheral tissues. One of the most remarkable changes induced by adaptation to a HP diet is an increase in AA oxidation, especially in the fed state (15). Although we did not specifically study AA oxidation, the production of urea that represents 80–85% of the nitrogen excreted in urine is highly correlated with AA catabolism. We observed a net increase in plasma uremia, which almost doubled when the HP group was compared with the AP group, and in total urea excretion over the experimental period. Similarly, the surplus nitrogen provided by dietary AA was mainly directed toward deamination when animals were adapted to a HP intake. This shows the stimulation of hepatic AA-metabolizing enzymes such as alanine and aspartate aminotransferases or glutamate dehydrogenase that was demonstrated in rats adapted to a 51% protein diet (8). Rémy et al. (34) reported an increase in mitochondrial glutamine hydrolysis in hepatocytes isolated from animals fed a 70% casein diet, this being an important step in urea production. However, because the gut mucosa metabolizes >90% of the luminal Glu and Gln (33a, 43a), the liver is likely to be less important in the catabolism of those AA. The lower baseline concentration of Gln in animals fed the HP diet could also be explained by its high level of utilization in kidneys, since this AA is the major precursor of urinary ammonia (5), and by a diminution in de novo synthesis from BCAA in muscle (25). This probably also leads to a decrease of plasma Ala (45). As a result, with the remarkable exception of BCAA and Arg, plasma concentrations of numerous AA were diminished in the rats of the HP group, whereas the protein intake of those animals was almost three times that of animals in the AP group. The increase in Arg concentration must reflect the intestinal and hepatic activation of the urea cycle, and it has clearly been shown that the expression of branched-chain aminotransferase was not regulated in the liver but rather in the kidneys, muscle, and heart (43). All of these adaptive processes result in the remarkable stability of plasma AA concentrations in the postprandial state.

Is the Muscle Nitrogen Metabolism Regulated?

Our study showed that the dietary nitrogen content in the nonprotein fraction of muscle was considerably increased, whereas less dietary nitrogen (even though it was not significant) was incorporated in the protein pool. Several studies have supported the idea that HP consumption does not enhance muscle protein synthesis (2, 23, 40). For instance, Masanes et al. (24) recently reported a decreased FSR in the muscles of Zucker rats fed a 36% protein diet vs. rats receiving a 9% protein diet. This observation agrees with the assumption made by Abumrad et al. (1), who suggested that, when skeletal muscle is exposed to excessive amounts of BCAA, most of what is incorporated is not used for protein synthesis but sequestrated in a free AA pool, which may be mobilized later. If the apparent increased availability of AA in the intracellular pool is in fact mainly the result of an in-
crease in BCAA, as indicated by the plasma AA concentrations, this suggests that muscle weakly reacts to modifications of the AA pattern, since the oxidation of BCAA in the muscle does not seem to be enhanced. Because the availability of other AA is depressed, protein synthesis must be slowed through the paradoxical reduction in substrate availability. We should note the fact that, in our study, the isocaloric exchange between diets was performed on carbohydrates. It can be argued that the daily insulin secretion in HP rats was lower than that of AP rats, as supported by the work of Takahashi et al. (41). However, although insulin is known to exert an anabolic effect on protein, several authors have suggested that this effect is stronger in splanchnic proteins, especially albumin, than in peripheral proteins (10, 42). It also can be argued that, because an HP-diet consumption results in an energy loss through enhanced thermogenesis (16) and because protein synthesis is energy demanding, muscle protein synthesis is diminished when protein is isocalorically exchanged with carbohydrates. Very few data are available to support or refute this hypothesis because, in rat studies, isocaloric exchanges are mostly performed on carbohydrates because of the low amount of fat in the standard diets. However, Tail-landier et al. (40), who made an exchange between fat and protein, also reported a diminished FSR in the tibialis. Moreover, the limited energy availability of HP diets resulting from the cost of AA absorption and deamination and protein synthesis is highly controversial (4, 13, 17). In our view, muscle has a very low capacity of adaptation to a modification of the peripheral AA pattern. Because the surplus BCAA is not reduced by activated oxidation, they accumulate in the free pool and are proportionally less incorporated into protein. This would also be favored by low insulin secretion.

Minor Regulatory Pathways

Intestinal regulation. One way in which animals adapt to dietary change is by regulating the rates of intestinal nutrient absorption. Our study demonstrates that adaptation to a HP diet occurred also at the digestive level by changing the kinetics of nitrogen absorption kinetics. In fact, after 2 wk of adaptation, gastric evacuation was slightly delayed during the first 2 h after ingestion when compared with the rate in nonadapted rats receiving a HP meal. This result contradicts that of Shi et al. (37), but in this study the experimental meal...
consisted of soluble peptides, and the results were based on follow-up of the liquid phase. We also observed that dietary nitrogen remained longer in the lumen of rats adapted to the HP diet but not when HP food was presented for the first time, a finding consistent with an early observation by Peraino et al. (33). It has also been shown that intestinal enzymes and peptide and AA transporters adapt to diets containing different levels of protein (12, 20); this result is surprising and contradicts that of Karasov et al. (21). However, HP rations have been reported to modify different AA transport systems to varying degrees (12, 21, 22, 44).

Although luminal events appear to be involved, at least slightly, in adaptation to HP diets, the intestinal mucosa is not affected by a chronic increase in protein intake. Indeed, incorporation of the tracer into mucus protein was very similar whether the HP diet was given for the first time or after 2 wk of adaptation. The FSR can be calculated assuming that luminal AA were the direct precursors of protein synthesis in the mucus, an assumption that is sustained in the light of recent reports in the literature (3, 39). We reported FSR values ranging from 38 to 66%/day in the proximal intestine and from 10 to 23%/day in the distal portion; these values are consistent with those published elsewhere (30, 31). The mucosal FSR is insensitive to chronic consumption of a HP diet, thus confirming the results published by Masanes et al. (24), who showed that the 36% protein diet had no effect on the FSR in the small intestine.

**Hepatic anabolism.** In the liver, the stimulation of dietary nitrogen incorporation in constitutive hepatic protein was higher in the HP-50 group than in the AP-50 group during the first 3 h of the postprandial period, as if the protein synthesis system was “ready” to respond to the large influx of dietary AA after the meal. This can result from both a stimulated synthesis rate, as shown by several authors (11, 19), and an increased amount of available dietary nitrogen for synthesis. Indeed, the amount of dietary nitrogen recovered in the hepatic nonprotein fraction was found to be higher. Last, our study throws new light on the possible role of plasma protein, especially albumin, in peripheral AA homeostasis. Indeed, we were surprised to observe a higher level of dietary nitrogen incorporation in the plasma protein when rats were given the HP meal for the first time than when they had chronically ingested an HP diet. Plasma protein could thus be interpreted as a pool capable of reacting quickly to an AA surplus when the catabolic capacities of the liver are overloaded, as was the case in the AP-50 group.

**Regulation of Dietary Nitrogen Metabolism: A Synthesis**

Because our study allowed us to determine the immediate distribution of dietary nitrogen after a single meal, we intended to extrapolate our results to identify the daily regulations of dietary and endogenous nitrogen metabolism when the chronic ingestion of protein is increased three times. For this purpose, the single 15N-labeled meal was considered to be representative of the daily protein ingestion; the protein ingested with the experimental meal is 13.7 and 12.3% of the daily protein ingestion in the HP and the AP group, respectively. Figure 7 shows how dietary nitrogen is distributed through the different compartments. The most spectacular increase was that of dietary AA nitrogen transferred to urea, which was enhanced 11-fold, whereas the transfer of endogenous nitrogen to urea was only increased by 2.7-fold. An interesting finding of this work was that the splanchnic zone tended to maintain the transfer of dietary AA to the periphery at a constant level. As a direct consequence, the daily incorporation of dietary nitrogen in the protein fraction of muscles was almost identical whether the rats ingested 3 or 10 g of protein/day. Although it increased nitrogen intake threefold, splanchnic protein incorporated two times as much dietary nitrogen resulting principally from the liver (2 times), whereas muscles incorporated the same amount whether rats ingested either 3 or 10 g of protein/day.

In conclusion, our study, although confirming the predominant role of liver in the adaptation processes to a HP diet, throws new light on the acute and midterm mechanisms that ensure a relatively constant amount of dietary nitrogen reaching the periphery. The enhancement of catabolic and (to a lesser extent) anabolic mechanisms in the liver induces a reduction of the availability of all peripheral plasma AA except BCAA and Arg, which results in the absence of hyperaminoacidaemia during the postprandial phase. Our study suggests that a habitual HP intake could both dramatically increase the nonprotein nitrogen pool and, in the context of a diluting effect, reduce the incorporation of dietary nitrogen in muscle protein.

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**REFERENCES**


