Dietary protein regulates hepatic constitutive protein anabolism in rats in a dose-dependent manner and independently of energy nutrient composition

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THE CONSUMPTION OF HIGH–PROTEIN (HP) diets is common, particularly in association with weight reduction, weight loss maintenance, or exercise. At the whole body level in humans, an increase in protein intake is associated with marked increases in amino acid oxidation, as well as with a stimulation in the fasted state and a strong inhibition in the fed state of the protein breakdown rate. Body protein synthesis rates, however, are little affected by only moderate stimulation in the fed state (11, 28, 33). Specific tissues respond differently to an increase in protein intake (8, 12, 13, 20). In the fed state, in humans (15) but not in rats (8), muscle protein turnover is increased by an HP diet. In the fasted state in rats, an HP diet has no effect on protein synthesis in muscle or on visceral organs, such as the intestine, stomach, or kidneys, whereas the protein synthesis rate is decreased in the liver (8, 12, 19, 37).

An increased protein intake is associated with a concomitant decrease in dietary carbohydrate (CHO) (8, 20), fat (37), or both CHO and fat (12) in the diet, which makes it impossible to specifically ascribe the metabolic effects observed to manipulations of the protein level alone. Indeed, each macronutrient, and particularly protein and CHO, is likely to influence levels of plasma amino acids (AA), glucose, and insulin in both the short and long term. Insulin and AA differently regulate protein metabolism in individual tissues (36). In piglets, the stimulation of muscle protein synthesis rates requires both insulin and amino acids (29, 36), whereas visceral organs, such as the liver, mainly respond to amino acids (30, 36). In rats, liver protein synthesis appears to be insensitive to both insulin and AA (25).

Our previous study was performed using high levels of protein of up to 50% of energy (8), whereas 35% energy is already a very high protein level in humans (15). Thus, the possibility to extrapolate these findings to more realistic levels consumed by humans ingesting high-protein diets is questionable. In this context, our aim was, therefore, to determine the individual role of macronutrients in the tissue protein turnover response to increased protein and decreased CHO levels, and concomitantly to explore whether tissue metabolic changes were affected dose dependently by a rise in the protein level.

MATERIALS AND METHODS

Animals and diets. The 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 (license no. 75–1562). Male Wistar rats (n = 104, 180–200 g) were purchased from Harlan (Horst, The Netherlands) and housed under controlled environmental conditions (temperature, 12-h dark period starting at 0700). The rats had free access to water and commercial laboratory chow for 5 days before the start of dietary adaptation to experimental diets. The animals were allocated at random to one of eight experimental diets (n = 13 per group, no differences in initial body weights). The diets contained three different levels of protein: normal (NP: 14% energy as protein), intermediate (IP: 32 to 36% of protein), or high (HP: 50 to 52% of protein). For each level of protein, two or three different ratios of CHO to fat were tested (Table 1). The diets were supplied in a semiliquid form, so as to prevent spillage and improve the recording of the quantities ingested. The rats were accustomed to receiving their food following...
Fig. 1. Food intake (A) and growth (B) of rats subjected to isoenergetic diets containing variable protein (NP, normal protein; IP, intermediate protein; HP, high protein) and carbohydrate (CHO) levels (LC, low CHO; IC, intermediate CHO; HC, high CHO). Values are expressed as means ± SD; *n* = 13 rats per group.

There were significant global effects on energy consumption of the dietary protein level (*P* < 0.0001), time (*P* < 0.0001), and their interaction (*P* < 0.005), but not of the CHO content of the diet (mixed models for repeated-measures analysis). There were significant global effects of time (*P* < 0.0001) and of the interaction between protein level and time (*P* < 0.0001) but not of the CHO content of the diet on changes to body weight.

### Table 1. Composition of the experimental diets

<table>
<thead>
<tr>
<th>Protein level CHO level</th>
<th>NP</th>
<th>IP</th>
<th>HP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total milk protein*</td>
<td>150</td>
<td>358</td>
<td>521</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>620</td>
<td>441</td>
<td>338</td>
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<tr>
<td>Sucrose</td>
<td>89</td>
<td>63</td>
<td>49</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>44</td>
<td>40</td>
<td>1</td>
</tr>
<tr>
<td>Minerals mix†</td>
<td>10.0</td>
<td>10.0</td>
<td>9.4</td>
</tr>
<tr>
<td>Vitamin mix†</td>
<td>35</td>
<td>35</td>
<td>42</td>
</tr>
<tr>
<td>Cellulose</td>
<td>50</td>
<td>50</td>
<td>60</td>
</tr>
<tr>
<td>Choline</td>
<td>2.3</td>
<td>2.3</td>
<td>47</td>
</tr>
<tr>
<td>g/kgDM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of total energy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metabolizable energy</td>
<td>3.57</td>
<td>3.59</td>
<td>3.52</td>
</tr>
</tbody>
</table>

*R* Nutrinov, Rennes, France. †AIN-93M, ICN Biochemicals, Cleveland, OH. DM, dry matter; NP, normal protein; IP, intermediate protein level; HP, high protein; HC, high carbohydrate (CHO); IC, intermediate CHO level; LC, low CHO.
a pattern that consisted of a first meal that occurred between 0900 and 1000, which provided 6 g of dry matter and then free access to food between 1400 and 1800. This pattern was adopted so as to habituate the animals to completely consuming a standard meal within 1 h, so as to standardize the physiological state of the animals studied in the fed state on the experimental day.

**Experimental protocol.** The body weights and food intakes of the rats were measured daily during the first 3 days and then every other day for the next 12 days of the adaptation period. On days 12 or 13, a blood sample was collected from the tail vein of animals fasted overnight, in EDTA-prefilled tubes, to measure plasma metabolites and hormones. On day 15, half of the rats in each group was studied in the fasted state (16-h fast), and half was studied in the fed state (2 h after the start of the 6-g calibrated meal, supplied after a 16-h fast). To measure fractional protein synthesis rates, the animals were injected subcutaneously with 300 μmol/kg body wt of a flooding dose of L-[1-13C]-valine (50 mol%; Cambridge Isotope Laboratories, Andover, MA) 30 min before death. The subcutaneous flooding dose method has been developed and validated by Mosoni et al. (26, 27). Rats were then anesthetized with an intraperitoneal injection of pentobarbital sodium (50 mg/kg body wt) before incision of the abdomen. They were killed by rupture of the caudal vena cava and aorta after a heparin injection (Choay Laboratoire, Gentilly, France). Under sterile conditions, the liver and gastrocnemius muscle were removed, rinsed, weighed, and immediately frozen in liquid nitrogen until analysis. Blood from the vena cava was collected and centrifuged. Standards (norvaline and guanidopropionic-acid, 20 μmol/ml of plasma) were added to the sample used for amino acid measurements. In each group, one of the eight animals did not receive the 13C-valine flooding dose, so that baseline levels of 13C-valine in tissues could be determined (control animals).

**Biochemical measurements.** To determine tissue protein contents, the tissues were freeze-dried and weighed, and their total N content was assessed by the Dumas method using an elemental analyzer (Euro Elemental Analyzer 3000, EuroVector) with atropine as the standard. Plasma amino acid concentrations were determined using ion exchange chromatography with postcolumn ninhydrin derivatization (Biotech Instrument, St. Quentin-en-Yvelines, France). Plasma samples were first deproteinized with an intraperitoneal injection of sulfosalicylic acid. Blood glucose concentrations were determined using a portable reflectometer (Accu-check, Roche Diagnostics, Mannheim, Germany). Insulin, glucagon, and leptin concentrations were analyzed using a rat endocrine panel (RENDO; Linco Research, St. Charles, MO) on a Bioplex 200 system (Bio-Rad Laboratories, Hercules, CA). Insulin, glucagon, and leptin concentrations were analyzed using a rat endocrine panel (RENDO; Linco Research, St. Charles, MO) on a Bioplex 200 system (Bio-Rad Laboratories, Hercules, CA). Under sterile conditions, the liver and gastrocnemius muscle were removed, rinsed, weighed, and immediately frozen in liquid nitrogen until analysis. Blood from the vena cava was collected and centrifuged. Standards (norvaline and guanidopropionic-acid, 20 μmol/ml of plasma) were added to the sample used for amino acid measurements. In each group, one of the eight animals did not receive the 13C-valine flooding dose, so that baseline levels of 13C-valine in tissues could be determined (control animals).

**Gene expression of proteolytic enzymes.** Total RNA (0.05 to 0.1 g) was extracted from frozen tissues (liver, muscle, and kidney) using TRIzol Reagents (Invitrogen). The total RNA amounts were quantified at 260 nm, and ethidium bromide staining was used to confirm RNA integrity. The synthesis of first-strand complementary DNA (cDNA) was performed using a reverse transcription kit (Applied Biosystems, Courtaboeuf, Les Ulis, France) with a PTC-200 thermocycler (MJ Research, Waltham, MA). Real-time PCR was carried out using the power SYBR green PCR master mix (Applied Biosystems, Foster City, CA) on a 7300 real-time PCR system (Applied Biosystems, Foster City, CA) on a Bioplex 200 system (Bio-Rad Laboratories, Hercules, CA). Biochemical measurements.

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the same procedure as that described for the free amino acid fraction. Plasma albumin was extracted from 1 ml plasma with 2 ml of 10% trichloroacetic acid. After mixing, the samples were centrifuged at 650 g for 15 min at 4°C. The pellet was rinsed, and 2 ml of 100% ethyl alcohol and 100 µl of 10% trichloroacetic acid were added. The supernatant was collected after a 1200 g, 10 min 4°C centrifugation and then dried by evaporation. The resulting sample was treated in the same way as the pellet containing the tissue proteins.

Isotopic measurements. To determine 13C-valine enrichment, amino acids were derivatized according to the silylation method: acetonitrile (Acros Organics, Fair Lawn, NJ) was mixed with N-tert-Butyldimethylsilyl-N-methyltrifluoroacetamide (Sigma-Aldrich, Steinheim, Switzerland) at a proportion of 1:1. Eighty microliters of this mix was added to 2 mg of the dried eluate containing free amino acids and then heated at 100°C during 30 min. The enrichment levels of free amino acid derivatives were measured using GC-MS (6890 N; Hewlett-Packard, Bethesda, MD) with electron impact ionization. Derivatives of 13C-valine from protein-bound amino acid pools were analyzed by GC interfaced with a combustion system and coupled with an Isotopic Ratio Mass Spectrometry (GC-C-IRMS HP5890/Isoprime; VG Instruments, Manchester, UK) using a 50-m apolar column (HP5MS; Hewlett-Packard). The baseline enrichment of free 13C-valine in tissues was determined on samples from the control animals.

Calculations. The total protein content (g) of tissues was determined as 
\[ P = \text{TM} \times \frac{\% \text{DM} \times \% \text{N}}{6.25/10,000}, \]
where TM refers to dry tissue mass (g), DM is the dry matter, and N is the percentage of nitrogen. The total muscle mass was not measured but was estimated as 45% of body weight (18). The fractional synthesis rates (FSR, %/day) of tissue proteins were calculated as 
\[ \text{FSR} = \frac{\text{Ebound val}}{(\text{Efree val} \times r) \times 100}, \]
where Ebound val and Efree val are protein-bound and free 13C-valine enrichments in tissues, respectively. Absolute synthesis rates (ASR, g/day) were calculated as 
\[ \text{ASR} = \text{FSR} \times P, \]
where P is the total tissue protein content. The homeostatic model assessment (HOMA) index was calculated using plasma insulin and glucose concentrations using the following equation: 
\[ \text{HOMA} = \frac{\text{insulin (pmol/l)} \times \text{glucose (mmol/l)}}{22.5}. \]

Statistics. Data are expressed as means ± SD. The effects on food intake and growth of the different diets with respect to their protein and CHO levels were analyzed using mixed models for repeated-measures analysis (version 9.1; SAS Institute, Cary, NC). The global effects of dietary protein and CHO levels on endpoint measures were analyzed using ANOVA with the CHO level (low, intermediate, or high) as a nested factor and the protein level (normal, intermediate, or high) and nutritional state (fasted or fed) as the main factors. The CHO level effect was chosen to be analyzed rather than CHO/lipid ratio effect since the study was designed to determine the specific effects of protein and CHO intake on protein metabolism in the tissues. Post hoc Tukey tests for multiple comparisons were performed to enable pairwise comparisons. Regression analyses were performed to assess the relationships between dietary protein levels and tissue mass, N content, and FSR. Differences were considered to be significant at \( P < 0.05 \).

RESULTS

Food intake and animal growth. The total food intakes of the rats subjected to the different diets are presented in Fig. 1A. Overall, ingested energy decreased as protein levels increased (\( P < 0.0001 \)), independently of the other macronutrients.
2 wk of dietary adaptation, the cumulative energy intakes of IP and HP rats were 15% and 21% less than those of NP rats, respectively (NP: 4,622 ± 413 kJ, IP: 3,943 ± 319 kJ and HP: 3,631 ± 292 kJ, P < 0.0001 for pairwise comparisons). The time-course of rat growth was influenced by the protein level (protein level-by-time interaction; P < 0.0001) but not by the CHO level (Fig. 1B). The 16-day body weight gain was significantly higher in the groups consuming less protein (P < 0.0001), irrespective of the CHO level in the diet. Weight gains were 15% and 28% lower in the IP and HP groups than in the NP groups, respectively (NP: 71.1 ± 14.1 g, IP: 60.2 ± 13.2 g and HP: 51.3 ± 14.2 g, P < 0.0001 for pairwise comparisons). The food efficiency ratio (weight gain/energy ingested) did not differ between groups (data not shown). An increase in the dietary protein level was significantly correlated to the mass of the liver and gastrocnemius muscle, expressed on a body weight basis (Fig. 2A). In the liver, but not in muscle, the dietary protein level was also associated with a higher protein content (Fig. 2B). These effects were independent of the CHO level in the diet.

**Plasma glucose, hormones, urea, and amino acids.** Fasting plasma glucose concentrations did not differ significantly between groups and ranged from 0.95 ± 0.14 to 1.07 ± 0.09 g/l (Fig. 3). Fasting insulin concentrations were influenced significantly by the CHO level in the diet. On average, insulin concentrations tended to decrease as the protein level increased (NP: 471 ± 148, IP: 461 ± 142, HP: 384 ± 121 pmol/l), whereas they increased in parallel with the CHO level (LC: 324 ± 121, IC: 441 ± 130, HC: 501 ± 148 pmol/l). The CHO level exerted a significant effect on the HOMA index (P = 0.02), which fell with decreasing CHO levels: 129 ± 42 in HC groups, 109 ± 37 in IC rats and 84 ± 33 in LC rats, with no significant effect of the protein level (P = 0.09, HP: 120 ± 42, IP: 115 ± 38, NP: 83 ± 35). There was a significant global effect of the protein level on glucagon, which increased in line with the protein level. The CHO level was also significant (P < 0.001), but without a clear response. Dietary protein had a pronounced effect on fasting leptin concentrations, which reached 723 ± 379, 590 ± 278, and 426 ± 147 pmol/l in rats subjected to the NP, IP, and HP diets, respectively.

In the fasted state, plasma AA concentrations were influenced neither by the protein level nor by the CHO level. Total and indispensable AA concentrations were increased after the meal (fasted vs. fed). Total AA levels were not influenced significantly by the macronutrient composition of the diet (Fig. 4), in contrast to indispensable AA levels that were globally significantly higher with an increased protein intake. Gluconeogenic AA were globally significantly decreased when the dietary protein content increased, independently of the CHO level. As for individual AA, the leucine concentration rose significantly under the influence of both the dietary protein level and meal ingestion. Glycine concentrations fell significantly with both an increased protein intake and meal ingestion but rose when the CHO level increased. AA responses to the dietary intervention displayed five profiles: 1) increased concentrations in parallel with the protein level and meal intake (isoleucine, leucine, phenylalanine, and tyrosine); 2) decreased concentrations with an increased protein intake, with or without an influence of feeding status (threonine, tryptophan, and serine); 3) modulation of concentrations by the meal only, independently of macronutrient levels (methionine, alanine, arginine, tyrosine, and glutamate). Total AA levels were increased in parallel with the CHO level (LC: 121 pmol/l, IC: 121 pmol/l, HC: 148 pmol/l), whereas they decreased in parallel with the protein level (NP: 384 pmol/l, IP: 461 pmol/l, HP: 471 pmol/l). Protein level exerted a significant effect on the HOMA index (P = 0.02), which fell with decreasing CHO levels: 129 ± 42 in HC groups, 109 ± 37 in IC rats and 84 ± 33 in LC rats, with no significant effect of the protein level (P = 0.09, HP: 120 ± 42, IP: 115 ± 38, NP: 83 ± 35). There was a significant global effect of the protein level on glucagon, which increased in line with the protein level. The CHO level was also significant (P < 0.001), but without a clear response. Dietary protein had a pronounced effect on fasting leptin concentrations, which reached 723 ± 379, 590 ± 278, and 426 ± 147 pmol/l in rats subjected to the NP, IP, and HP diets, respectively.
arginine, asparagine, and taurine); 4) AA concentrations that were influenced by both protein and CHO levels (lysine and glycine); and 5) no effect of protein or CHO levels or feeding status (histidine, aspartic acid, citrulline, glutamic acid, glutamine, and ornithine).

Plasma urea concentrations were markedly influenced by the level of protein intake and meal ingestion (Fig. 5). Uremia was 71% higher in IP rats than in NP rats and 24% higher in HP rats than in IP rats. Within each level of protein intake (NP, IP, or HP), feeding elicited the same rise in plasma urea levels compared with fasting values (+25%).

**Tissue protein synthesis rates.** In the liver, the dietary protein level and meal ingestion exerted a marked effect on the FSR, with no additional significant influence of the CHO level (Table 2). Compared with NP rats, IP and HP rats displayed liver FSR that were 16% and 25% lower, respectively. Independently of feeding status, the liver FSR of NP, IP, and HP rats reached 101 ± 25, 88 ± 20, and 74 ± 13%/day, respectively. Fed rats had a liver FSR that was 20% higher than fasted rats, whatever the protein level. There was a negative relationship between liver FSR and the level of protein in the diet in both the fed state ($R^2 = 0.88$; $P < 0.001$) and the fasted state ($R^2 = 0.78$; $P < 0.05$). The liver ASR (mg/day) was influenced by both the protein level of the diet and meal ingestion, without the CHO level having any effect. Rats subjected to NP, IP, and HP diets synthesized 2.0 ± 0.5, 1.7 ± 0.4, and 1.70 ± 0.3 g of hepatic protein per day, respectively (Table 3). In contrast to constitutive liver proteins, the dietary protein and CHO levels, as well as the feeding status had no significant influence on albumin FSR (Table 2). The muscle protein FSR was only influenced significantly by the meal with an average stimulation of 20% by feeding. The muscle protein ASR was influenced by neither dietary protein nor by the CHO level (Table 3). There was a trend toward a stimulation of the muscle ASR by meal feeding.

**Gene expression of proteolysis pathways.** In the liver, ubiquitin mRNA (Fig. 6) was significantly influenced by the dietary protein level and by the nutritional state. In the fed state, ubiquitin mRNA was lowered compared with the fasted state ($P < 0.0001$), the lowest value being observed in the HP group (33% reduction). m-Calpain mRNA was also significantly affected by the dietary protein level, but there was an interaction with the nutritional state. The CHO content of the diet also influenced m-Calpain mRNA, which was lower when the CHO content was high. The expression of genes encoding other proteins involved in proteolytic pathways in the liver, i.e., E2

### Table 2. Fractional synthesis rates of liver constitutive proteins, albumin, and muscle proteins in rats subjected to isoenergetic diets differing in terms of their protein level and CHO levels

<table>
<thead>
<tr>
<th>Protein Level</th>
<th>Fasted</th>
<th>Fed</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Liver</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HC</td>
<td>95.7 ± 30.3</td>
<td>120.3 ± 19.0</td>
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<tr>
<td>IC</td>
<td>105.5 ± 18.9</td>
<td>131.3 ± 40.8</td>
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<tr>
<td>IP</td>
<td>85.5 ± 19.4</td>
<td>95.7 ± 14.1</td>
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<tr>
<td>IC</td>
<td>89.5 ± 15.4</td>
<td>88.8 ± 21.0</td>
</tr>
<tr>
<td>LC</td>
<td>90.2 ± 26.0</td>
<td>105.7 ± 14.6</td>
</tr>
<tr>
<td>HP</td>
<td>75.0 ± 11.9</td>
<td>106.2 ± 25.5</td>
</tr>
<tr>
<td>IC</td>
<td>79.2 ± 13.9</td>
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</tr>
<tr>
<td>LC</td>
<td>69.0 ± 13.8</td>
<td>85.5 ± 16.5</td>
</tr>
<tr>
<td><strong>Albumin</strong></td>
<td></td>
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</tr>
<tr>
<td>Protein</td>
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</tr>
<tr>
<td>NP</td>
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<td></td>
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</tr>
<tr>
<td>LC</td>
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</tbody>
</table>

Statistical effects:
- Protein: $P < 0.0001$
- CHO: NS
- Meal: $P = 0.055$

Values expressed as means ± SD; $n = 6$. Fractional synthesis rates are given as %/day. NP, normal protein; IP, intermediate protein; HP, high protein; LC, low carbohydrate; IC: intermediate carbohydrate, HC, high carbohydrate; NS, not significant. Rats were sacrificed in the fasted or fed state. The global effects of the protein and CHO levels are indicated [ANOVA with CHO level (low, intermediate, or high) as a nested factor and protein level (normal, intermediate, or high) as the main factors].

Fig. 5. Urea concentrations in the fasted and fed state in rats subjected to isoenergetic diets containing variable protein (NP, IP, and HP) and CHO levels (LC, IC, and HC). Values are expressed as means ± SD: $n = 8$ per group. The global effects of the dietary protein and CHO levels, as well as the nutritional state, are indicated [ANOVA with CHO (low, intermediate, or high) as a nested factor and protein (normal, intermediate, or high) level and nutritional state (fasted or fed) as the main factors].
enzyme and cathepsin D, was affected by neither the protein content of the diet nor by the nutritional state (results not shown).

In muscle, the expression of ubiquitin was only affected by the nutritional state, with weaker expression in the fed state ($P < 0.01$). m-Calpain expression was influenced by the CHO content of the diet. Enzyme E2 and cathepsin D mRNA were affected by neither the nutritional state nor by the CHO content of the diet (results not shown). Moreover, no effect of the protein content in the diet was observed with respect to gene encoding muscle proteolytic enzymes. In the kidney, dietary manipulations or the nutritional state had no effect on the expression of the proteolysis genes studied except an effect of CHO on m-calpain ($P = 0.04$, results not shown).

**DISCUSSION**

The findings of this study confirmed the slowing down of hepatic protein synthesis rates by an increase in the habitual protein intake in rats, and also demonstrated that this effect was directly related to dietary protein, with no additional influence of parallel changes in CHO or fat. In addition, the hepatic response to increased protein was dose dependent, and an intermediate level of 33–35% protein was sufficient in rats to elicit the significant metabolic effects that are likely to occur in humans consuming HP diets. We also showed that the muscular anabolism was not sensitive to an increase of protein intake, even at a level as high as 50% (i.e., twofold above the recommendation); this level can easily be achieved in humans by using protein supplements.

The present study showed that an increase in protein intake elicited a significant reduction of protein synthesis rates in the liver, with no effect on skeletal muscle. This confirms our previous findings (8) that yielded a lower liver FSR ($-34\%$) in rats adapted to an NP diet (with a high CHO content) than to an HP diet (with a low CHO content). Few other studies have produced conflicting results since the data available show either an absence of changes (12, 13), a slight decrease (10, 14), or an increase (20) in liver protein synthesis when rats were switched from a normal to a high-protein diet. However, this last study was performed using a leucine flooding dose, which could impair the results due to the signal effect of this amino acid on protein synthesis. Among the other studies cited above, none of them is really comparable to ours regarding the model used. Our results show that the reduction in the protein FSR was dose dependent and more marked when switching from NP to IP ($-18\%$) than from IP to HP ($-10\%$) (Fig. 7). This dose-dependent deceleration was observed in both the fasting and fed states, which was at variance with our previous findings when we observed a much more pronounced effect in the fed state (8). Moreover, the positive linear relationship that we described between the dietary protein level and both liver mass and liver protein content are totally in agreement with numerous other reports in rats (10, 14, 16).

Unlike liver constitutive proteins, which appear to play a major role in regulating protein metabolism in the event of a large protein supply, the albumin synthesis rate was not sensitive to dietary protein manipulations. This was in line with previous results obtained in rats and dogs (14, 42) and was also consistent with data in humans showing the lack of effect of diets supplying 9% or 24% of energy as protein, but containing identical levels of energy and fat, on albumin, fibrinogen, or fibronectin synthesis rates (6). These results also agree with the weak association demonstrated between albumin FSR and the level of dietary protein, when these ranged from the recommended daily allowance to an excess value of 25% (39). Thus, it can be inferred that the decelerating effect of HP diets on the liver constitutive protein synthesis rate is not compensated for by a parallel modulation of albumin synthesis and presumably of other exported hepatic proteins. Furthermore, no effect of CHO and fat levels on the albumin FSR could be observed in the diets, a finding consistent with the same albumin synthesis rates obtained following a meal that contained protein only or a complete mix of macronutrients (7), or the lack of influence of the dietary CHO-to-fat ratio on albumin FSR under a given protein intake (1).
In muscle, the level of dietary protein did not modulate protein metabolism. We only observed a slight increase in the relative mass of the gastrocnemius muscle in line with a rise in protein intake, which probably resulted from the lower adiposity of HP-fed rats, as previously reported (3, 32). This is consistent with our previous studies in which the carcass mass was not higher in HP than in NP-fed rats (3, 17, 18), confirming that in normal physiological conditions, muscle mass cannot be enhanced by increasing the protein intake above the requirement, even at a very high level, i.e., twofold higher than...
observed. Rats consuming the same level of protein were pooled. An effect of the protein 12 in the IP and HP groups. As there was no effect of the CHO level in the diet, concentrations were much more sensitive to the changes to intake within each dietary protein level. On the other hand, AA although this effect was not very marked. As expected, insulin been reported by other (12, 15, 18), but not all (31) groups, the latter usually fell when the protein intake increased, as has occasionally been found to elicit an abrupt rupture of the metabolic response (4). The present finding thus suggests that the adaptation of hepatic protein metabolism parallels an elevation of dietary protein levels.

The paradoxical situation in which liver FSR decreases while liver protein pool increases may be explained by a strong inhibition of protein breakdown in response to HP feeding in the liver, which is supported by the gene expression level of key enzymes involved in proteolytic pathways, especially the ubiquitin-proteasome system. Previous studies have described a stimulation of proteolysis with long time periods, at least of 24 h, fasting, and an inhibition after feeding at the whole body level (11, 31), a regulation commonly attributed to both muscle and hepatic protein intakes is likely to mainly concern specific protein pools, such as hepatic proteins, whereas muscle proteolysis was not significantly affected. In addition, the Ca2+ dependent system seems to be affected by the CHO content in the diet in both liver and muscle, with a general trend for an enhanced expression of calpain when CHO decreases, suggesting a possible additional role of insulin on calpain expression.

Because of the satiating effect of dietary proteins (40), the rats subjected to high-protein diets reduced their energy intake compared with rats receiving normal protein levels. One might question whether this could have been responsible for the metabolic responses we observed. However, as discussed in our previous paper (8), energy intake is mainly affected during the first days of a diet, and the difference fades away during the last days of the experimental period that precede the measurements of tissue protein synthesis rates. Moreover, as shown by other studies, food deprivation leads to decreased muscle FSR and hepatic protein content (21, 22, 26), which was the opposite to our observations. Finally, the difference in energy intake between the HP and IP groups was not very great, but despite this, they displayed significantly different hepatic protein synthesis rates.

In conclusion, these findings suggest that a high-protein diet exerts a metabolic impact on liver constitutive protein,
but not albumin, and that these metabolic effects are primarily due to the greater supply of amino acids and probably also to the different pattern of circulating amino acids induced as a result and characterized by a higher proportion of indispensable AA (particularly BCAA and aromatic AA) and a lower proportion of neogluconeic AA (particularly, glycine and threonine). Changes to other macronutrients had no further impact.

Finally, while muscle proteins are not sensitive to an increase of the protein intake above the requirements, we demonstrated a dose-response effect and the occurrence of hepatic metabolic effects at protein intakes of ~33–35%, a level that is comparable to that which can be achieved in humans using HP diets. Further studies now need to examine the functional consequences of such diet-induced modulations to hepatic anabolism.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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