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ORIGINAL ARTICLE

The postprandial use of dietary amino acids as an energy substrate is delayed after the deamination process in rats adapted for 2 weeks to a high protein diet

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Abstract The aim of this study was to determine the contribution of dietary amino acids (AA) to energy metabolism under high protein (HP) diets, using a double tracer method to follow simultaneously the metabolic fate of α-amino groups and carbon skeletons. Sixty-seven male Wistar rats were fed a normal (NP) or HP diet for 14 days. Fifteen of them were equipped with a permanent catheter. On day 15, after fasting overnight, they received a 4-g meal extrinsically labeled with a mixture of 20 U-[^{15}N]-[^{13}C] AA. Energy metabolism, dietary AA deamination and oxidation and their transfer to plasma glucose were measured kinetically for 4 h in the catheterized rats. The transfer of dietary AA to liver glycogen was determined at 4 h. The digestive kinetics of dietary AA, their transfer into liver AA and proteins and the liver glycogen content were measured in the 52 other rats that were killed sequentially hourly over a 4-h period. [^{15}N] and [^{13}C] kinetics in the splanchnic protein pools were perfectly similar. Deamination increased fivefold in HP rats compared to NP rats. In the latter, all deaminated AA were oxidized. In HP rats, the oxidation rate was slower than deamination, so that half of the deaminated AA was non-oxidized within 4 h. Non-oxidized carbon skeletons were poorly sequestrated in glycogen, although there was a significant postprandial production of hepatic glycogen. Our results strongly suggest that excess dietary AA-derived carbon skeletons above the ATP production capacity, are temporarily retained in intermediate metabolic pools until the oxidative capacities of the liver are no longer overwhelmed by an excess of substrates.

Keywords HP diet · Dietary amino acids · Oxidation · Glycogen · Stable isotopes

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Introduction

The consumption of high protein (HP) diets is still contentious in terms of their effects on energy pathways and glycemic control (Krezowski et al. 1986; Farnsworth et al. 2003; Khan et al. 1992; Krebs et al. 2003; Linn et al. 2000), and particularly in relation to type 2 diabetes (Linn et al. 1996; Gannon et al. 2003). The principal mechanism of adaptation to an increase in protein intake is the stimulation of both dietary amino acids (AA) deamination and urea production (Morens et al. 2000, 2001), which is usually interpreted as an increase in dietary AA oxidation. However, the precise fate of the dietary AA carbon skeletons derived from deamination under HP feeding has been the subject of little study, and there is no direct evidence that the body of deaminated dietary AA is also oxidized and not converted to other metabolic substrates.

Ingestion of an HP diet also leads to a postprandial decrease in the insulin/glucagon ratio, in both animals (Khan et al. 1992; Baum et al. 2006; Gannon 1993; Gannon and Nuttall 1995; Nuttall and Gannon 1990; Westphal et al.

1990) and humans (Khan et al. 1992; Krebs et al. 2003). Depending on the carbohydrate level (CHO) in the diet, ingestion of an HP diet is associated with either a relative maintenance of hepatic glycogen (HP-low CHO diets) (Obeid et al. 2006; Baum et al. 2006) without a rise in hepatic glucose release (Rossetti et al. 1989) or with a slight depletion of hepatic glycogen (HP–CHO free diets) (Gannon 1993; Gannon and Nuttall 1995; Krebs et al. 2003). Under HP feeding, the concomitant increase in dietary AA supply and decrease in the insulin/glucagon ratio has been shown to up-regulate the first cytosolic step of hepatic gluconeogenesis during the postprandial phase but not its final step (Azzout-Marniche et al. 2007), suggesting a channeling to glycogen. Furthermore, the bulk of the carbon skeleton derived from the deamination of ketogenic and gluconeogenic AA can also be used as an energy substrate, either directly in the citrate cycle through acetyl CoA or indirectly after conversion to glucose through hepatic gluconeogenesis.

Study of the metabolic fate of carbon skeletons originating from the deamination of dietary AA requires simultaneous monitoring of the metabolic fate of α-amino groups and carbon skeletons during the postprandial period. For this purpose, a mix of 20 AA uniformly labeled with [^{13}C] and [^{15}N] was used as an oral tracer. This approach made it possible to characterize the metabolic fate of dietary AA in the splanchnic area and to ascertain that [^{13}C] and [^{15}N] displayed similar kinetics until deamination. It also allowed us to quantify the utilization of dietary AA separately during the deamination and decarboxylation processes, as well as the transfer of the AA-derived carbon skeleton into glycogen and glucose.

Materials and methods

Experimental diets

The experimental diets were produced by the "*Atelier de Production des Aliments Expérimentaux*" (INRA, Jouy-en-Josas, France) (Table 1). The normal protein (NP) diet was an AIN-93 modified diet containing 140 g total milk protein/kg food instead of casein and cysteine. NP and HP diets supplied 14 and 53% of energy in dried matter (DM) as total milk protein, respectively. In the HP diet, the protein level was increased at the expense of carbohydrates only (33% of DM vs. 73% of DM). Fat, cellulose, mineral and vitamin contents were similar. Both diets were moistened to prevent spillage. The experimental meals were labeled with a 6 mg mixture of 20 uniformly and doubly labeled [^{15}N]-[^{13}C]-AA (CortecNet, Paris, France). The composition of the mixture was (%): ala (9.9), arg (5.1), asn (3.6), asp (8.7), cys (3.6), gln (3.6), glu (9.2), gly (5.8), his (2.1), ile (2.8), leu (7.6), lys (10.9), meth (1.4), phe (7.5), pro (5.1), ser (4), thr (4.3), trp (0.2), tyr (3.4) and val (4.1).

Table 1 Diet composition

	NP (g kg^{-1})	HP (g kg^{-1})
Total milk protein[a]	140	530
Sucrose[b]	100.3	45.7
Corn starch[c]	622.4	287.0
Soybean oil[d]	40	40
AIN 93 M mineral mixture[e]	35	35
AIN 93 M vitamin mixture[e]	10	10
Cellulose[f]	50	50
Choline[e]	2.3	2.3
Metabolizable energy (kJ g^{-1})	14.6	14.6
Protein (% energy)	14	55
Carbohydrate (% energy)	76	38
Fat (% energy)	10	10

[a] IDI, Arras, France

[b] Cerestar, Haubourdin, France

[c] Eurosucre, Paris, France

[d] Bailly SA, Aulnay-sous-bois, France

[e] ICN Biochemicals, Ohio, USA

[f] Medias filtrants Durieux, Torcy, France

Animals and experimental design

Experiments were carried out in accordance with the guidelines of the French Committee for Animal Care and the European Convention on Vertebrate Animals Used for Experimentation. Sixty-seven male Wistar rats (Harlan-France, Gannat, France), weighing 180–199 g on arrival, were housed individually in a room with controlled temperature (22 ± 1°C) and humidity, under a 12:12-h reversed light–dark cycle (lights on from 18:00 to 06:00). After adaptation to the laboratory conditions, 15 rats were implanted with a chronic venous catheter as described by Nicolaidis et al. (1974). All rats received the NP diet for 1 week before the experiments were started. They were then randomly assigned to two groups, in which they received either the NP diet ($n = 33$, including 7 rats implanted with a chronic venous catheter) or the HP diet ($n = 34$, including 8 rats implanted with a chronic venous catheter), for 14 days (adaptation period). Every day, the rats received the experimental diet in two periods: one calibrated meal of 4 g given between 09:00 and 09:30 and free access to food between 12:00 and 18:00. The morning calibrated meal was given in order to train the rats to quickly consume a standardized amount of food.

At the end of the dietary adaptation, body weight was similar in NP rats (246 ± 18 g) and HP rats (247 ± 25 g).

Sample collection to determine the splanchnic kinetics of [U- ^{13}C, ^{15}N] AA

On day 15, rats that were not implanted with a chronic venous catheter ($n = 26$ in both the NP and HP groups) received the 4 g calibrated meal at 09:00 supplemented with the 6 mg mixture of 20 uniformly and doubly labeled [^{15}N]-[^{13}C]-AA. They were then sequentially anesthetized 0, 1, 2, 3 or 4 h after meal ingestion ($n = 2$ at t_0, $n = 6$ at 1, 2, 3 and 4 h) with an intraperitoneal injection of 45 mg/kg of pentobarbital (Sanofi-Synthélabo Santé Animale, Libourne, France) and injected with 5,000 IU heparin (Laboratoire LEO, Saint-Quentin-en-Yvelines, France). The liver was removed, rinsed, and weighed before being sliced, aliquoted frozen in liquid nitrogen, and then stored at −20°C until the measurement of dietary AA transfer into liver proteins and determination of the total hepatic glycogen content. The contents of the stomach and small intestine were collected and kept at −20°C until analysis of their equivalent levels in dietary AA.

Sample collection to determine the energy metabolism and postprandial availability of [U- ^{13}C, ^{15}N] dietary AA carbon skeletons and their transfer to specific pools of CHO metabolism

On day 15, the rats implanted with a chronic venous catheter ($n = 7$ and 8 in the NP and HP groups, respectively) underwent an energy metabolism investigation at the beginning of the light cycle. They were placed in an open-circuit indirect calorimeter and continuously infused with a saline solution containing heparin (120 IU/h) via the permanent catheter. After fasting overnight in the calorimetric cage, they were given the usual 4 g calibrated meal at 09:00, supplemented with the 6 mg mixture of 20 uniformly and doubly labeled AA. During the 4 h following meal ingestion, data on both oxygen consumption (VO_2) and carbon dioxide production (VCO_2) were acquired by a computer at 10-s intervals. In order to determine dietary AA transfer into CO_2, expired gases were collected under water every 30 min from a derivation of the calorimetric circuit and maintained at room temperature until assay. Blood samples (0.5 mL) were collected via the catheter every 30 min, and centrifuged immediately (1,500*g*, 15 min, 4°C), and the plasma was then frozen at −20°C until the measurement of glucose levels and dietary AA transfer into glucose. Urine was collected continuously, from the beginning of the meal and during 4 h, in a fraction collector located beneath the cage, pooled every 2 h and then frozen at −20°C for subsequent measurements of the total urinary urea content and the transfer of dietary AA into urinary urea. Four hours after meal ingestion, the rats were anesthetized with a bolus of pentobarbital delivered via the catheter. After incising the abdomen, the aorta and vena cava were sectioned and total blood was collected and centrifuged (1,500*g*, 15 min, 4°C). Plasma was aliquoted and frozen at −20°C for the subsequent measurement of dietary AA equivalent in plasma urea. The liver was removed and processed in the same way as described above.

Urinary and plasma urea and liver protein analyses

Urinary and plasma urea concentrations were determined using a commercial kit (Bio-Mérieux, Marcy l'Etoile, France). Plasma urea was extracted by cation exchange chromatography on Dowex resin (AG 50W-X8 resin 100–200 Mesh Na form, Bio-Rad, Hercules, CA), as previously described (Gaudichon et al. 1999), and stored at 4°C until isotopic determination.

For liver protein analyses, about 250 mg of liver was homogenized in 3 volumes of 0.9% NaCl using an Ultra-Turrax (Janke & Kunkel, IKA Labortechnik, Staufen, Germany) on ice. After the addition of 5-sulfosalicylic acid (SSA) (10%, 700 µL/100 mg of liver, VWR-Prolabo, Fontenay-sous-Bois, France), the samples were centrifuged (5,000 rpm, 4°C, 15 min). The insoluble pellet was rinsed 3 times with 400 µL 10% SSA, centrifuged (5,000 rpm, 4°C, 15 min), freeze-dried, weighed and kept until isotopic determination.

Plasma glucose and liver glycogen content

Plasma glucose levels were determined using an enzymatic method (glucose oxidase–peroxidase) developed by Werner et al. (1970). For plasma glucose isotopic determinations, 500 µL perchloric acid ($HClO_4$, 1.2 mol/L, Sigma–Aldrich, St Louis, MO, USA) was added to 500 µL plasma. After centrifugation (10,000 rpm, 10 min, 4°C), the pH of the supernatant was neutralized to pH 5.5–6.2 by the addition of 110 µL potassium carbonate (K_2CO_3, 3.2 mol/l, Sigma–Aldrich, St Louis, MO, USA). After storage overnight without a cap at 4°C, the samples were centrifuged (2,500*g*, 10 min, 4°C), and the pH of the resulting supernatant was adjusted to between 5.5 and 7 by adding the appropriate quantity of K_2CO_3 or $HClO_4$. After centrifugation (2,500*g*, 10 min, 4°C), the supernatant was filtered through a 0.22 µm filter (Sartorius Stedim Biotech GmbH, Goettingen, Germany). Glucose in the residue was then passed through an anion (AG1-X8: formate form) and a cation (AG50-X8: hydrogen form) exchange column in order to trap organic acids and AA successively, as described by Lemosquet et al. (2004). Free glucose was eluted with 6 mL UHQ water in a 10 mL Pyrex tube and then dried overnight in a speed vacuum concentrator (Fisher Scientific Bioblock, Illkirch, France). The resulting

drop of glucose was derivatized as previously described (Luengo et al. 2009).

The liver glycogen content was determined as the difference between the glucose content before and after glycogen hydrolysis. Fifty milligram of liver was homogenized for 20 s in 1 mL of UHQ water using an Ultra-Turrax (Janke & Kunkel, IKA Labortechnik, Staufen, Germany) on ice. Two 50 µL aliquots of the homogenate were mixed with 450 µL acetate buffer (0.05 M, pH 4.8), while two others were mixed with 443 µL acetate buffer and 7 µL amyloglucosidase from *Aspergillus niger* (58 IU/mg, Sigma–Aldrich, St. Louis, MO, USA). After incubation at 55°C for 10 min in a water-bath, the aliquots were kept at ambient temperature for 30 min to achieve glycogen release, and then centrifuged (5,000 rpm, 10 min and 4°C). The glucose concentration in the supernatants was determined using an enzymatic method (glucose oxidase–peroxidase) developed by Werner et al. (1970). For liver glycogen isotopic analysis, glycogen was precipitated before hydrolysis with 1 mL absolute ethanol for 250 mg of liver, as previously described (Luengo et al. 2009).

Extraction of liver free AA

After the extraction of liver glycogen by ion exchange chromatography, the AA retained in the cation exchange column (AG50-X8: hydrogen form) were eluted with 4 M ammonium hydroxide (NH_4OH-ACS-Reagent, Sigma–Aldrich, St Louis, MO, USA) and rinsed with UHQ water. The resulting AA solution was dried overnight in a speed vacuum concentrator and kept dry at ambient temperature until determination of its [^{13}C]-enrichment. [^{15}N]-enrichment could not be determined in this fraction because of the interference with ammonium hydroxide, even after evaporation.

Isotopic determinations

[^{15}N]-enrichments in urinary and plasma urea were determined using an isotopic ratio mass spectrometer (IRMS, Isoprime, GV Instrument, Manchester, UK) coupled to an elemental analyzer (EA, Euro Elemental Analyser 3000, EuroVector). Both [^{15}N] and [^{13}C]-enrichments were measured in liver AA and proteins, as well as in gastrointestinal contents using the EA-IRMS system. [^{13}C]-enrichment in plasma glucose and liver glycogen was measured using gas chromatography-combustion-IRMS analysis, as described elsewhere (Luengo et al. 2009). The [^{13}C]-enrichment of CO_2 in expired gas was determined using GC-isotope-ratio MS (Multiflow/Isoprime, Micromass). Total nitrogen and carbon in the gastrointestinal contents and liver proteins were measured using the EA system, with, respectively, atropine (CE Instruments, Milan, Italy) and cyclohexanone (CE Instruments, Milan, Italy) as standards.

Calculations

Dietary AA transfer in different metabolic pools

The dietary AA present in the samples were expressed in grams of dietary proteins and calculated as follows:
For the nitrogen part

$$\text{Dietary protein} = N_{\text{tot-mmol}} \times 14 \times 6.25 \times \left(APE_{\text{sample}}/APE_{\text{meal}}\right),$$

where $N_{\text{tot-mmol}}$ is amount of total nitrogen (N) in the sample, APE_{sample} is [^{15}N]-enrichment percent excess of the sample = enrichment of the sample − basal enrichment, in atom percent (AP), APE_{meal} is [^{15}N]-enrichment percent excess of the meal = enrichment of the meal − basal enrichment of the diet. The conversion factor from N to protein was assumed to be 6.25 (16% of N in proteins).

The [^{15}N]-enrichment of the meal (in AP) was 1.28 and 0.6% in the NP and HP diet, respectively. The difference between meal enrichments was of similar amplitude to that obtained with intrinsic labeling of soy and milk proteins, which the postprandial fate was compared in previous metabolic studies (Morens et al. 2003; Fouillet et al. 2002; Fouillet et al. 2008; Bos et al. 2003).
For the carbon part

$$\text{Dietary protein} = C_{\text{tot-mmol}} \times 12 \times 2 \times \left(APE_{\text{sample}}/APE_{\text{meal}}\right),$$

where $C_{\text{tot-mmol}}$ is amount of total carbon (C) in the sample, APE_{sample} is [^{13}C]-enrichment percent excess of the sample = enrichment of the sample − basal enrichment, APE_{meal} is [^{13}C]-enrichment percent excess of the meal = enrichment of the meal − basal enrichment of the diet. The conversion factor from C to protein was assumed to be 2 (50% of C in proteins).

The [^{13}C]-enrichment of the meal was 2.01 and 1.33% in the NP and HP diet, respectively.

Total absorption of dietary AA

The percentage of dietary AA absorbed 4 h after meal ingestion was calculated as the difference between ingested proteins and the amount of dietary proteins recovered at 4 h in the stomach and small intestine.

Postprandial deamination and oxidation of dietary AA

The recovery of dietary AA in the urea body pool 4 h after the meal, expressed in grams of dietary protein, was calculated according to the following formula:

$$N_{exo-urea} = [urea] \times 2 \times 0.67/0.92 \times BW \times (APE_{urea}/APE_{meal}),$$

where [urea] is concentration of urea in the plasma, BW is body weight.

In the rat, the mean percentages of body water and water in plasma were assumed to be 67 and 92%, respectively (Sharp and La Regina 1998).

Finally, total deamination of dietary AA was calculated as the sum of the dietary protein recovered in urinary urea and the body urea pool during the 4 h after the meal.

The carbon from dietary proteins recovered in expired air was calculated by combining [$^{13}CO_2$] enrichment and CO_2 production, as follows:

$$\text{Dietary carbon recovery} = [(CO_2/22.4) \times 2 \times 12 \times (APE_{\text{expired air}}/APE_{meal})].$$

The conversion factor from C to protein was assumed to be 2 (50% of C in proteins).

Finally, the total oxidation of dietary AA was calculated as the sum of dietary carbon recovered in expired air at each time point, as described below:

$$\text{Total oxidation} = \sum_{t=0}^{4} \sum_{t}^{t+30} \text{dietary carbon recovery}.$$

Energy expenditure components and nutrient oxidation

Energy expenditure (EE) was calculated from O_2 consumption (VO_2, mL/min) and CO_2 production (VCO_2, mL/min) according to the formula developed by Weir (1949):

$$\text{TEE (Watt)} = (16.3\,VO_2 + 4.57\,VCO_2) \times 4.18/60.$$

The energy expended during spontaneous activity was quantified as the difference between total and resting energy expenditure (TEE and REE, respectively) measured throughout the study period in accordance with the Kalman filtering method, described elsewhere in detail (Even et al. 1991, 1994). The thermic effect of food (TEF) was calculated during the postprandial period from the increase in REE induced by the test meal (and was thus free from noise due to variations in spontaneous activity). Substrate oxidation was calculated using the classical stoichiometric formulae:

Protein oxidation Pox (mg) = 6.25 × N (mg)
Carbohydrate oxidation CHOox (Watt) = (4.57 VCO_2 − 3.23 VO_2 − 2.6 Pox) × (3.74 × 4.18/60)
Lipid oxidation Lox (Watt) = (1.69 VCO_2 − 1.69 VO_2 − 2.06 Pox) × (9.46 × 4.18/60)
For these equations, it was assumed that most deaminated AA were oxidized.

Hepatic net glycogen production

The hepatic net glycogen production was calculated as the difference between the maximal and the basal amounts of glycogen in the liver.

Statistics

Results were expressed as means ± standard deviation. For dietary AA deamination and oxidation balances and for data obtained through sequential euthanasia of rats, differences at each time point between groups (NP vs. HP) were tested using a pooled *T* test (Function *T* test, Microsoft Excel 2007, Microsoft, Redmond, WA, USA). Kinetics with time as a repeated factor, which were obtained in rats that underwent the investigations in the calorimetric cage, were analyzed using a mixed model (Proc Mixed, SAS 9.1, SAS Institute, Cary, NC, USA), with diet and time as main factors. Differences were considered to be statistically significant at $P < 0.05$.

Results

Energy metabolism and nutrient oxidation

In the metabolic cage, there were no significant differences in the speed of meal ingestion between the groups (not shown). After 2 h, 90 ± 20% and 83 ± 11% of the meal had been consumed by NP and HP rats, respectively. After 4 h, the amount of energy ingested was similar between groups.

There was no difference between the groups in terms of total and resting energy expenditure, or the thermic effect of feeding at 4 h after meal ingestion (Table 2). Compared to a NP diet, adaptation to an HP diet did not induce any difference in postprandial lipid oxidation, but led to both significantly higher protein oxidation and lower CHO oxidation over the 4 h after meal ingestion. As a result, the 4 h-postprandial CHO balance was dramatically decreased in HP rats compared to NP rats. The CHO balance in HP rats exhibited a slight excess of 5 kJ (i.e., 0.33 g), in favor of the protein balance (excess of 32 kJ, i.e., 1.8 g), while the CHO balance was 20 kJ (i.e., 1.12 g) in NP rats.

Digestive and splanchnic kinetics of dietary AA

There was a high similarity of the time course transfer of [^{13}C] and [^{15}N] tracers into both the stomach and intestinal contents after the NP and HP meals, indicating

Table 2 Components of energy metabolism, substrate oxidation and substrate balance during the 4 h after meal ingestion in rats adapted to an NP or HP diet

	NP (kJ)	HP (kJ)	Diet effect
Total energy expenditure	37.2 ± 4.9	36.4 ± 3.6	NS
Thermic effect of feeding	7.5 ± 2.1	4.4 ± 2.2	NS
Resting energy expenditure	31.6 ± 4.1	32.1 ± 2.9	NS
Carbohydrate oxidation	24.8 ± 8.5	17.9 ± 3.9	$P = 0.06$
Carbohydrate balance	20.2 ± 8.5	5.2 ± 3.9	$P = 0.0006$
Lipid oxidation	9.8 ± 5.5	11.7 ± 5.1	NS
Lipid balance	−3.9 ± 5.5	−5.8 ± 5.2	NS
Protein oxidation	2.6 ± 1.2	6.9 ± 2.6	$P = 0.0014$
Protein balance	7.5 ± 1.2	32.8 ± 2.5	$P < 0.0001$

Values are Mean ± SD, $n = 7$ for NP group and $n = 8$ for HP group. Substrate oxidation was determined from total energy metabolism. Substrate balance was the difference between oxidation and ingested macronutrients

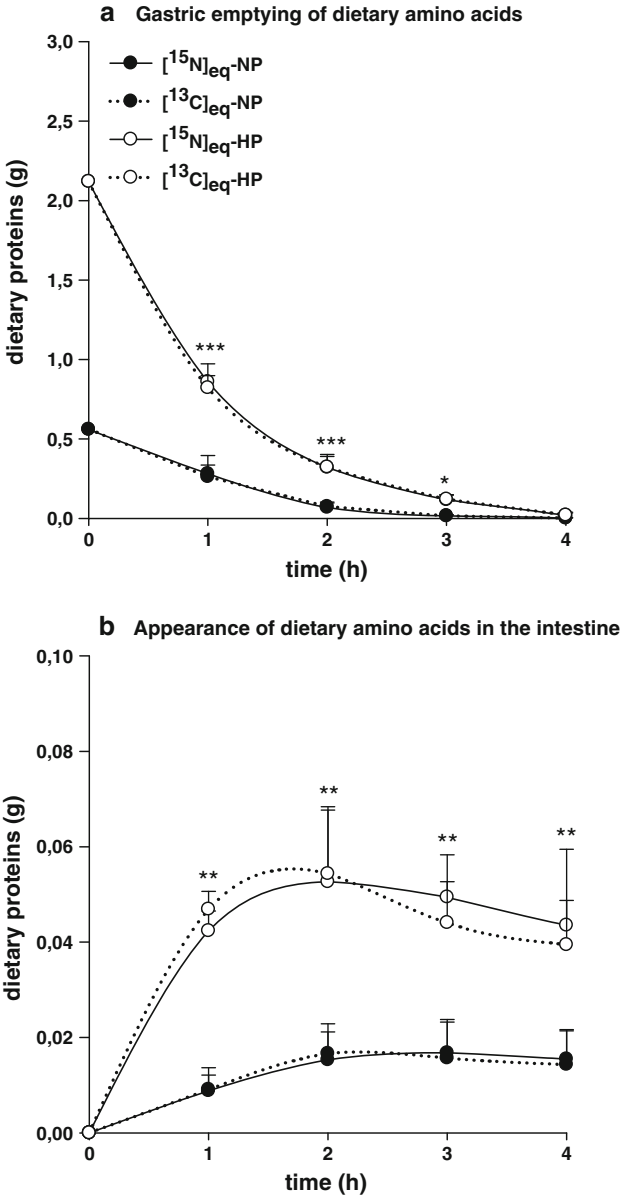

Fig. 1 Digestive kinetics of dietary AA of **a** gastric emptying and **b** transfer to the intestinal lumen in rats adapted for 14 days to high protein (HP) or normal protein (NP) diet. Rats ingested 4 g of an HP or a NP meal containing 6 mg U-[^{15}N]-[^{13}C]-labeled AA. They were killed after 1, 2, 3 or 4 h after the meal. Data are means ± SD ($n = 6$ at each time point). *Asterisks* indicates differences between groups at a specific time point (pooled T test): $^{*}P < 0.05$; $^{**}P < 0.001$; $^{***}P < 0.0001$

that there were no selective losses of dietary AA carbon skeletons or α-amino groups in the gastrointestinal tract (Fig. 1). As expected, the amount of dietary AA emptied by the stomach was significantly higher during the first 3 h after ingestion of the HP meal by comparison with the NP meal. However, at 4 h after the meal, there was no significant difference between the groups regarding the levels of dietary AA present in the stomach (Fig. 1a). The amount of dietary AA transferred to the intestine (Fig. 1b) in the HP group was more than threefold that seen in the NP group ($P < 0.01$). However, if expressed as a percentage of ingested proteins, the postprandial kinetics of dietary AA transfer to the intestine did not differ between the groups (data not shown). As a consequence, the percentage of ingested AA that was absorbed and was thus available for metabolic purposes at 4 h after the meal, reached 96.4 ± 1.8% in the HP group and 95.6 ± 2.7% in the NP group, there being no significant difference.

Dietary AA transfer into liver free AA was determined from [^{13}C]-enrichment (Fig. 2a). It was higher after the ingestion of HP meals, as indicated by the global diet effect ($P < 0.001$). Thus, at 1 h after meal ingestion, dietary AA transfer to liver free AA was 6 times higher in the HP versus the NP group. In liver proteins, there was a good fit of the kinetics of dietary AA α-amino groups and carbon skeleton transfer, although the latter always appeared to be slightly higher than that of α-amino groups after the HP meal (Fig. 2b). The amount of dietary AA transferred to liver proteins was twice higher in the HP group than in the NP group after 2 h and remained significantly higher in the HP than in the NP group during the 4 h following meal ingestion.

Dietary AA deamination and oxidation

The transfer of dietary AA α-amino groups to urinary urea was higher over the 4 h after the HP meal than after the NP meal, being significant at 4 h ($P < 0.0001$) (Fig. 3a). Moreover, the amount of dietary AA α-amino groups still in body urea 4 h after the meal was significantly higher in the HP group (125.3 ± 39.8 vs. 16.2 ± 5.7 mg). As a

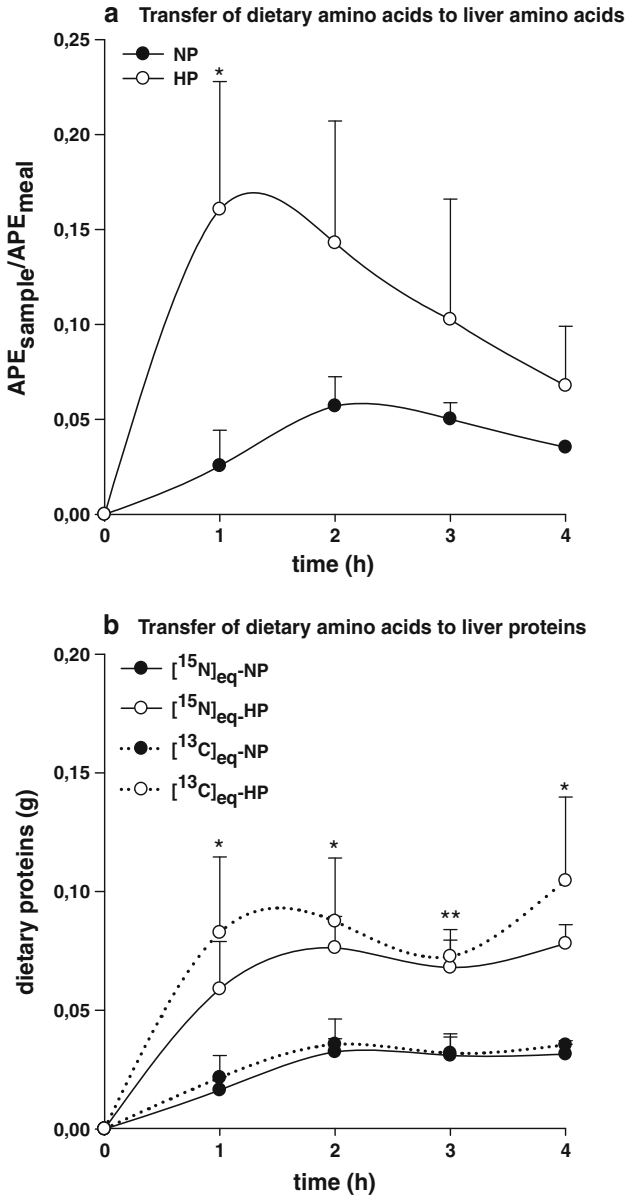

Fig. 2 Liver kinetics of dietary AA (**a**) appearance in liver AA and (**b**) transfer to liver protein in rats adapted for 14 days to an HP or NP diet. Rats ingested 4 g of an HP or a NP meal containing 6 mg U-[^{15}N]-[^{13}C]-labeled AA. They were killed after 1, 2, 3 or 4 h after the meal. Data are means ± SD ($n = 6$ at each time point). *Asterisks* indicates a difference between groups at a specific time point (pooled *T* test): *$P < 0.05$; **$P < 0.001$ (for [^{15}N]eq only)

consequence, cumulative dietary AA deamination 4 h after the meal, i.e., the sum of urinary output and dietary nitrogen still present in body urea at 4 h (not shown), was about 6 times higher in rats adapted to the HP diet, reaching 301.6 ± 110.2 mg of dietary N versus 50.1 ± 19.7 mg in the NP group (Fig. 3c). Over the 4 h following meal ingestion, the amount of dietary AA carbon skeletons transferred to expired CO_2 was higher in the HP versus NP group ($P < 0.0001$) (Fig. 3b). As a result, the cumulative oxidation of dietary AA 4 h after the HP meal was threefold that seen after the NP meal. However, in rats adapted to the HP diet, dietary AA oxidation was half that of their deamination, indicating that 50% of deaminated AA was not oxidized, which suggests a sequestration of carbon skeletons in metabolic pools (Fig. 3c). By contrast, the recovery rates of dietary AA α-amino groups and carbon skeletons were similar in NP rats, indicating that all deaminated AA were oxidized.

Postprandial time course of dietary AA transfer into plasma glucose and liver glycogen

Postprandial glycemia (Fig. 4a) did not differ between the groups but there was a significant time effect ($P < 0.0001$). Dietary AA transfer into plasma glucose was significantly higher ($P < 0.01$) in the HP group over the 4 h after meal ingestion (Fig. 4b). Thus, at the end of the experiment, the appearance of carbons from dietary AA in plasma glucose was 18 times higher in the HP group. A significant diet × time interaction resulted from an increase in dietary carbon appearance after 2 h in the HP group, while it decreased in NP animals. Assuming that the glucose flux was 4 mg/min on the basis of glucose oxidation in 4 h, the transfer of dietary AA to plasma glucose over 4 h was 4.5 ± 2.1% in HP rats.

The liver glycogen content tended to be lower over the 4 h in the HP versus NP groups, with a significant difference at 1 h (220 ± 52 and 435 ± 112 mg in HP and NP rats, respectively) (Fig. 5). Net hepatic glycogen production in rats adapted to the HP diet, although significant (230 mg/4 h), remained lower than in rats adapted to the NP diet (424 mg/4 h). In order to determine whether some of the non-oxidized carbon skeletons were sequestrated into glycogen, we measured [^{13}C] in liver glycogen 4 h after the meal. The levels of dietary [^{13}C] in liver glycogen were higher in HP rats than in NP rats ($APE_{glycogen}$/APE_{meal}: 0.03 ± 0.01 in the NP group and 0.12 ± 0.06 in the HP group), but amounted to less than 0.5% of dietary AA.

Discussion

The objective of this study was to follow the integration of dietary AA into the principal metabolic pathways during a 4 h period after the ingestion of a test meal in rats adapted to an NP or HP diet. For this purpose, we used uniformly labeled [^{15}N] and [^{13}C]-AA as extrinsic tracers of dietary protein in order to follow the metabolic fate of dietary AA α-amino groups and carbon skeletons The results showed that under NP conditions, all deaminated dietary AA-derived carbon skeletons were oxidized. By contrast,

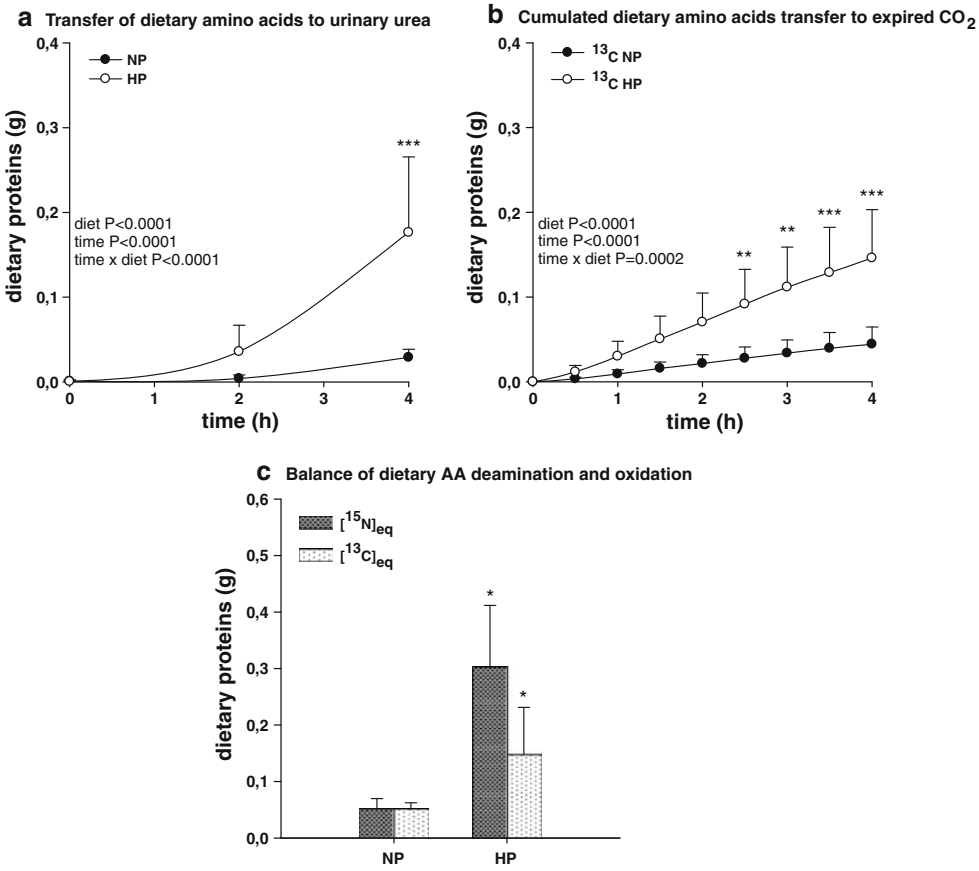

Fig. 3 Transfer kinetics of dietary AA to **a** urinary urea, **b** and expired CO_2, and **c** postprandial dietary AA total deamination and oxidation in rats adapted for 14 days to an HP or NP diet. Rats ingested 4 g of an HP or a NP meal containing 6 mg U-[^{15}N]-[^{13}C]-labeled AA. Data are means ± SD (HP $n = 8$, NP $n = 7$). Kinetics were analyzed using a mixed model. *Asterisks* indicates a difference between groups at a specific time point (**a** and **b** post hoc Fisher test, **c**: pooled T test): $*P < 0.05$; $**P < 0.001$; $***P < 0.0001$

under HP conditions, only half of the deaminated dietary AA-derived carbon skeletons were oxidized, indicating the generation of a pool of carbon skeletons. We determined that a small proportion of this pool was sequestrated in glycogen. In consequence, one has to consider the possibility that free AA-derived carbon skeletons may be retained temporarily in intermediate metabolic pools until further oxidation.

We used in this study a very high level (53%) of proteins to be consistent with previous studies from our laboratory, in which we intended to link the limiting adiposity effects, repeatedly observed in these conditions, with metabolic and physiologic parameters (Pichon et al. 2006; Morens et al. 2001; Blouet et al. 2006; Azzout-Marniche et al. 2007). Although protein levels in human diets rarely exceed 30% when using usual foods to formulate HP diets, a level of 50% of protein is frequently achieved in short-term programs for weight loss when protein isolates are consumed.

Our study showed that dietary AA carbon skeletons and α-amino groups displayed similar kinetics in the gastrointestinal tract after adaptation to HP and NP diets, indicating that about 97% of dietary AA were absorbed within 4 h of meal ingestion, in both HP and NP rats (Table 3). Our group had previously studied the absorption rate of dietary AA by monitoring the metabolic fate of α-amino groups under various conditions (Morens et al. 2001; Bos et al. 2005; Deglaire et al. 2009), but to our knowledge, the specific metabolic fate of dietary AA carbon skeletons in the gastrointestinal tract has rarely been studied. Because dietary AA can undergo numerous reactions in the gastrointestinal tract, including transamination in enterocytes (Sève and Le Floc'h 2000; Wu 1998), a separation of dietary AA α-amino groups from carbon skeletons might have occurred. However, during the present study, we did not find any evidence of their partial sequestration in the gut, especially in epithelial cells. In addition, and as already reported (Morens et al. 2000, 2001; Fouillet et al. 2008), our results showed that adaptation to the HP diet led to a fivefold increase in the postprandial deamination of dietary AA. Indeed, after 4 h, dietary AA deamination reached 50 mg (9% of ingested proteins) and 300 mg (14.2%), with 492 mg (88%) and 1,756 mg (82.8%) of ingested AA remaining available for protein metabolic use in NP and HP rats, respectively (Table 3). Accordingly, more dietary proteins were incorporated in liver proteins after adaptation to the HP diet, which agrees with a previous observation (Morens et al. 2001). The increase in postprandial dietary AA deamination after adaptation to an HP diet was considered to be accompanied by a parallel increase in their oxidation (Morens et al. 2000, 2001).

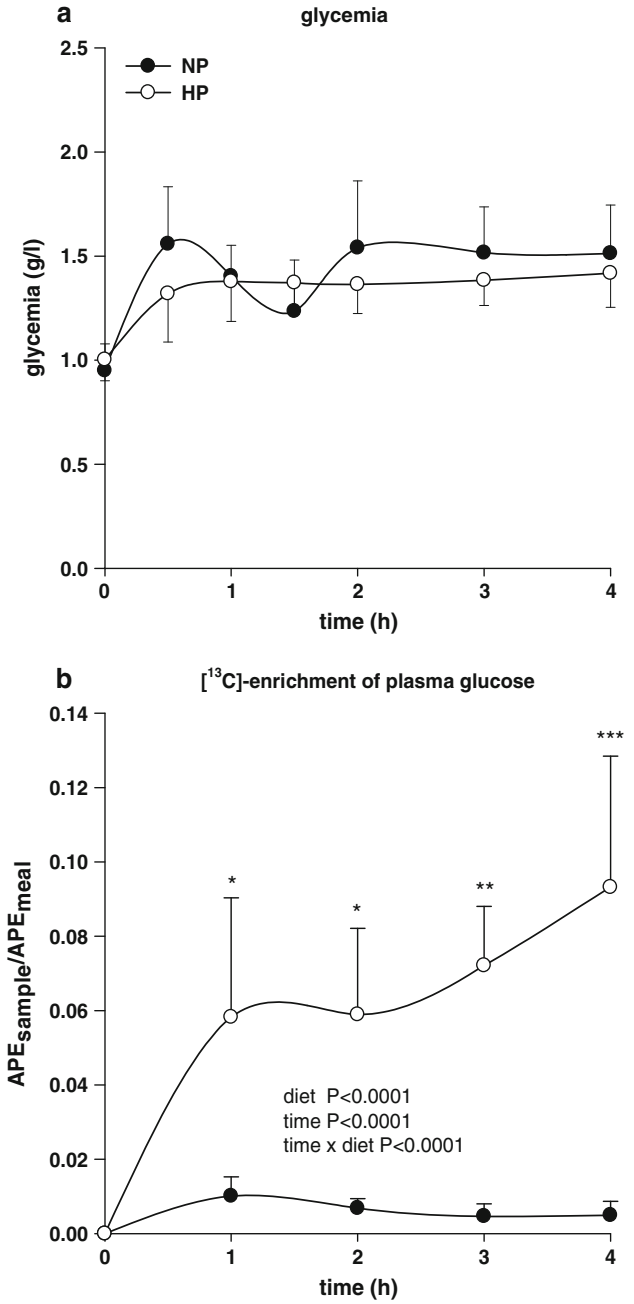

Fig. 4 **a** Postprandial glycemia, **b** dietary carbon appearance in plasma glucose in rats adapted for 14 days to an HP or NP diet. Rats ingested 4 g of an HP or a NP meal containing 6 mg U-[^{15}N]-[^{13}C]-labeled AA. Data are means ± SD (HP $n = 8$, NP $n = 7$). Kinetics were analyzed using a mixed model. *Asterisks* indicates a difference between groups at a specific time point (post hoc Fisher test): $^{*}P < 0.05$; $^{**}P < 0.001$; $^{***}P < 0.0001$

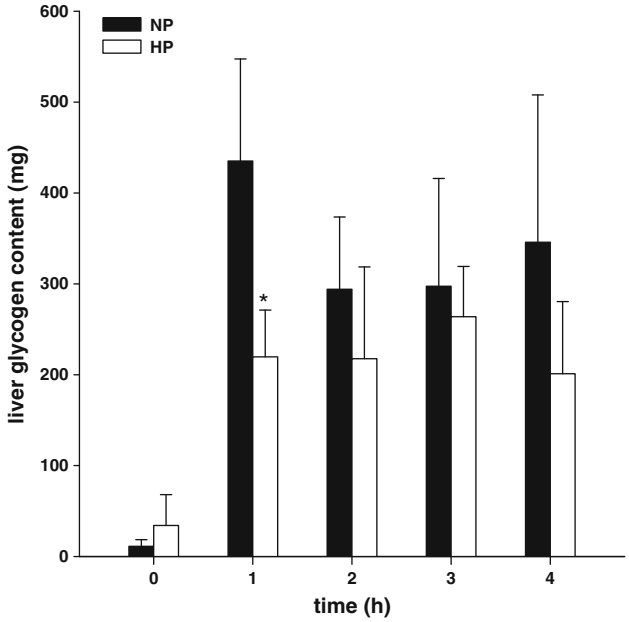

Fig. 5 Time course of total liver glycogen levels in rats adapted for 14 days to an HP or NP diet. Rats ingested 4 g of an HP or a NP meal. They were sacrificed after 1, 2, 3 or 4 h after the meal. Data are means ± SD ($n = 6$ at each time point). *Asterisks* indicates a difference between groups at a specific time point (pooled *T* test): $^{*}P < 0.05$

This study has indeed confirmed that under NP feeding conditions, postprandial AA deamination was directly related to oxidation. By contrast, under HP feeding, the results showed that the transfer of dietary AA-derived carbon skeletons to energy pathways increased direct oxidation through acetyl CoA threefold, but that 50% (150 mg, i.e., 7% of ingested proteins) remained in a pool of non-oxidized dietary AA-derived carbon skeletons. As shown in Fig. 3, the cumulated recovery of [^{13}C] through CO_2 output was far from achieving a plateau after 4 h in HP animals. Interestingly, it clearly appears that the slopes for the urinary (Fig. 3a) and CO_2 (Fig. 3b) outputs of dietary proteins were quite similar in NP rats but displayed a marked difference in HP rats. This strongly suggests that the oxidation of dietary AA-derived carbon skeletons was delayed from the deamination process under HP feeding.

A critical methodological aspect of our study is that we used a commercial mix of free AA as tracers of dietary proteins. As a previous study in rats showed that the gastric emptying of free AA was faster than for intact proteins (Daenzer et al. 2001), free AA may not perfectly mimic the postprandial metabolic fate of dietary proteins in the gastrointestinal tract. Comparing our results to some obtained previously using intrinsically [^{15}N]-labeled milk proteins (Morens et al. 2001), it appeared that the gastric emptying rate of free AA is moderately increased. Indeed, the half-time of gastric emptying was 1 and 1.5 h in NP and HP groups, respectively, while we obtained values of 0.8 and 1 h in the present study. Consequently, a part of free AA must reach the liver earlier than whole proteins, probably increasing their hepatic catabolism. Indeed, it was previously showed that fast digestion of proteins increased the postprandial utilization of dietary nitrogen for urea

Table 3 Balance of the metabolic fate of dietary AA over the 4 h after meal ingestion in rats adapted to an NP or HP diet

Metabolic fate of dietary nitrogen and carbon	NP diet		HP diet	
	Dietary protein (mg)	% of dietary N or C (mg)	Dietary protein	% of dietary N or C
Ingestion	560	100	2,120	100
Absorption	542	96.7	2,058	97.1
Deamination	50	8.9	302	14.2
Oxidation	50	9.0	148	7.2
Availability of dietary AA for protein metabolism	492	87.8	1,756	82.8
Non-oxidized dietary AA-derived carbon skeletons	0	0	153	7.5

synthesis in humans (Lacroix et al. 2006; Deglaire et al. 2009). As a consequence, the use of free AA as tracers of dietary protein may have led to an overestimation of the postprandial deamination. Further investigations are necessary to ascertain our results, requiring the use of uniformly and intrinsically labeled proteins with [^{15}N] and [^{13}C]. However, while [^{15}N] proteins are relatively easy to obtain, the production for uniformly labeled [^{13}C] proteins, with acceptable cost, is unlikely to be performed. We thus used the only available mix containing uniformly labeled [^{15}N] and [^{13}C] AA.

The present results show that the chronic consumption of an HP diet led to an increase in dietary AA transfer to plasma glucose, but in the absence of determining blood flow or glucose fluxes it was not possible to precisely quantify the contribution of dietary AA to gluconeogenesis. In order to assess approximately this contribution, we assumed that the plasma glucose flux was quite similar to oxidized glucose, i.e., 4 mg/min in HP rats in the postprandial state. This value (although probably underestimated because it omits that part of the glucose which is stored), appeared to be acceptable given the rate of glucose appearance reported in the postabsorptive state in rats (2 mg/min) (Saadatian et al. 2000; Podolin et al. 1999). Under these conditions, we estimated that 75 mg of dietary AA contributed to plasma glucose synthesis after adaptation to a HP diet, which represents less than 5% of ingested protein. However, all AA do not participate equally in gluconeogenesis. If only carbons ingested from the main gluconeogenic AA were taken into account, i.e., alanine, glycine, serine, threonine and aspartate, then 73% of these AA were probably transferred to plasma glucose in HP rats.

In previous publications, we and others suggested that after adaptation to HP diets, a significant part of dietary AA was channeled toward glycogen synthesis (Peret et al. 1975; Azzout-Marniche et al. 2007). Some studies have also measured the postprandial kinetics of liver glycogen in rats adapted to HP versus NP diets for 90 min after meal ingestion (Baum et al. 2006). Although this study found lower glycogen contents at all times after the meal, the relative kinetic profiles for HP and NP groups were similar to those found in the present study. However, none of these studies addressed specifically the question of the transfer of dietary AA to glycogen. In our study, the measurement of [^{13}C] in glycogen revealed than even though a higher transfer of dietary carbon skeletons to glycogen occurred in HP rats than in NP rats, this accounted for less that 0.5% of ingested AA. As a consequence, this could not explain the non-oxidative disposal of carbon skeletons in HP rats, and further suggests that other sources of glucose are substrates for glycogen synthesis in HP-fed rats. In this context, the slight but positive postprandial CHO balance (5 kJ, ~300 mg glucose) was sufficient to account for the 200 mg of glycogen synthesized during the postprandial period. However, there are other likely precursors of liver glycogen, in particular glycerol, lactate and endogenous AA. The glycerol released with fatty acids during lipolysis is considered to be a major glucose precursor during fasting (Baba et al. 1995; Peroni et al. 1997) but its contribution to glucose synthesis is very small in the fed state (Baba et al. 1995; Wahren and Ekberg 2007). Some in vitro studies have pointed out that lactate was used efficiently for gluconeogenesis in fed rats adapted to an HP diet (Kaloyianni and Freedland 1990), but in vivo studies showed that lactate extraction by the liver was not affected by the quantity of protein ingested in adapted animals (Remesy et al. 1978). On the contrary, it was established that the liver extraction of endogenous AA was higher after HP versus NP diets (Remesy et al. 1978).

It is possible that an excess of AA-derived carbon skeletons may be stored in body fat through the metabolic crossroad of acetyl CoA. However, adaptation to an HP diet is associated with a marked decrease in postprandial fatty acid synthesis (Schmid et al. 1984; Botion et al. 1992) and with the down-regulation of liver fatty acid synthase (Baum et al. 2006; Pichon et al. 2006; Blouet et al. 2006). Moreover, we checked for the presence of dietary carbon above baseline in liver and adipose tissue triglycerides, but failed to find any (data not shown). Transfer to ketone bodies could be another possibility that buffers this non-recovered pool. Few studies have focused on the contribution of AA to ketogenesis, and they are mainly about the postabsorptive phase or fasting state (Thomas et al. 1982; Kulaylat et al. 1988;

Noda and Ichihara 1974). Other studies have suggested that both β-hydroxybutyrate and acetoacetate plasma levels are higher in animals adapted to HP-CHO free versus high CHO diets (Peret et al. 1981; Robinson et al. 1981). However, one study reported that postprandial hydroxybutyrate hepatic–portal vein differences did not change significantly in rats adapted to HP versus NP diets (Remesy et al. 1978). This apparent inconsistency may have been due to the presence of CHO in the HP diet of later studies. Indeed, it was shown that both insulin and CHO status repressed ketogenesis (Williamson 1981; Fukao et al. 2004; Hegardt 1998) and that the addition of AA to glucose did not significantly modify the in vitro production of hepatic ketone bodies (Baquet et al. 1991). Taken together, these considerations, and the marked difference in the slopes for the urinary and CO_2 outputs of dietary proteins, suggest that the excess of carbon skeletons from dietary AA, above the ATP production capacity (Jungas et al. 1992), are temporarily retained in intermediate metabolic pools until the oxidative capacities of the liver are no longer overwhelmed by an excess of substrates. If future studies confirm that nitrogen excretion is dissociated from the protein oxidation, the validity of this parameter to calculate nutrient oxidation in the stoichiometric equations should be reassessed, at least in the case of HP diets. Considering our result that Pox is only half of the value expected from urinary urea production, the new values would then be 14.4 ± 4 and 19.3 ± 4 kJ for Lox and CHOox, respectively, indicating a 2–3 kJ (10–20%) underestimation of nutrient oxidation when non-adjusted equations are used.

In conclusion, this study highlights the fact that, after HP diet adaptation, half of deaminated AA was not oxidized during the postprandial phase. These carbon skeletons were not sequestrated in hepatic glycogen. Together with the apparently high capacity of the body to store excess dietary AA temporarily in the pool of body protein, the weak ability of deaminated AA to be transferred to energy stores probably participates in the limiting effect of HP diets on adiposity.

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References

Azzout-Marniche D, Gaudichon C, Blouet C, Bos C, Mathe V, Huneau JF, Tome D (2007) Liver glyconeogenesis: a pathway to cope with postprandial amino acid excess in high-protein fed rats? Am J Physiol Regul Integr Comp Physiol 292(4):R1400–R1407

Baba H, Zhang XJ, Wolfe RR (1995) Glycerol gluconeogenesis in fasting humans. Nutrition (Burbank, Los Angeles County Calif 11(2):149–153

Baquet A, Lavoinne A, Hue L (1991) Comparison of the effects of various amino acids on glycogen synthesis, lipogenesis and ketogenesis in isolated rat hepatocytes. Biochem J 273(Pt 1):57–62

Baum JI, Layman DK, Freund GG, Rahn KA, Nakamura MT, Yudell BE (2006) A reduced carbohydrate, increased protein diet stabilizes glycemic control and minimizes adipose tissue glucose disposal in rats. J Nutr 136(7):1855–1861

Blouet C, Mariotti F, Azzout-Marniche D, Bos C, Mathe V, Tome D, Huneau JF (2006) The reduced energy intake of rats fed a high-protein low-carbohydrate diet explains the lower fat deposition, but macronutrient substitution accounts for the improved glycemic control. J Nutr 136(7):1849–1854

Bos C, Metges CC, Gaudichon C, Petzke KJ, Pueyo ME, Morens C, Everwand J, Benamouzig R, Tome D (2003) Postprandial kinetics of dietary amino acids are the main determinant of their metabolism after soy or milk protein ingestion in humans. J Nutr 133(5):1308–1315

Bos C, Juillet B, Fouillet H, Turlan L, Dare S, Luengo C, N'Tounda R, Benamouzig R, Gausseres N, Tome D, Gaudichon C (2005) Postprandial metabolic utilization of wheat protein in humans. Am J Clin Nutr 81(1):87–94

Botion LM, Kettelhut IC, Migliorini RH (1992). Reduced lipogenesis in rats fed a high-protein, carbohydrate-free diet: Participation of liver and four adipose depots. Brazilian journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas/Sociedade Brasileira de Biofisica [et al. 25(4): 419-428

Daenzer M, Petzke KJ, Bequette BJ, Metges CC (2001) Whole-body nitrogen and splanchnic amino acid metabolism differ in rats fed mixed diets containing casein or its corresponding amino acid mixture. J Nutr 131(7):1965–1972

Deglaire A, Fromentin C, Fouillet H, Airinei G, Gaudichon C, Boutry C, Benamouzig R, Moughan PJ, Tome D, Bos C (2009) Hydrolyzed dietary casein as compared with the intact protein reduces postprandial peripheral, but not whole-body, uptake of nitrogen in humans. Am J Clin Nutr 90(4):1011–1022

Even PC, Perrier E, Aucouturier JL, Nicolaidis S (1991) Utilisation of the method of Kalman filtering for performing the on-line computation of background metabolism in the free-moving, free-feeding rat. Physiol Behav 49(1):177–187

Even PC, Mokhtarian A, Pele A (1994) Practical aspects of indirect calorimetry in laboratory animals. Neurosci Biobehav Rev 18(3):435–447

Farnsworth E, Luscombe ND, Noakes M, Wittert G, Argyiou E, Clifton PM (2003) Effect of a high-protein, energy-restricted diet on body composition, glycemic control, and lipid concentrations in overweight and obese hyperinsulinemic men and women. Am J Clin Nutr 78(1):31–39

Fouillet H, Mariotti F, Gaudichon C, Bos C, Tome D (2002) Peripheral and splanchnic metabolism of dietary nitrogen are differently affected by the protein source in humans as assessed by compartmental modeling. J Nutr 132(1):125–133

Fouillet H, Juillet B, Bos C, Mariotti F, Gaudichon C, Benamouzig R, Tome D (2008) Urea-nitrogen production and salvage are modulated by protein intake in fed humans: results of an oral stable-isotope-tracer protocol and compartmental modeling. Am J Clin Nutr 87(6):1702–1714

Fukao T, Lopaschuk GD, Mitchell GA (2004) Pathways and control of ketone body metabolism: on the fringe of lipid biochemistry. Prostaglandins Leukot Essent Fatty Acids 70(3):243–251

Gannon MC (1993) The effect of oral casein on hepatic glycogen metabolism in fasted rats. Metab Clin Exp 42(5):649–653

Gannon MC, Nuttall FQ (1995) Physiological doses of oral casein affect hepatic glycogen metabolism in normal food-deprived rats. J Nutr 125(5):1159–1166

Gannon MC, Nuttall FQ, Saeed A, Jordan K, Hoover H (2003) An increase in dietary protein improves the blood glucose response in persons with type 2 diabetes. Am J Clin Nutr 78(4):734–741

Gaudichon C, Mahe S, Benamouzig R, Luengo C, Fouillet H, Dare S, Van Oycke M, Ferriere F, Rautureau J, Tome D (1999) Net postprandial utilization of [15n]-labeled milk protein nitrogen is influenced by diet composition in humans. J Nutr 129(4): 890–895

Hegardt FG (1998) Transcriptional regulation of mitochondrial HMG-COA synthase in the control of ketogenesis. Biochimie 80(10):803–806

Jungas RL, Halperin ML, Brosnan JT (1992) Quantitative analysis of amino acid oxidation and related gluconeogenesis in humans. Physiol Rev 72(2):419–448

Kaloyianni M, Freedland RA (1990) Contribution of several amino acids and lactate to gluconeogenesis in hepatocytes isolated from rats fed various diets. J Nutr 120(1):116–122

Khan MA, Gannon MC, Nuttall FQ (1992) Glucose appearance rate following protein ingestion in normal subjects. J Am Coll Nutr 11(6):701–706

Krebs M, Brehm A, Krssak M, Anderwald C, Bernroider E, Nowotny P, Roth E, Chandramouli V, Landau BR, Waldhausl W, Roden M (2003) Direct and indirect effects of amino acids on hepatic glucose metabolism in humans. Diabetologia 46(7):917–925

Krezowski PA, Nuttall FQ, Gannon MC, Bartosh NH (1986) The effect of protein ingestion on the metabolic response to oral glucose in normal individuals. Am J Clin Nutr 44(6):847–856

Kulaylat MN, Frexes-Steed M, Geer R, Williams PE, Abumrad NN (1988) The role of leucine in hepatic ketogenesis. Surgery 103(3):351–360

Lacroix M, Bos C, Leonil J, Airinei G, Luengo C, Dare S, Benamouzig R, Fouillet H, Fauquant J, Tome D, Gaudichon C (2006) Compared with casein or total milk protein, digestion of milk soluble proteins is too rapid to sustain the anabolic postprandial amino acid requirement. Am J Clin Nutr 84(5):1070–1079

Lemosquet S, Thibault JN, Thomas A, Debras E, Hurtaud C (2004) Validation of the measurement of glucose appearance rate with [6, 6–2h2]glucose in lactating dairy cows. Reprod Nutr Dev 44(1):17–27

Linn T, Geyer R, Prassek S, Laube H (1996) Effect of dietary protein intake on insulin secretion and glucose metabolism in insulin-dependent diabetes mellitus. J Clin Endocrinol Metab 81(11): 3938–3943

Linn T, Santosa B, Gronemeyer D, Aygen S, Scholz N, Busch M, Bretzel RG (2000) Effect of long-term dietary protein intake on glucose metabolism in humans. Diabetologia 43(10):1257–1265

Luengo C, Azzout-Marniche D, Fromentin C, Piedcoq J, Lemosquet S, Tome D, Gaudichon C (2009) [13c] GC-C-IRMS analysis of methylboronic acid derivatives of glucose from liver glycogen after the ingestion of [13C] labeled tracers in rats. J Chromatogr 877(29):3638–3644

Morens C, Gaudichon C, Metges CC, Fromentin G, Baglieri A, Even PC, Huneau JF, Tome D (2000) A high-protein meal exceeds anabolic and catabolic capacities in rats adapted to a normal protein diet. J Nutr 130(9):2312–2321

Morens C, Gaudichon C, Fromentin G, Marsset-Baglieri A, Bensaid A, Larue-Achagiotis C, Luengo C, Tome D (2001) Daily delivery of dietary nitrogen to the periphery is stable in rats adapted to increased protein intake. Am J Physiol 281(4):E826–E836

Morens C, Bos C, Pueyo ME, Benamouzig R, Gausseres N, Luengo C, Tome D, Gaudichon C (2003) Increasing habitual protein intake accentuates differences in postprandial dietary nitrogen utilization between protein sources in humans. J Nutr 133(9): 2733–2740

Nicolaidis S, Rowland N, Meile MJ, Marfaing-Jallat P, Pesez A (1974) A flexible technique for long term infusions in unrestrained rats. Pharmacol Biochem Behav 2(1):131–136

Noda C, Ichihara A (1974) Control of ketogenesis from amino acids. Ii. Ketone bodies formation from alpha-ketoisocaproate, the keto-analogue of leucine, by rat liver mitochondria. J Biochem 76(5):1123–1130

Nuttall FQ, Gannon MC (1990) Metabolic response to egg white and cottage cheese protein in normal subjects. Metab Clin Exp 39(7):749–755

Obeid OA, Boukarim LK, Al Awar RM, Hwalla N (2006) Postprandial glycogen and lipid synthesis in prednisolone-treated rats maintained on high-protein diets with varied carbohydrate levels. Nutrition (Burbank, Los Angeles County Calif 22(3):288–294

Peret J, Chanez M, Cota J, Macaire I (1975) Effects of quantity and quality of dietary protein and variation in certain enzyme activities on glucose metabolism in the rat. J Nutr 105(12): 1525–1534

Peret J, Foustock S, Chanez M, Bois-Joyeux B, Robinson JL (1981) Hepatic metabolites and amino acid levels during adaptation of rats to a high protein, carbohydrate-free diet. J Nutr 111(10): 1704–1710

Peroni O, Large V, Diraison F, Beylot M (1997) Glucose production and gluconeogenesis in postabsorptive and starved normal and streptozotocin-diabetic rats. Metab Clin Exp 46(11):1358–1363

Pichon L, Huneau JF, Fromentin G, Tome D (2006) A high-protein, high-fat, carbohydrate-free diet reduces energy intake, hepatic lipogenesis, and adiposity in rats. J Nutr 136(5):1256–1260

Podolin DA, Wei Y, Pagliassotti MJ (1999) Effects of a high-fat diet and voluntary wheel running on gluconeogenesis and lipolysis in rats. J Appl Physiol 86(4):1374–1380

Remesy C, Demigne C, Aufrere J (1978) Inter-organ relationships between glucose, lactate and amino acids in rats fed on high-carbohydrate or high-protein diets. Biochem J 170(2):321–329

Robinson JL, Foustock S, Chanez M, Bois-Joyeux B, Peret J (1981) Circadian variation of liver metabolites and amino acids in rats adapted to a high protein, carbohydrate-free diet. J Nutr 111(10): 1711–1720

Rossetti L, Rothman DL, DeFronzo RA, Shulman GI (1989) Effect of dietary protein on in vivo insulin action and liver glycogen repletion. Am J Physiol 257(2 Pt 1):E212–E219

Saadatian M, Peroni O, Diraison F, Beylot M (2000) In vivo measurement of gluconeogenesis in animals and humans with deuterated water: a simplified method. Diabetes Metab 26(3): 202–209

Schmid H, Kettelhut IC, Migliorini RH (1984) Reduced lipogenesis in rats fed a high-protein carbohydrate-free diet. Metab Clin Exp 33(3):219–223

Sève B, Le Floc'h N (2000) Le devenir des protéines et des acides aminés dans l'intestin du porc: De la digestion à l'apparition dans la veine porte. INRA Production Animale 13(5):11

Sharp PE, La Regina MC (1998) The laboratory rat, 1st edn. CRC Press, Boca Raton

Thomas LK, Ittmann M, Cooper C (1982) The role of leucine in ketogenesis in starved rats. Biochem J 204(2):399–403

Wahren J, Ekberg K (2007) Splanchnic regulation of glucose production. Annu Rev Nutr 27:329–345

Weir JB (1949) New methods for calculating metabolic rate with special reference to protein metabolism. J Physiol 109(1–2):1–9

Werner W, Rey HG, Wielinger H (1970) Über die eigenschaften eines neuen chromogens für die blutzuckerbestimmung nach der god/pod-methode Fresenius'. J Anal Chem 252(Numbers 2-3): 224–228

Westphal SA, Gannon MC, Nuttall FQ (1990) Metabolic response to glucose ingested with various amounts of protein. Am J Clin Nutr 52(2):267–272

Williamson DH (1981) Mechanisms for the regulation of ketogenesis. Proc Nutr Soc 40(1):93–98

Wu G (1998) Intestinal mucosal amino acid catabolism. J Nutr 128(8):1249–1252