Splanchnic and whole-body leucine kinetics in young and elderly men\textsuperscript{1–3}

Yves Boirie, Pierre Gachon, and Bernard Beafrère

**ABSTRACT** Whole-body and splanchnic protein metabolism were determined in six young (mean age: 22.7 y) and six old (68.2 y) men before and during a standardized meal (41.8 kJ/kg) containing 15.6\% protein, by using a combination of intravenous (\textsuperscript{13}C)leucine) and oral (\textsuperscript{2}H\textsubscript{1}leucine) tracers. In the postabsorptive state, leucine flux and oxidation were similar in both groups when corrected for lean body mass (\(\bar{x} \pm \text{SEM}: 1.80 \pm 0.09 \) compared with 1.79 ± 0.07 \(\mu\)mol \(\cdot\) kg\(^{-1}\) \(\cdot\) min\(^{-1}\) and 0.55 ± 0.02 compared with 0.49 ± 0.04 \(\mu\)mol \(\cdot\) kg\(^{-1}\) \(\cdot\) min\(^{-1}\) for young and old men, respectively, NS). The pattern of response to the meal was also similar in young and old men: increased flux and oxidation, decreased protein breakdown, and unchanged protein synthesis. Splanchnic extraction of dietary leucine was twice as high in elderly men (50 ± 11\% compared with 23 ± 2\%, \(P < 0.05\)), was inversely related to plasma leucine concentration (\(r = -0.771\), \(P < 0.01\)), and was positively related to body mass index (\(r = 0.861\), \(P < 0.001\)). In conclusion, in elderly men there is higher leucine extraction by the gut, liver, or both during feeding, which could lead to a lower peripheral availability of dietary leucine. *Am J Clin Nutr* 1997;65:489–95.

**KEY WORDS** Protein metabolism, stable isotopes, meal, aging, splanchnic tissues, muscle, liver, body composition, body mass index

**INTRODUCTION**

Reduction of the amount of body protein is a major characteristic of aging (1, 2). It involves mostly muscle proteins, the loss of which leads to decreased muscle strength and to limited amino acid storage, which could result in an inadequate response to stresses such as sepsis or trauma (3, 4). The causes for this protein loss remain unclear but necessarily result from alterations in protein metabolism kinetics, which could occur either in the postabsorptive state, during feeding, or both.

Protein kinetics, studied in the postabsorptive state by using a constant \(\textsuperscript{13}C\)leucine infusion, are similar in young and elderly adults when the results are adjusted for lean body mass (5–8). Studies using \(^{15}\)N labels lead to the same conclusions, although this model provides an integrated estimate of total daily nitrogen turnover (ic, postabsorptive and fed state) (9–11). By contrast, few studies have specifically examined the response of protein kinetics to feeding in elderly humans or animals, although the fed state represents \(\geq 50\%\) of the 24-h metabolic activity and corresponds to the reconstitution of the protein mass lost during fasting. Welle et al (6) reported that nonoxidative leucine disposal, an index of whole-body protein synthesis, is normal during feeding in elderly persons, but provided no data on protein breakdown or amino acid oxidation. In the same study, muscle protein synthesis was decreased but normally responsive to feeding, whereas recent data in old rats found the opposite (12). These two studies focused on muscle protein synthesis. However, gut and liver are two other quantitatively important tissues for protein metabolism and could be of particular importance in elderly people because the relative weight of these organs and their contribution to total protein synthesis increases with age in rodents (13, 14). Still, protein turnover in these tissues is more difficult to study in vivo in humans, although it can be approached by determining the splanchnic (ie, gut plus liver) extraction of an orally administered tracer by using dual-tracer methodology (15, 16).

Therefore, the purpose of this study was to compare the postprandial response of protein kinetics to a standardized meal in young and elderly adults with a special emphasis on splanchnic extraction, which was assessed with a combination of deuterated leucine given orally and intravenous \(\textsuperscript{13}C\)leucine.

**SUBJECTS AND METHODS**

**Subjects**

Two groups of six volunteers each, young men aged (\(\bar{x} \pm \text{SEM} 22.7 \pm 0.4\) y and old men aged 68.2 ± 0.8 y, participated in the study. Each subject had a normal physical examination without any medical history of renal, cardiovascular, endocrine, or currently evolving disease. All subjects maintained their usual physical activity before the study. The physical characteristics of the subjects are listed in Table 1.

The purpose and the potential risks of the study were fully explained to the subjects and written informed consent was obtained from each participant. The experimental protocol was approved by the Ethical Committee of Clermont-Ferrand.

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\textsuperscript{1} From the Laboratoire de Nutrition Humaine, Université Clermont Auvergne, Clermont-Ferrand, France.

\textsuperscript{2} Supported by grants from INRA (Nutriage AIP 93/4930), MESR (Alliment demain 94 G0079), and University Clermont Auvergne.

\textsuperscript{3} Address reprint requests to Y Boirie, Laboratoire de Nutrition Humaine, PO Box 321, 58 rue Montalembert, 63009 Clermont-Ferrand Cedex 1, France.

Received August 3, 1995.

Accepted for publication October 22, 1996.
TABLE 1
Physical characteristics of the subjects

<table>
<thead>
<tr>
<th></th>
<th>Young men (n = 6)</th>
<th>Old men (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>22.7 ± 0.4</td>
<td>68.2 ± 0.8</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>63.2 ± 3.9</td>
<td>72.8 ± 4.1</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.3 ± 1.0</td>
<td>26.1 ± 1.4</td>
</tr>
<tr>
<td>FFM (kg)†</td>
<td>54.0 ± 3.6</td>
<td>50.4 ± 2.1</td>
</tr>
<tr>
<td>Fat (%)†</td>
<td>14.5 ± 1.0</td>
<td>30.1 ± 2.9</td>
</tr>
</tbody>
</table>

1 ± SEM. FFM, fat-free mass.
2 Significantly different from young men: *P < 0.001, †P < 0.05.

Experimental protocol

To check that the elderly men had no nitrogen malabsorption and were not in negative nitrogen balance, the isotopic study was preceded by a 5-d period of controlled diet composed of ≥117.2 kJ·kg⁻¹·d⁻¹ and 1.2 g protein·kg⁻¹·d⁻¹, which was close to their usual intake as assessed by a previous dietary inquiry. The meals were prepared in the dietary kitchen of our laboratory and consumed in the laboratory. Nitrogen balances were measured during the controlled diet period by collection of 24-h urine and feces samples.

To correct protein metabolism data for fat-free mass (FFM), body composition was estimated by a monofrequency (50 kHz) bioelectrical impedance analyzer (BIA-101A; RJL Systems, Inc, Detroit). Equations supplied by the manufacturer were used for the young men, but a specific equation was applied for fat and FFM estimations in the elderly men (17; see Discussion).

Materials

L-[1-¹³C]Leucine (99 mole percent excess, MPE), L-[5,5,5-²H₃]leucine (97 MPE), and sodium [¹³C]bicarbonate (99 MPE) were obtained from MassTrace Inc (Woburn, MA). The isotopic and chemical purity of the leucine compounds were checked by gas chromatography–mass spectrometry (GC-MS). Solutions of tracers were tested for sterility and pyrogenicity before use and were prepared in sterile nonpyrogenic saline. During each experiment, the tracers were filtered through 0.22-µm filters.

On the day of the experiment, a catheter was inserted retrogradely into a dorsal vein of the hand for arterialized blood sampling after introduction of the hand into a 70 °C heated, ventilated box. A second catheter was inserted into a vein of the contralateral arm for tracer infusions. After a prime dose of [¹³C]bicarbonate (6 mg), a primed (4.2 µmol/kg), continuous (0.07 µmol·kg⁻¹·min⁻¹) infusion of L-[l-¹³C]leucine was begun and continued for 7 h. After 160 min, a semiliquid diet was administered for the four remaining hours (from 160 to 400 min). The diet provided 41.8 kJ/kg, 15.6% as protein (in the form of whey protein concentrate, containing 10% leucine by wt), 50% as carbohydrate (dextrose maltose hydrolyzed from potato starch of low natural [¹³C] abundance), and 35% as fat (in the form of vegetable oil). The liquid meal was prepared on the day of the protocol, just before the beginning of the study, and was ingested in small (50-mL) aliquots given every 20 min. Deuterated leucine was added to the meals to obtain an oral administration rate of 0.07 µmol·kg⁻¹·min⁻¹. In two additional young subjects, we checked that this diet did not induce any modification of the natural [¹³C] abundance in expired carbon dioxide (data not shown).

Blood and breath samples were taken before any infusion and at 20-min intervals during the last hour of each plateau in the postabsorptive state (ie, from 100 to 160 min) and in the fed state (from 340 to 400 min). The plasma supernate was separated, an internal standard was added, and the sample was kept at −20 °C until analyzed further. Breath samples were kept in 10-mL evacuated containers (Vacutainer; Becton Dickinson, Grenoble, France). Total carbon dioxide production rates were measured at isotopic plateau during the last hour of the two plateaus by open-circuit indirect calorimetry (Deltatrac; Datex, Geneva).

Finally, three of the elderly subjects were studied on a second occasion with an identical protocol except that nitrogen balance was not measured and the routes of tracer administration were reversed, ie, deuterated leucine was infused over 7 h whereas [¹³C]leucine was infused intravenously during the first 160 min and then given with the meals during the feeding period.

Analytical methods

Nitrogen analysis on duplicated meals, urine, and feces was performed by using a pyrochemiluminescence method (Antek 7000; Antek Instruments Inc, Houston). Nitrogen balances were calculated as nitrogen intake − (stool and urine nitrogen) − 6 mg·kg⁻¹·d⁻¹ (other obligatory losses).

Plasma [¹³C]- and [²H₃]leucine and ketoisocaproate (KIC) enrichments were measured by selected ion monitoring–electron impact GC-MS (Hewlett-Packard 5971A; Hewlett-Packard, Palo Alto, CA) with tertiary-butyldimethylsilyl derivatives as described previously (18). Plasma leucine concentrations were measured on the same runs with norleucine as the internal standard. Corrections for the [¹³C] and [²H₃] enrichments were applied according to Biolo et al (19). [¹³CO₂] enrichments were measured on a gas isotope ratio–mass spectrometer (μGas system; Fisons Instruments, VG Isotech, Middlewich, England). Plasma insulin concentrations were measured by radioimmunoassay (CIS, Gif-sur-Yvette, France).

Calculations

As described previously (18), leucine kinetics were calculated as follows:

\[ \text{Leu Ra} = F [¹³C] \text{leu}/([¹³C] \text{leu MPE} \times 0.01) \] (1)  

where Leu Ra (µmol·kg⁻¹·min⁻¹) is total leucine systemic flux. F[¹³C]leu is the [¹³C]leucine infusion rate (µmol·kg⁻¹·min⁻¹) corrected for isotopic purity, and [¹³C]leu MPE is the plasma [¹³C]leucine enrichment. This flux includes the tracers infusions.

Leucine splanchnic extraction (%) was calculated as follows:

\[ \text{Leu splanchnic extraction} = \left[ 1 - \left( \frac{\text{Leu Ra}[¹³C] / \text{Leu Ra}[²H₃]}{100} \right) \right] \] (2)  

where Leu Ra [¹³C] and [²H₃] are total leucine fluxes calculated according to equation 1 with either the intravenous [¹³C] or oral [²H₃] tracer. Splanchnic extraction represents the fraction of ingested leucine taken up by the gut or liver during its
first pass. It can also be expressed in absolute terms: leucine splanchnic extraction \( \times \) leucine intake in \( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \). Leucine oxidation (Leu Ox, \( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \)) was calculated as follows:

\[
\text{Leu Ox} = \frac{^{13}\text{CO}_2 \text{ excretion}}{[^{13}\text{C}]\text{KIC MPE} \times 0.01}
\]

where \(^{13}\text{CO}_2 \) excretion (\( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \)) is the product of carbon dioxide production and \(^{13}\text{CO}_2 \) atoms percent excess (APE), corrected for incomplete recovery by a factor of 0.70 in the postabsorptive state and 0.82 in the fed state according to Hoerr et al (20). For leucine oxidation, \( [^{13}\text{C}]\text{KIC} \) enrichments were used rather than \( [^{13}\text{C}]\text{leucine} \) enrichments because KIC is the immediate precursor of irreversible leucine decarboxylation (21).

Nonoxidative leucine disposal (NOLD, \( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \)), an index of whole-body protein synthesis, is the difference between total leucine flux (Leu Ra) and leucine oxidation (Leu Ox). Endogenous leucine production (\( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \)), an index of whole-body protein catabolism, is the difference between total leucine flux (minus the intravenous tracer infusion rate) and either leucine intake (Endo Leu Ra) or leucine intake corrected for splanchnic extraction (Corrected Endo Leu Ra) calculated as follows:

Corrected Leu intake
\[
= \text{Leu intake} \times [1 - (\text{Leu splanchnic extraction} \times 0.01)]
\]

Finally, net leucine balance is the difference between total leucine intake (including the tracers) and leucine oxidation.

**Statistical analysis**

Results are expressed as means ± SEMs. Amino acid kinetic parameters were compared between the two groups by a one-way analysis of variance (ANOVA), age being the classifying factor. Paired \( t \) tests were performed for the comparisons of fed with postabsorptive states in each subject. Relations between splanchnic extraction and leucine kinetics were analyzed by simple-linear-regression analysis.

**RESULTS**

Body composition was different between the two groups, with an increased percentage of fat mass \((P < 0.001)\) and a lower FFM in the old men (NS; Table 1). Energy and protein intakes obtained from dietary histories in the elderly men did not differ from the intakes during the controlled diet. Mean nitrogen balances in the old men were not significantly different from zero (\(-0.012 \pm 0.014 \text{ g N} \cdot \text{kg}^{-1} \cdot \text{d}^{-1} \)).

Insulin concentrations were not different between the groups, either in the postabsorptive (58 ± 5 compared with 47 ± 6 pmol/L, young and old men respectively) or in the fed state (263 ± 52 compared with 249 ± 43 pmol/L).

As shown in Figure 1, steady state leucine and KIC enrichments were obtained for the two tracers. The ratio of \( [^{13}\text{C}]\text{KIC} \) to \( [^{13}\text{C}]\text{leucine} \) enrichment was identical in the two groups both before and during the meal (0.71 ± 0.09 compared with 0.72 ± 0.08, young and old men, respectively). By contrast, the ratio of \( [^3\text{H}]\text{KIC} \) to \( [^3\text{H}]\text{leucine} \) enrichment (i.e., for the oral tracer) was higher in the elderly than in the young men (0.91 ± 0.04 compared with 0.77 ± 0.03, \( P < 0.01 \)).

In the postabsorptive state, leucine flux and oxidation were significantly lower in elderly than in young men when expressed per kilogram body weight (both \( P < 0.05; \text{Table 2} \)). NOLD tended to be lower, although the difference was not significant. Plasma leucine concentrations were the same in the two groups (117 ± 4 and 115 ± 2 \( \mu \text{mol/L} \)). When adjusted for FFM, these fluxes became similar in both groups (Table 2).

During feeding, leucine intake per kilogram body weight including the oral tracer was the same in all subjects (1.31 ± 0.00 and 1.32 ± 0.01 \( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \)), but when expressed per kg FFM was higher in the old men (1.90 ± 0.09 compared with 1.54 ± 0.02 \( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \), \( P < 0.001 \)). The pattern of qualitative changes in response to the meal was similar in both groups: feeding resulted in increased leucine flux, increased oxidation, decreased endogenous leucine flux, and a positive leucine balance (all \( P < 0.05 \), fed compared with postabsorptive state in each group), whereas NOLD was unchanged. Results were similar whether they were expressed per kg body wt or per kg FFM. Plasma leucine concentrations increased with feeding in both groups (167 ± 9 compared with 147 ± 11 \( \mu \text{mol/L} \), young compared with old men, NS). Splanchnic extraction was twice as high in old than in young men both in relative (50.1 ± 10.8% compared with 22.7 ± 2.2%, \( P < 0.05 \)) and absolute terms (\( P < 0.05 \), see Table 2). Noticeably, splanchnic extraction was much more variable in
TABLE 2
Leucine kinetics during fasting and feeding in young and elderly men

<table>
<thead>
<tr>
<th>Leucine kinetics</th>
<th>Young men (n = 6)</th>
<th>Old men (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Postabsorptive state</td>
<td>Fed state</td>
</tr>
<tr>
<td>Leucine flux</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(μmol · kg body wt⁻¹ · min⁻¹)</td>
<td>1.54 ± 0.09</td>
<td>1.99 ± 0.07</td>
</tr>
<tr>
<td>(μmol · kg FFM⁻¹ · min⁻¹)</td>
<td>1.80 ± 0.09</td>
<td>2.32 ± 0.07</td>
</tr>
<tr>
<td>Splanchnic extraction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(μmol · kg body wt⁻¹ · min⁻¹)</td>
<td>—</td>
<td>0.30 ± 0.03</td>
</tr>
<tr>
<td>(μmol · kg FFM⁻¹ · min⁻¹)</td>
<td>—</td>
<td>0.35 ± 0.03</td>
</tr>
<tr>
<td>Leucine oxidation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(μmol · kg body wt⁻¹ · min⁻¹)</td>
<td>0.47 ± 0.02</td>
<td>0.98 ± 0.04</td>
</tr>
<tr>
<td>(μmol · kg FFM⁻¹ · min⁻¹)</td>
<td>0.55 ± 0.02</td>
<td>1.15 ± 0.06</td>
</tr>
<tr>
<td>Nonoxidative leucine disposal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(μmol · kg body wt⁻¹ · min⁻¹)</td>
<td>1.07 ± 0.08</td>
<td>1.00 ± 0.07</td>
</tr>
<tr>
<td>(μmol · kg FFM⁻¹ · min⁻¹)</td>
<td>1.25 ± 0.09</td>
<td>1.17 ± 0.07</td>
</tr>
<tr>
<td>Endogenous leucine flux</td>
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<tr>
<td>(μmol · kg body wt⁻¹ · min⁻¹)</td>
<td>1.45 ± 0.09</td>
<td>0.59 ± 0.07</td>
</tr>
<tr>
<td>(μmol · kg FFM⁻¹ · min⁻¹)</td>
<td>1.70 ± 0.09</td>
<td>0.69 ± 0.07</td>
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<tr>
<td>Corrected endogenous leucine flux</td>
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<tr>
<td>(μmol · kg body wt⁻¹ · min⁻¹)</td>
<td>—</td>
<td>0.88 ± 0.07</td>
</tr>
<tr>
<td>(μmol · kg FFM⁻¹ · min⁻¹)</td>
<td>—</td>
<td>1.03 ± 0.07</td>
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<tr>
<td>Leucine balance</td>
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<tr>
<td>(μmol · kg body wt⁻¹ · min⁻¹)</td>
<td>-0.38 ± 0.02</td>
<td>0.42 ± 0.04</td>
</tr>
<tr>
<td>(μmol · kg FFM⁻¹ · min⁻¹)</td>
<td>-0.45 ± 0.02</td>
<td>0.48 ± 0.04</td>
</tr>
</tbody>
</table>

1 SEM. FFM, fat-free mass.
2,4 Significantly different from the fed state: 2 P < 0.001, 4 P < 0.05, 5 P < 0.01.
6 Significantly different from young men: 6 P < 0.05, 8 P < 0.01.

elderly men (CV of 48% in elderly men compared with 21% in young men).

Leucine flux per kg body wt was moderately lower in elderly men (P < 0.05), this difference being corrected when expressed per kg FFM as during the postabsorptive state. Despite identical leucine intakes, leucine oxidation expressed per kg body wt was lower in elderly men (P < 0.01). This difference persisted (P < 0.05) when leucine oxidation was expressed per kg FFM, even though leucine intake was higher when referred to this unit. NOLD was unchanged and similar in the two groups, whatever the mode of expression (per kg body wt or FFM). Finally, all fluxes were also calculated by using the KIC enrichments, and although the absolute values differed by 20–30%, the qualitative modifications were the same (data not shown).

Statistical analysis of the relations between splanchnic extraction and leucine kinetics revealed an inverse correlation between splanchnic extraction and leucine concentration (r = -0.771, P < 0.01; Figure 2), which was stronger when only the elderly men were considered (r = -0.930, P < 0.001). Splanchnic extraction was also related to the ratio of [3H]leucine to [3H]KIC (r = 0.663, P < 0.02). Leucine oxidation was positively related to plasma leucine concentration (r = 0.726, P < 0.01). Splanchnic extraction was negatively related to

FIGURE 2. Relations between splanchnic extraction of leucine and plasma leucine, between leucine oxidation and plasma leucine, and between splanchnic extraction of leucine and body mass index (in kg/m²) in young (○) and elderly men (●).
leucine oxidation expressed per unit body wt (r = -0.926 per kg body wt, P < 0.001), and unexpectedly, positively related to fatness as assessed by body mass index (r = 0.861, P < 0.001, Figure 2) or by percentage fat mass (r = 0.851, P < 0.001).

The three older men studied on two occasions had similar leucine fluxes during both protocols (postabsorptive: 1.18 ± 0.07 and 1.29 ± 0.07 μmol·kg⁻¹·min⁻¹, fed: 1.50 ± 0.24 and 1.56 ± 0.10 μmol·kg⁻¹·min⁻¹, calculated with the intravenous tracer). Their splanchnic extraction was high (36 ± 8%). Their basal leucine oxidation was lower (0.37 ± 0.08 compared with 0.26 ± 0.02 μmol·kg⁻¹·min⁻¹) and their postprandial leucine oxidation was marginally higher (0.64 ± 0.15 compared with 0.71 ± 0.05 μmol·kg⁻¹·min⁻¹) in the second study. The increase of leucine oxidation over baseline was 73% in the first study and 173% when the [13C]leucine was given orally.

**DISCUSSION**

In the postabsorptive state, whole-body leucine flux, an index of protein turnover, and leucine oxidation decreased with age when expressed per unit of body wt, confirming previous observations (5–8). A usual problem encountered with studies comparing young and old subjects is the way to express the results, per kg body wt or kg FFM. Although bioelectrical impedance analysis is well validated in young adults, data on body-composition assessment in elderly subjects are still rare (17, 22, 23). We chose to use the age-specific equation proposed by Hughes and Evans (17), because in our experience, and as was recently shown by Reilly et al (23), the equation of Deurenberg et al (22) systematically overestimates fat mass. A preliminary study conducted in our laboratory on six subjects comparing total body water estimated by 18O dilution and by various bioelectrical impedance analysis equations, confirmed that Hughes and Evans’ equation gives the best approximation. In addition, our values are in good agreement with FFM obtained by other methods in recent studies (6–8). When the fluxes are expressed per unit of lean body mass, they become similar in young and elderly subjects, indicating that the metabolic activity of lean body mass does not change with age, at least in terms of protein turnover. This does not preclude a possible redistribution of this activity between various tissues.

Feeding resulted in increased flux and oxidation, decreased protein breakdown, unchanged protein synthesis, and a positive leucine balance. Although the inhibition of protein breakdown by feeding is reported by all researchers (18, 24–26), the variations of protein synthesis are more controversial. Most authors, including ourselves (18, 25, 26), find no modification of whole-body protein synthesis in response to feeding, except when protein intake is particularly high (27, 28). This was confirmed recently in a study comparing postabsorptive- and fed-state protein kinetics in elderly subjects receiving growth hormone (29). However, Welle et al (6) reported a 25% stimulation of NOLD by feeding both in young and old men, which might be attributed to a slightly higher protein intake, to a different study design, or both; leucine kinetics were measured on 2 separate days in the postabsorptive and fed state in this latter study.

In addition, important methodologic issues must be raised concerning the accurate measurement of leucine oxidation (on which NOLD and balance depend). First, carbon dioxide recovery has not been measured by us or others in elderly subjects, and possible differences in bone mass could result in a different carbon dioxide recovery in this population. Second, leucine oxidation was initially assessed with an intravenous tracer during feeding. It was shown previously that little (~2%) dietary leucine is oxidized on its first splanchnic pass in young adults who had splanchnic extractions of 20% (16). Then, leucine oxidation is similar whether the [13C] tracer is given orally or intravenously.

Things might be different in elderly subjects who had a high splanchnic extraction: if a significant fraction of dietary leucine is oxidized on its first pass, this would not be assessed by the intravenous [13C] tracer and would lead to an underestimation of total leucine oxidation. Therefore, to test this hypothesis we studied three of the older men again, giving the [13C] leucine orally during the meal. However, it was not possible to study the subjects who had the highest splanchnic extraction and who would have been the most informative. In addition, and for unknown reasons, the postabsorptive oxidation values of these subjects were somewhat lower in the second study despite similar fluxes. However, the results support the initial hypothesis: with the oral [13C] tracer, the increase over baseline of leucine oxidation during feeding was much larger and, in the subject who had the highest splanchnic extraction (ie, 50%), postprandial leucine oxidation was doubled (0.75 compared with 0.43 μmol·kg⁻¹·min⁻¹). Therefore, we would conclude that postprandial leucine oxidation in the elderly subjects was probably underestimated by the intravenous [13C] tracer, thus leading to an overestimated leucine balance in this group, which is in keeping with the observed neutral nitrogen balance, and 2) the negative relation between splanchnic extraction and leucine oxidation may well be artifactual due to the uncertainty of the oxidation measurement.

The main finding of this study is that splanchnic extraction of dietary leucine increases with age. We chose, as most authors have (15, 16, 18), to calculate splanchnic extraction using the leucine and not the KIC enrichments. Because most (65% in dogs; 30) of the dietary leucine that appears in the systemic circulation is not transaminated in the splanchnic area, the labeled KIC derived from the enteral tracer is not representative of the splanchnic fate of the dietary leucine. In any case, splanchnic extraction calculated with KIC enrichments gave similar qualitative results (15.3 ± 3.7% compared with 39.3 ± 11.5%, young compared with old men, respectively, P < 0.05). Our values in young subjects are consistent with previously published values, ie, 15–25% of the ingested leucine being taken up by the liver or gut (16). By contrast, splanchnic extraction was twice as high in the elderly men and also much more variable. This certainly corresponds to an uptake by the gut or liver and not to a malabsorption of the ingested amino acids; none of the subject had clinical evidence of nitrogen malabsorption, and fecal nitrogen represented 14 ± 3% of the mixed-protein diet during the period of nitrogen balance.

Such a high splanchnic extraction could have three possible explanations. First, it could result from an increased leucine oxidation in the gut, liver, or both in elderly men. A second possibility is increased dietary leucine utilization for hepatic protein synthesis in elderly men. The age-related changes in total hepatic protein synthesis are equivocal, with data indicat-
ing either an age-related decline (31), no change (14), or an increase (32). Several reports also suggested that albumin synthesis increases in aged animals (33, 34), although the only study examining albumin synthesis in a limited number of elderly humans showed little age-related variations (35). This question could be addressed by using the $\text{C}^{13}$ tracer method available now (36). Finally, it is possible that an increased amount of dietary leucine is transaminated in the gut or liver. An indirect measurement of splanchnic leucine transamination is the ratio of KIC to leucine for the oral tracer. Our value in young men (0.77) is slightly lower than other reported values (ranging from 0.78 to 0.95: 15, 16, 19, 20, 37). Still, it is higher than the corresponding ratio for the intravenous tracer, as reported by us and others (15, 16, 28). Interestingly, the ratio of deuterated KIC to leucine enrichment is higher in old than in young men, which supports the hypothesis of a higher splanchnic transamination in elderly men. To our knowledge, transaminase activity, which is predominant in the gut rather than in the liver, was never assessed in old animals.

Whatever the fate of dietary leucine within the splanchnic bed, a high splanchnic extraction implies that less dietary leucine reaches the systemic circulation. This fact has potential consequences for the modeling of leucine kinetics and particularly for the estimation of whole-body protein breakdown. For example, in two of our elderly subjects, total leucine flux increased so little during feeding that protein breakdown estimated as flux − intake turned out to be negative. In these two subjects, splanchnic extraction was very high (63% and 88%) and only the difference between total leucine flux and leucine intake corrected for splanchnic extraction yielded a meaningful index of protein breakdown. Thus, we believe that under circumstances in which splanchnic extraction is high, only this corrected flux gives a reliable index of protein breakdown (Table 2).

It is also noticeable that the higher the splanchnic extraction, the lower the peripheral leucine concentration (Figure 2). Systemic leucine concentration results from an equilibrium between leucine production (protein breakdown and dietary leucine escaping the splanchnic tissues) and utilization (for protein synthesis and oxidation). A high splanchnic extraction could therefore be responsible for a lower postprandial leucine concentration. This would in turn induce a lower peripheral oxidation because a strong relation between leucine concentration and oxidation was shown previously by us (28) and others (38), and because branched-chain amino acids are potent stimulators of the branched-chain keto acid dehydrogenase (39), the rate-limiting enzyme for leucine oxidation. Furthermore, it is tempting to speculate, in keeping with data in rodents (12), that a lower systemic amino acid concentration could be responsible for a lesser stimulation of muscle protein synthesis during feeding because high hyperaminoacidemia is the main stimulating factor for protein synthesis (27).

Finally, the reason for an age-related increase in splanchnic extraction remains to be determined. We found a positive correlation between splanchnic extraction and body fatness as assessed by body mass index ($r = 0.861, P < 0.001$). We have no explanation for this fact, and, to our knowledge, splanchnic extraction of dietary amino acids has never been examined in obese patients. However, a study indicated that amino acid extraction by the liver is elevated in genetically obese rats, which might be due to the large liver size of these animals (40).

In conclusion, the main finding of this study is a higher leucine splanchnic extraction in elderly men, which might result in a lower availability of dietary leucine to the peripheral tissues. It could be speculated that in the case of low protein intake or increased protein requirement, this limited systemic availability of dietary amino acids could contribute to decreased muscle protein synthesis during feeding in elderly men.

We thank Michel Genest, Paulette Roussel, Liliane Morin, Elisabeth Verdier, Guy Manlihot, and Josyane Moinard for technical assistance, and Philippe Patureau Mirand for helpful discussion.

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