Postprandial metabolism and aversive response in rats fed a threonine-devoid diet

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1Institut National de la Recherche Agronomique de Paris Arignon, Laboratoire de Nutrition Humaine et Physiologie Intestinale, 75231 Paris Cedex 05; and 2Centre Européen des Sciences du Goût, Centre National de la Recherche Scientifique, Unité Propre de Recherche 9054, 21000 Dijon, France
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Even, P. C., V. Rolland, S. Feurté, G. Fromentin, S. Roseau, S. Nicolaidis, and D. Tomé. Postprandial metabolism and aversive response in rats fed a threonine-devoid diet. Am J Physiol Regulatory Integrative Comp Physiol 279: R248–R254, 2000.—Lack of an indispensable amino acid in the diet induces a rapid reduction in food intake. In this study, we assessed whether the anorectic signal after ingestion of a meal lacking threonine originated from either direct perception of the decrease in plasma threonine or from an indirect effect related to increased postprandial amino acid catabolism and energy expenditure. We observed that 3 g of such a meal was sufficient to induce an aversive response to the diet within 2 h. Postprandial changes to plasma ammonia and urea, urinary urea, and energy metabolism did not differ from those measured after a control meal. In contrast, plasma threonine levels fell within 1 h after the meal. It is concluded that an increase in postprandial energy expenditure is not involved in the anorectic response to eating a threonine-devoid diet. The drop in plasma threonine levels may be a potential signal, but the fact that the decrease in food intake occurred 1 h after the decrease in plasma threonine questions a direct causal relationship.

energy expenditure; urea; ammonia; dietary proteins

Lack of an indispensable amino acid in the diet induces a decrease in food intake that leads to impaired body weight gain and growth, and in the long term, may result in death (13, 14, 26). In animals, however, physiological mechanisms exist to prevent repeated ingestion of an amino-acid-deficient diet, the most obvious being the rapid detection of the amino acid deficiency. This induces the acquisition of an aversion for the deficient food and, by opposition, a neophilia for any novel food (9, 17).

An important characteristic of the behavioral response to the lack of an essential amino acid is that the delay of the establishment of the aversive response depends on the nature of the limiting amino acid. However, the behavioral response may be much more subtle than the acceptance or rejection of food. Indeed, the sensitivity and precision of the mechanisms of recognition of the amino acid composition of a meal is such that rats are able to adjust the relative intake of two foods or drinks lacking different essential amino acids to equilibrate their amino acid intake (10, 16, 25, 28).

Whatever its sensitivity and efficacy, the initial signal that is responsible for the recognition by specific brain structures of the amino acid deficiency and that is effective at decreasing food intake in the short term remains unclear. The most often quoted hypothesis is a direct brain detection of the decrease in the plasma and brain level of the limiting amino acid that usually follows the ingestion of a meal lacking this amino acid (11, 12, 14). An alternative mechanism could proceed through alterations in postprandial protein turnover, but the ingestion for the first time of a meal deficient in an indispensable amino acid usually failed to induce significant changes in postprandial protein synthesis (15, 29). Another hypothesis is that alterations in the postprandial catabolism of amino acids may generate signals sensed in the brain to stop food intake. These signals may also be endotoxic signals through production of large amounts of ammonia (24; reviewed in Ref. 17) or physiological satiety signals produced through an increased postprandial amino acid oxidation, generating an aminostatic (21), an energostatic (3), or an ischymetric signal (22).

The objective of the present study was to assess postprandial changes in some metabolic parameters potentially modified by the ingestion of a threonine-devoid (Thr-dev) diet. In the first experiment, the behavioral response of rats was analyzed to define the amount of the Thr-dev diet sufficient to induce an aversive response. In a second study, this amount was used as a calibrated stimulus to measure postprandial changes in plasma threonine, urea and ammonia, protein oxidation (Pox), and energy expenditure. The results showed that 3 g of a Thr-dev diet were sufficient...
to induce both an aversion for the diet and a fall in plasma threonine levels, whereas it did not induce any significant activation of amino acid catabolism and energy expenditure.

MATERIALS AND METHODS

Diet. During the adaptation or prefeeding periods, rats were fed a standard diet for laboratory rats (Powdered Extralabo M25, Ets Pietrement, France, 13.4 kJ/g; by weight: 24% protein, 48.1% carbohydrate, 5% fat, 3.3% cellulose, 7.4% minerals and vitamins, and 12% moisture). Three other diets were used depending on the experimental conditions (9): (1) a protein-free diet (PO); (2) a diet in which the amino acids were brought under the form of a balanced mixture of free amino acids (Thr-dev); (3) a diet in which the essential amino acid threonine was removed and replaced with an equivalent amount of glucose and starch (Thr-dev). All diets were supplemented with cellulose (20 k/kg), vitamins (10 g/kg), and minerals (17.1; potassium phosphate, 10.8; calcium carbonate, 8.1; magnesium carbonate, 6; L-cystine, 12; L-histidine, 15; L-leucine, 15; L-lysine, 15; L-isoleucine, 15; L-lysine, 15; L-threonine, 6; L-isoleucine, 15; L-leucine, 21; L-phenylalanine, 15.5; L-tryptophan, 4; L-valine, 16; and L-tyrosine, 9.5; total, 124; Thr-cor provided L-methionine, 10; L-cystine, 6; L-histidine, 12; L-lysine, 15; L-threonine, 6; L-isoleucine, 15; L-leucine, 21; L-phenylalanine, 15.5; L-tryptophan, 4; L-valine, 16; and L-tyrosine, 9.5; total, 130 (Degussa, Ridgefield Park, NJ). The Thr-dev diet provided L-methionine, 10; L-cystine, 6; L-histidine, 12; L-lysine, 15; L-isoleucine, 15; L-leucine, 21; L-phenylalanine, 15.5; L-tryptophan, 4; L-valine, 16; and L-tyrosine, 9.5; total, 124 (Degussa, Ridgefield Park, NJ). The Thr-dev diet provided L-methionine, 10; L-cystine, 6; L-histidine, 12; L-lysine, 15; L-isoleucine, 15; L-leucine, 21; L-phenylalanine, 15.5; L-tryptophan, 4; L-valine, 16; and L-tyrosine, 9.5; total, 124 (Degussa, Ridgefield Park, NJ). The Thr-dev diet provided L-methionine, 10; L-cystine, 6; L-histidine, 12; L-lysine, 15; L-isoleucine, 15; L-leucine, 21; L-phenylalanine, 15.5; L-tryptophan, 4; L-valine, 16; and L-tyrosine, 9.5; total, 124 (Degussa, Ridgefield Park, NJ). The Thr-dev diet provided L-methionine, 10; L-cystine, 6; L-histidine, 12; L-lysine, 15; L-isoleucine, 15; L-leucine, 21; L-phenylalanine, 15.5; L-tryptophan, 4; L-valine, 16; and L-tyrosine, 9.5; total, 124 (Degussa, Ridgefield Park, NJ). The Thr-dev diet provided L-methionine, 10; L-cystine, 6; L-histidine, 12; L-lysine, 15; L-isoleucine, 15; L-leucine, 21; L-phenylalanine, 15.5; L-tryptophan, 4; L-valine, 16; and L-tyrosine, 9.5; total, 124 (Degussa, Ridgefield Park, NJ). The Thr-dev diet provided L-methionine, 10; L-cystine, 6; L-histidine, 12; L-lysine, 15; L-isoleucine, 15; L-leucine, 21; L-phenylalanine, 15.5; L-tryptophan, 4; L-valine, 16; and L-tyrosine, 9.5; total, 124 (Degussa, Ridgefield Park, NJ). The Thr-dev diet provided L-methionine, 10; L-cystine, 6; L-histidine, 12; L-lysine, 15; L-isoleucine, 15; L-leucine, 21; L-phenylalanine, 15.5; L-tryptophan, 4; L-valine, 16; and L-tyrosine, 9.5; total, 124 (Degussa, Ridgefield Park, NJ).

Table 1. Composition of the diets used in the experiments

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>PO</th>
<th>Thr-Dev</th>
<th>Thr-Cor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dispensable AAa</td>
<td>77.0</td>
<td>77.0</td>
<td>77.0</td>
</tr>
<tr>
<td>Indispensable AAa</td>
<td>124.0</td>
<td>130.0</td>
<td>130.0</td>
</tr>
<tr>
<td>Salt mixture</td>
<td>45.0</td>
<td>45.0</td>
<td>45.0</td>
</tr>
<tr>
<td>Vitamin mixtureb</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>African peanut oilc</td>
<td>20.0</td>
<td>20.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Rapeseed oild</td>
<td>30.0</td>
<td>30.0</td>
<td>30.0</td>
</tr>
<tr>
<td>Cellulosef</td>
<td>20.0</td>
<td>20.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Cornstarchg</td>
<td>437.5</td>
<td>337.0</td>
<td>334.0</td>
</tr>
<tr>
<td>Glucoseh</td>
<td>437.5</td>
<td>337.0</td>
<td>334.0</td>
</tr>
<tr>
<td>Total</td>
<td>1,000</td>
<td>1,000</td>
<td>1,000</td>
</tr>
</tbody>
</table>

All values are in g/kg diet. PO, protein-free diet; AA, amino acid; Thr-dev, threonine-dev void diet; Thr-cor, threonine-corrected diet.
aProvided glutamic acid, 30; bL-glutamic acid, 10; cL-arginine, 10; dL-alanine, 3.5; eL-asparagine, 10; fL-proline, 10; gL-serine, 3.5; total, 77 (Degussa, Ridgefield Park, NJ). hL-proline, 10; L-glutamic acid, 10; L-arginine, 10; L-alanine, 3.5; L-asparagine, 10; L-proline, 10; L-serine, 3.5; total, 77 (Degussa, Ridgefield Park, NJ). iL-glutamic acid, 10; L-arginine, 10; L-alanine, 3.5; L-asparagine, 10; L-proline, 10; L-serine, 3.5; total, 77 (Degussa, Ridgefield Park, NJ).

Animals and surgery. All the experiments were done according to the guidelines of the French Committee for Animal Care. Adult male Wistar rats (Ifsa-Credo, France) weighing 200-250 g at the start of the experiment and individually housed in stainless steel wire cages were used after 10 days of adaptation to the laboratory conditions (12:12-h light-dark cycle; lights on at 0600; temperature 22 ± 2°C). In some experiments, blood samples or intravenous infusions were done on conscious, unrestrained animals. For these experiments, the rats were surgically implanted with a soft catheter (Silastic 105 from Dow Corning) chronically fitted in the right jugular vein according to a technique previously described (23, 27) and allowed at least 1 wk to recover.

Blood collection and blood samples analysis. During the experiments, the intracardiac catheter was connected to a syringe by a Silastic tubing suspended on a balanced gallow tree at the top of the cage (23). Blood samples (400 µl) were collected in heparinized tubes and were immediately centrifuged (4°C, 3,000 rpm, 10 min). Plasma was separated and stored at −20°C until analysis. Urea (5 µl/assay) and ammonia (100 µl/assay) in the plasma were assayed by spectrophotometry (kit 66-UV and 171-UV; Sigma Diagnostics, Saint Quentin Fallavier, France). For threonine determination, plasma (200 µl/assay) was deproteinized by adding 10 mg of S-sulfosalicylic acid (4°C, 60 min) and centrifuged (4°C, 3,000 rpm, 10 min), and the supernatant was analyzed with the use of an amino acid analyzer (Pharmacia LKB Alpha Plus, Cambridge, UK).

Measurements of glucose, lipid, and protein oxidation. Glucose (Gox) and lipid (Lox) oxidation and Pox were computed from measurement of respiratory exchanges and urea production in a metabolic cage designed to allow for the utilization of the techniques of blood samplings/perfusions on free-moving rats (for details on this device, see Ref. 4). Oxygen consumption (Vo2) and carbon dioxide production (VCO2) (using differential gas analyzers), spontaneous activity (using piezo-electric strain gauges located beneath the cage), and food intake (using a microscale weighing the food cup) were recorded at 10-s intervals with a computer-assisted program of data acquisition. During the experiment, the rats received a continuous infusion of hypotonic saline (0.45%) at a rate of 5 ml/h to increase urine production and to allow collection of regular samples at 30-min intervals. Urine was collected on 10 µl of 5N HCl in a fraction collector located under the calorimetric device. Urea in urine was assayed with the use of a commercial kit (Sigma Medical kit 171-UV).

A computer-assisted modeling of the changes induced on total Vo2 and VCO2 by the occurrence of bursts of spontaneous activity was used to compute the oxygen consumed (Vo2-act) and the carbon dioxide released (VCO2-act), specifically in relation to activity. Subtraction of Vo2-act and VCO2-act from total Vo2 and VCO2 gave the values of resting Vo2 (Vo2-rest) and VCO2 (VCO2-rest) throughout the time (4, 6). Computation of Pox from urinary nitrogen excretion was done with the use of a standard conversion factor of 6.25 g protein oxidized/g nitrogen excreted. Pox was assumed to be unaffected by spontaneous activity. Because Pox values were obtained at 30-min intervals, whereas respiratory exchanges were recorded at 10-s intervals, Pox values at 10-s intervals were computed by linear extrapolation between two measured Pox values. Meal-induced changes in Gox, Lox, and Pox rates and in energy metabolism were computed from Vo2-rest, VCO2-rest, and urinary nitrogen excretion according to standard stoichiometric formulas (4).

Statistics. Results are shown as means ± SE. Statistical significances were determined by ANOVA with the use of the statistical package Statgraf (Statistical Graphic), introducing diets, days, and groups as factors. Statistical significance was
set at $P < 0.05$. When differences were detected by ANOVA, differences between individual means were determined with the use of the signed-rank test for unpaired samples.

RESULTS

Anorectic response to unrestricted access to Thr-dev diet. Two groups of 10 rats were given unrestricted access to the standard diet from 1730 to 0930 the next day. This slight temporal limitation had the advantage of inducing a regular daily pattern of food intake starting at 1730 and thus favoring comparison of food intake and feeding pattern from 1 day to another. Adaptation to this scheduled feeding was done during 8 days. On the 9th day, the standard diet was replaced with the Thr-cor diet in one group and with the Thr-dev diet in the other group (Fig. 1).

At 90 min after the onset of feeding, the same amount [50 kJ ($\sim 3$ g)] of food was ingested in both groups. Thereafter, ingestion of the Thr-dev diet decreased so that at 150 min, energy intake was significantly reduced in the rats fed the Thr-dev diet. At this time, 80 kJ (5 g) of the Thr-cor diet and 58 kJ (3.7 g) of the Thr-dev diet had been ingested. Thereafter, energy intake continued to be smaller in the Thr-dev fed rats and finally leveled at 112 kJ. At 0930 the next day, energy intake was reduced by as much as 63% in the rats fed the Thr-dev diet.

Conditioned taste aversion response induced by a 3-g Thr-dev meal. This experiment was done as a control so that, according to the results of the above study, a 3-g meal could be used as a calibrated stimulus to study the metabolic signals underlying aversion (Table 2).

Forty-eight rats were divided into four groups of 12 rats. Three groups had free access to the standard diet, and one group had free access to the Thr-dev diet from 1730 to 0930 the next day. In addition, a 3-g meal of the usual diet was made available daily between 1430 and 1500. This additional meal was offered daily to habituate the rats to ingesting a 3-g meal at this time. The purpose of this additional meal was to get the rats used to ingesting a 3-g meal at this time of the day and thus to have 2.5 to 3 h to analyze the postingestive consequences of this meal before the food cups were returned to the cages. Such a delay is known well to be more than sufficient for the rats to learn the postingestive consequences of a meal.

On the 9th day, one of the groups fed the standard diet received the standard meal given at 1430 as usual (control/control group), whereas the second group received a Thr-cor meal (control/thr$^-$ group), and the third group received a Thr-dev meal (control/thr$^+$ group). The fourth group, maintained on the Thr-dev diet, received as usual a Thr-dev meal (Thr$^-$/Thr$^-$ group). At 1730, instead of their usual diet, all the rats were offered a choice between a P0 and a Thr-cor diet, and the intake of each diet was measured the morning after.

During the adaptation period, the group fed the Thr-dev diet had a depressed energy intake ($P < 10^{-3}$) due to the lack of threonine in the maintenance diet (9, 18). When offered the choice between the P0 and the Thr-cor diet, these rats rejected the Thr-cor diet and showed a clear preference for the inadequate P0 diet ($P < 10^{-3}$). In the rats fed the standard diet, ingestion of the 3-g test meal of either the standard, Thr-dev, or Thr-cor diet was complete. Thereafter, the rats that ingested the standard test meal or the Thr-cor test meal did not eat different amounts of the Thr-cor or the P0 diet. By opposition, the rats that ingested the Thr-dev test meal showed a clear preference for the P0 diet ($P < 0.01$). This result indicated that the 3-g test meal of the Thr-dev diet had induced a taste aversion.

Postprandial plasma threonine, urea, and ammonia could be the mechanisms responsible for the taste aversion.

Twenty-six rats implanted with a venous canula were given unrestricted access to the standard diet.

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Table 2. Energy intake in rats given a choice between a protein-free diet and a threonine-corrected diet

<table>
<thead>
<tr>
<th>Test day</th>
<th>Standard/Standard</th>
<th>Standard/thr$^+$</th>
<th>Standard/thr$^-$</th>
<th>Thr$^-$/Thr$^-$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prefeeding</td>
<td>397.7 ± 9.9*</td>
<td>416.7 ± 8.3*</td>
<td>407.9 ± 11.0*</td>
<td>167.7 ± 9.3t</td>
</tr>
<tr>
<td>Test day with P0</td>
<td>193.8 ± 18.5*</td>
<td>187.0 ± 20.3*</td>
<td>261.5 ± 12.5*</td>
<td>267.1 ± 17.9t</td>
</tr>
<tr>
<td>Test day with Thr-cor</td>
<td>217.4 ± 22.2*</td>
<td>234.8 ± 22.2*</td>
<td>53.3 ± 11.9t</td>
<td>51.7 ± 14.1†</td>
</tr>
</tbody>
</table>

Values are means ± SE for energy intake in kJ/day, Thr$^-$, diet corrected for the threonine deficiency; Thr$^+$, diet devoid of threonine; Standard/Standard, prefeeding with standard diet and test meal of standard diet; Standard/thr$^+$, prefeeding with standard diet and test meal of Thr$^+$; Standard/thr$^-$, prefeeding with standard diet and test meal of Thr$^-$; Thr$^-$/Thr$^-$, prefeeding with Thr$^-$ and test meal of Thr$^-$

*Values in the same line with the same superscript are not significantly different.

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Fig. 1. Cumulative energy intake ($\pm$ SE) after unrestricted access to a threonine-devoid (Thr-dev) or a threonine-corrected (Thr-cor) diet for the first time. Precise measurement of food intake was carried out with the use of computer recording of the weight of the food cups (sensitivity ± 0.1 g) at 1-min intervals.
from 1730 to 0930 the next day during at least 8 days. On the experimental day, instead of the usual standard diet, they were given a 3-g meal of either the Thr-dev \( n = 13 \) or the Thr-cor \( n = 13 \) diet. Blood samples were taken just before and then at intervals after ingestion of the meals for assay of plasma threonine, urea, and ammonia (Fig. 2).

Plasma threonine was not significantly affected by ingestion of the Thr-cor meal. In contrast, the Thr-dev meal induced a decrease in plasma threonine that reached significance within 30 min \( (P < 0.05) \). Thereafter, plasma threonine levels continued to decrease rapidly. Three hours after the meal, plasma threonine level in the rats that ingested the Thr-dev meal was only 30% of the threonine level in the rats that ingested the Thr-cor meal. Plasma urea increased after ingestion of both test meals, and no differences were observed between the Thr-cor and the Thr-dev meals, though plasma urea showed a tendency to be higher after the Thr-dev meal in the 2–4 h after meal ingestion. Contrary to urea, plasma ammonia did not increase after the meals and remained <50 \( \mu \text{M} \) in all the samples. No differences were observed in plasma ammonia between the rats fed the Thr-cor or the Thr-dev meals.

Postprandial responses in the rates of substrate oxidation and energy expenditure to a 3-g Thr-dev meal. The postprandial energy expenditure in response to the 3-g test meal of the Thr-dev diet was also evaluated as a possible mechanism responsible for the taste aversion.

Twelve rats were prepared as described in the above study. On the experimental day, they were housed in the calorimeter at 1100. Recording of respiratory exchanges and collection of urine were started immediately as described in the section Measurements of glucose, lipid, and protein oxidation and were continued without interruption until 0900 the next day. At 1730, a 3-g test meal of the Thr-dev \( (n = 6) \) or the Thr-cor diet \( (n = 6) \) was introduced in the food cup of the metabolic cage. Changes on Gox, Lox, and Pox induced by the meals were computed as described in MATERIALS AND METHODS.

Continuous weighing of the food cup in the metabolic cage showed that all the food was ingested within 90–120 min in all rats for both the Thr-cor and the Thr-dev diet; i.e., following a pattern close to the one observed in the behavioral study. Changes in Pox assessed from urinary nitrogen excretion showed that Pox was increased in both groups between 3 and 5 h after feeding (Fig. 3). No significant differences were observed after feeding the Thr-cor or the Thr-dev meals, although Pox was always higher after the Thr-dev meal after 2 h. Changes in Gox and Lox were also similar after the Thr-cor or the Thr-dev meals. Meal-induced changes in energy expenditure (the thermic effect of the meal) were comparable between Thr-cor and Thr-dev rats (Fig. 4). The amount of extra energy expended over baseline (3.7 \( \text{kJ} \) ) measured at 6 h after the meal accounted for 6.7% of the ingested calories.

![Fig. 2. Changes in plasma ammonia, urea, and threonine after ingestion for the first time of 3 g of a Thr-dev or Thr-cor meal in rats. Blood samples (400 µl) were taken in 26 unrestrained rats implanted with a chronic venous canula. * \( P < 0.05 \) between the Thr-Cor and the Thr-Dev rats. Nos. in parentheses, no. of subjects.](http://ajpregu.physiology.org/)

![Fig. 3. Cumulative protein oxidation (Pox) measured from 2 h before until 10 h after ingestion of the 3-g Thr-cor and Thr-dev test meals given in the metabolic cage. The line without symbols shows the linear extrapolation of the rate of urea production measured during the 2 h that preceded the meal.](http://ajpregu.physiology.org/)

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**THREONINE-DEVOID DIET FOOD INTAKE AND METABOLISM**

R251

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R251
Nine-deficient diet induced a reduction in food intake as the physiological signal controlling food intake. The relevance of the changes in the plasma amino acid concentration to amino acid metabolism, but rather followed a decrease in plasma threonine rather than to less specific signals more generally related to whole body energy metabolism. However, the temporal relationship between changes in plasma threonine and changes in feeding is rather weak, which may question the relevance of the changes in the plasma amino acid pool as the physiological signal controlling food intake.

In the first experiment, we observed that the threonine-deficient diet induced a reduction in food intake that was well marked within 90 min and reached significance at 150 min of unrestricted access to the food. At the time when the difference in food intake appeared, 3 g of the Thr-dev diet had been ingested, which suggested that this amount was sufficient to induce a taste aversion. It was the goal of the second experiment to verify this point. It is well known that contrary to well-fed rats that present some form of careful neophobia for a novel food, amino-acid-deficient rats exhibit a strong avoidance for the deficient diet and a neophilia for any novel food, even if this novel food is also an amino-acid-deficient or a P0 diet. This specific behavior is a confident tool to reveal that rats have developed an aversion for a given food. Our experiments clearly showed that one single 3-g meal of a Thr-dev diet was sufficient to inhibit food intake, i.e., to produce the signal(s) responsible for the recognition of the threonine deficiency by specific brain structures. Such results confirmed previous studies (2). It was also remarkable to observe that the rats fed only one single 3-g meal of the Thr-dev diet showed an aversion for the diet as strong as the aversion exhibited by the rats fed the Thr-dev diet during several days.

An important result was the observation that hypothetical postprandial signals, i.e., increased circulating plasma ammonia, were certainly not involved in the decrease in food intake induced by the ingestion of a Thr-dev meal because plasma ammonia remained well below 50 µM, i.e., below values that may be considered as neurotoxic (20). Indeed, one potential mechanism by which ingestion of the 3-g Thr-dev test meal can have decreased food intake is an increase in amino acid catabolism due to an excess in other free essential amino acids coming from the diet and not used for protein synthesis because of the absence of the indispensable amino acid threonine. The consequence of such an increased catabolism would be an overproduction in urea and possibly ammonia when urea production is saturated (24). In fact, plasma urea levels and urinary urea output increased slightly but not sufficiently to reach significance. This observation indicates that decreased food intake occurred, whereas postprandial amino acid oxidation was probably slightly stimulated, but to an extent not likely to significantly affect postprandial energy metabolism or, as demonstrated by the stability of plasma ammonia levels, to saturate the processes involved in the deamination of the amino acids. These results agree with previous results (24; reviewed in Ref. 17) and confirm and extend them if we consider that in the present study, measurements were done also before food intake started to decrease.

Another hypothesis that was challenged in this study was that the decrease in food intake that follows ingestion of a meal lacking an essential amino acid could be supported by physiological satiety signals generated by an increase in the postprandial catabolism of the ingested nutrients, amino acids, but also carbohydrates and lipids. In this context, it has long been demonstrated that the satiating effect of nutrients is strongly dependent on their oxidation and the energy released from this oxidation rather than on their blood
concentration (5, 19). Therefore, in the ischymetric (22), energostatic (3), or metabolic (8) hypotheses, the energy released from the postprandial oxidation of the ingested nutrients is assumed to participate in the control of food intake by generating satiety signals that inhibit food intake as long as substrates are provided by the intestinal tractus in amounts sufficient to fuel ATP production and to maintain basal metabolism at a high level. To test the hypothesis that the aversion generated by the 3-g Thr-dev test meal was sustained by an energy-based satiety signal, we combined the utilization of sequential collection of urinary nitrogen excretion and the measurement of VO2 and VCO2 on free-moving, free-feeding rats. These measurements clearly demonstrated that neither Pox, Gox, or Lox rates, nor overall postprandial energy expenditure were significantly modified by the lack of threonine in the food. As already suggested by the similar changes in plasma urea concentrations, Pox estimated from urinary urea excretion of saline-infused rats showed a tendency to increase, but in no way was this slight increase sufficient to affect significantly overall energy expenditure or the parallel oxidation of glucose and lipids. As a result, physiological satiety signals as those proposed in the aminostatic, energostatic, ischymetric, and more generally metabolite control of feeding are probably not involved in the short-term recognition of the threonine deficiency.

In conclusion, the utilization of a calibrated 3-g Thr-dev test meal to induce a taste aversion ruled out the possibility that increased levels of plasma ammonia could reach toxic levels and refuted the hypothesis that an increased postprandial metabolism could generate satiety signals. In contrast, ingestion of the Thr-dev test meal induced a rapid and pronounced depression in circulating threonine, a result that confirms that plasma amino acid pattern is rapidly modified in the rat after ingestion of an amino-acid-imbalanced diet (7).

Perspectives

This study also demonstrated that the fall in plasma amino acid level occurs before the decrease in food intake and thus can participate in the anorectic response. Such a signal is strongly suspected to act directly on specific brain structures [Anterior piriform cortex (11)], but whether this signal also acts indirectly via intestinal or hepatic pathways remains to be established. It is known that amino acids come into contact with inner sensors in the circulatory system before uptake into cells and utilization in metabolic pathway and protein synthesis, either in peripheral or central organs. These sensors are either amino acid transport systems, amino acid receptors, or intracellular effectors sensitive to the amino acid levels. Other peripheral signals such as changes in gut and metabolic hormones [cholecystokinin, insulin, glucagon (1)] as well as peripheral or central mediators (11) are also suspected to act in response to an amino-acid-deficient diet and to play a role in the transmission of the anorectic signal.

The authors thank Sophie Daré for skillful technical assistance in analysis of plasma amino acids.

REFERENCES