Duodenal vs. gastric administration of labeled leucine for the study of splanchnic metabolism in humans

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Creenn, Pascal, Francois Thuillier, Benjamin Rakatoaminina, Monique Rongier, Dominique Darmaun, and Bernard Messing. Duodenal vs. gastric administration of labeled leucine for the study of splanchnic metabolism in humans. J Appl Physiol 89: 573–580, 2000.—Low-rate (6 ml/h) intragastric infusion of stable, isotopically labeled amino acids is commonly used to assess the splanchnic handling of amino acids in humans. However, when used in the postabsorptive state, this method yields unreliable plasma isotopic enrichments, with a coefficient of variation >10%. In this metabolic condition, we confirmed in six subjects that an intragastric infusion of L-[1-13C]leucine at 6 ml/h yields an unreliable isotopic steady state in plasma amino acids with a coefficient of variation of 43 ± 12% (mean ± SD). In five additional subjects, we assessed the effects of 1) increasing the rate of delivery of a leucine tracer in an isotonic plasmalike solution at 240 ml/h into the gastric site, and 2) changing the site of infusion from gastric to duodenal with this same high rate of delivery. In contrast to the gastric route, and regardless of the rate of delivery, only the intraduodenal route allowed 1) isotopic plasma steady state (i.e., coefficients of variation were <10%: 5 ± 3%), and 2) reproducible leucine extraction coefficients (22 ± 5%). We conclude that an infusion site that bypasses the gastric emptying process, i.e., the duodenal route, along with delivery of a plasmalike solution, is necessary to reach isotopic steady state in plasma when labeled leucine is infused into the gastrointestinal tract in the postabsorptive state.

duodenum; stomach; stable isotopes

THE PRIMED, CONSTANT INTRAVENOUS (iv) infusion of amino acids labeled with stable isotopes has become the reference method for studying whole body protein kinetics in humans (23). L-[1-13C]leucine is the most commonly used tracer because it is possible to estimate rates of protein oxidation from expired breath 13CO2 measurement, whereas plasma [13C]leucine enrichment at steady state allows for the calculation of the leucine appearance rate, an index of protein breakdown. Indeed, during iv infusion, a steady state reflected by a coefficient of variation [CV = (SD/mean) × 100] <10% in plasma isotopic enrichment allows for the calculation of the kinetic parameters of protein metabolism using steady-state equations. The use of splanchnic catheterization, either in animals or humans, has demonstrated the important contribution of intestinal and hepatic tissues to whole body protein metabolism (33, 34). To assess splanchnic amino acid metabolism in a noninvasive fashion, the constant gastric infusion of a labeled amino acid has been used in conjunction with the iv infusion of another tracer of the same amino acid. This dual route of infusion allows the calculation of “first pass splanchnic extraction,” a reflection of the intestinal and hepatic utilization of the amino acid. With the assumption that there is no malabsorption of the tracer, which is unlikely because <1% of radioactivity of [14C]phenylalanine was found in stools after oral [14C]phenylalanine administration (5), the comparison between the plasma enrichments of the enterally and intravenously infused tracers will allow calculation of the splanchnic extraction coefficient (f). Nevertheless, there are difficulties in obtaining isotopic steady state via the gastric route, as evidenced by a CV >10% for plasma enrichment, especially in the postabsorptive state. For example, in one study, the CV was 40% with intragastric (ig) L-[1-13C]leucine (10), and Hoerr et al. (15) acknowledged that the variability of plasma enrichment tracer is higher with the ig than with the iv route. In most studies, an ig tracer infusion route was chosen, with the exception of two studies, in which duodenal routes of infusion were used (17, 19). In all but one of these studies (17), the flow rate of tracer solution infusion was similar to the iv tracer rate (i.e., 6–12 ml/h). When the oral route was chosen, the tracer was administered at 15- (24, 27) or 20-min (5, 6, 30, 31) intervals in a 200-ml glass of water in the postabsorptive state. The latter mode of oral tracer administration failed to achieve a satisfactory isotopic steady state as well, and the authors noted that unreliable plasma enrichment plateaus were obtained (24).

To 1) obtain reproducible f values and 2) compare different metabolic situations, it is important to achieve a robust steady state after enteral infusion of tracer. The aim of the present study was, therefore, to determine whether more reliable isotopic plateaus,

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with CVs ≤10% (i.e., similar to those obtained via the iv route), can be achieved in the postabsorptive state through modifications in the mode of enteral tracer delivery by either increasing the rate of delivery (240 vs. 6 ml/h) in the gastric site, or by changing the site of infusion from ig to intraduodenal (id) with this same high rate of delivery. Labeled leucine, a reference tracer for in vivo protein metabolism study, was used.

METHODS

Subjects

A total of 11, healthy male volunteers (n = 6, subjects 1–6 in protocol A, ig and iv administration of tracers; n = 5, subjects 7–11 in protocol B, ig, iv, and id administration of tracers), without previous metabolic or digestive diseases, ages 21–47 yr [32.0 ± 6.9 (SD) yr] were studied at the Saint-Lazare Hospital Nutrition Unit. They weighed 67–95 kg (72.0 ± 11.0 kg) with a height of 163–190 cm (177 ± 6.6 cm). Body mass index was 20.1–26.3 kg/m² (23.0 ± 2.3 kg/m²). The subjects were told of the purpose and risks of the study and gave their written consent in accordance with a protocol approved by the Paris-VII University ethics committee.

Materials

L-[1-13C]leucine (99% 13C), L-[5,5,5-2H₃]leucine (99% 2H₃), and NaH¹³CO₃ (99% 13C) were obtained from Tracer Technologies (Somerville, MA). Before each infusion, sterile solutions of the tracers, without previous metabolic or digestive diseases, ages 21–47 yr [32.0 ± 6.9 (SD) yr] were studied at the Saint-Lazare Hospital Nutrition Unit. They weighed 67–95 kg (72.0 ± 11.0 kg) with a height of 163–190 cm (177 ± 6.6 cm). Body mass index was 20.1–26.3 kg/m² (23.0 ± 2.3 kg/m²). The subjects were told of the purpose and risks of the study and gave their written consent in accordance with a protocol approved by the Paris-VII University ethics committee.

Fig. 1. Protocols for tracer infusion studies.

- Protocol A (6 subjects studied with simultaneous iv and ig infusions):

- Protocol B (5 subjects studied on separate periods: interval between infusions was 1-2 weeks):

iv: intravenous, ig: intragastric, id: intraduodenal.

Experimental Design

The protocol designs are depicted in Fig. 1. The night before each infusion, each subject ate dinner at 2000 and then fasted until the next morning. Before infusion of the isotopic tracers, which began at 0800, two short iv catheters were placed, one in a forearm vein for isotope infusion and the other in a superficial vein of the contralateral hand for blood sampling. During the sampling periods, the hand was placed in a heated box (air temperature = 60–65°C) to produce arterialized venous blood (8). At the beginning of each experiment, priming doses of NaH¹³CO₃ (0.2 mg/kg), to saturate the bicarbonate pool, and the equivalents of infusion for each amino acid tracer were injected iv. For the gastrointestinal experiments of protocol B, the priming doses were given via the gastrointestinal route. In every experiment, the subject’s head was placed under the canopy of an indirect calorimeter (MMC-Horizon; Beckman Instruments, Anaheim, CA), allowing continuous measurement of gas flows and carbon dioxide and oxygen pressures (18). Before tracer infusion was started, blood samples and three expired air samples were obtained for determination of basal, natural abundance of isotopic enrichment in plasma leucine, the α-ketoadic of leucine (KIC), and breath ¹³CO₂. Gas sampling was performed directly from the ventilated canopy as previously described (18), and carbon dioxide production was measured over 20-min periods. Expired air and arterialized blood samples were collected for the measurement of steady-state breath ¹³CO₂ and plasma leucine enrichments, respectively. Each blood sample was immediately centrifuged at 4°C and frozen at −20°C until analysis.
Intravenous administration of tracer. A routine, 4-h priming constant infusion (6 ml/h) of a saline solution with L-[1-13C]leucine at 4 μmol·kg⁻¹·h⁻¹ was given in protocols A and B. The tracer was administered through calibrated syringe pumps (Roucaire Laboratory, Vélizy-Villacoublay, France), and blood samples were taken every 20 min during the final 2 h of tracer infusion.

Digestive administration of tracers. To access the gastric and duodenal sites, we used a silicone feeding tube (3.3 mm or 10-Fr diameter, length = 125 cm; Vygon, Ecouen, France) that was weighted by 20 g of mercury metal in the duodenal studies. The position of the feeding tube tip, either in the stomach antrum or into the duodenum at Treitz’s flexure, was controlled by X-ray just before the beginning of the study, with the tube being fixed to the nostril to prevent movement. In these digestive experiments, tracers were infused in an isotonic plasmalike saline solution (14) (composed of 135 meq/l Na, 5 meq/l K, 110 meq/l Cl, 30 meq/l HCO3, osmolality = 280 mosmol/kgH2O, osmolality was verified before each infusion). In protocol A (n = 6), L-[2H₃]leucine was administered by the ig route and simultaneously infused at 6 ml/h with iv L-[1-13C]leucine. In protocol B (n = 5), the iv, ig, and id studies were separated by 1 or 2 wk, in random order. In protocol B, enteral tracer leucine was administered via either the ig or id routes and was infused at 240 ml/h. In protocol A, the gastric tracer L-[2H₃]leucine was administered at 4 μmol·kg⁻¹·h⁻¹ for 4 h. In protocol B, the gastric and duodenal tracer L-[1-13C]leucine was administered at 6 μmol·kg⁻¹·h⁻¹ for 6 h. To obtain a minimum of four data points for better calculation of the CV, blood samples were taken every 20 min during the ig studies of protocol A and every 30 min in the ig and id studies of protocol B.

In addition, after 4 h of iv infusion in three subjects of protocol A, L-[2-15N]glutamine (99% ¹⁵N; Tracer Technologies) was switched to ig infusion for 4 h and infused at 6 ml/h at 6 μmol·kg⁻¹·h⁻¹. In three subjects of protocol B, L-[2-15N]glutamine was separately administered iv, switched as in protocol A (12 μmol·kg⁻¹·h⁻¹ for 6 h) to the digestive tract (in the stomach antrum or into the duodenum at Treitz’s flexure), and infused at 240 ml/h in the isotonic plasmalike solution. Results of splanchnic L-[2-15N]glutamine metabolism on these preliminary experiments are presented in the DISCUSSION.

Analytic Methods

Stable isotope enrichments in plasma leucine and KIC were determined by electron-impact gas chromatography–mass spectrometry (GC-MS; model R1010T, Nermag, Argenteuil, France). Plasma leucine was analyzed as its N-trifluoroacetyl, n-butyl (TFAB) derivative and KIC as its quinoxalinol-trimethylsilyl (Q-TMS) derivative (25). Separate injections were used for leucine and KIC in the GC-MS. Isothermal programs at 150 and 190°C were used for TFAB-leucine and Q-TMS-KIC, respectively. Injections were made into a 0.22 mm × 25 m OVI capillary gas chromatography column (Spiral, Dijon, France) with a split ratio of 1/25 and helium as a carrier gas. Ions at mass-to-charge ratios (m/z) of 228 and 227, 185 and 182, and 233 and 232 were selectively monitored to quantitate the molar ratios of L-[1-13C]leucine to natural leucine isotope, L-[2H₃]leucine to natural leucine, and [¹³C]KIC to natural KIC, respectively. Plasma glutamine was analyzed as its N-acetyl, n-propyl (NAP) ester derivative (11). Isothermal program at 230°C was used for NAP-glutamine. Ions at m/z 187 and 186 were selectively monitored to quantitate the molar ratios of L-[2-¹⁵N]glutamine to natural glutamine. Enrichments were calculated from the background-corrected isotope ratios as previously described (23). The CV of the analytic method (repeatability, n = 10) was <2% for L-[1-¹³C]leucine, L-[2H₃]leucine, and L-[2-¹⁵N]glutamine, and <3% for [¹³C]KIC. Analyses of breath ¹³CO₂ were performed by automated gas chromatography-isotope ratio mass spectrometer (GC-IRMS; Tracer Mass, Europa Scientific, Crewe, UK).

Calculations

Leucine f (fₗₑᵤₕ) was calculated with plasma enrichment (Ep; mol %excess) after iv and digestive infusion with normalization by tracer infusion rate (22)

\[ fₗₑᵤₕ = 1 - \frac{Ep_{diq}/i_{siq}}{Ep_{iv}/i_{iv}} \]

in which Ep_{diq} is plasma enrichment after digestive infusion; i_{siq} is digestive tracer infusion rate (μmol·kg⁻¹·h⁻¹); Ep_{iv} is plasma enrichment after iv infusion and i_{iv} is iv tracer infusion rate.

Leucine splanchnic oxidation (fₗₐₓ) was calculated according to Matthews et al. (21)

\[ fₗₐₓ = \left[ \frac{F¹³C₇O₂_{siq}}{i_{diq}} - \frac{F¹³C₇O₂_{iv}}{i_{iv}} \right] \times (1 - fₗₑᵤₕ) \]

in which F¹³C₇O₂ is ¹³CO₂ flux from [¹³C]leucine oxidation

\[ F¹³C₇O₂ = \frac{ECO₂ \times VCO₂ \times 44.6 \times 60}{wt \times r \times 100} \]

in which ECO₂ is the ¹³C enrichment (mol % excess) in expired carbon dioxide; wt is the subject’s body weight; VCO₂ is total carbon dioxide production in milliliters; 44.6 converts ml CO₂/min to μmol/min; 60 is min/h; r is the estimated fraction of carbon recovered in expired air [i.e., 0.72 in the fasting state (15)]; and 100 converts enrichment to fractions of unity.

Statistical Analysis

To test the reliability of plateau enrichments in plasma and in expired ¹³CO₂, two methods were used: 1) CV of enrichment [(enrichment SD/mean enrichment) × 100], in which a CV ≤10% was considered consistent with the existence of a steady state (2, 34), and 2) linear regression analysis of enrichment vs. time, in which a slope different from zero was considered inconsistent with steady state. To test for differences in enrichment variances at plateau between the iv and ig methods in protocol A and the iv, ig, and id methods in protocol B, a two-way (subject × method) ANOVA (SPSS 7.5, Chicago, IL) was used. The significance level for statistical tests was established at P ≤ 0.05.

RESULTS

Enrichments in iv Experiments

A steady state was observed between 140 and 240 min, with a mean plasma enrichment CV of 7% (range: 2–13%) for L-[1-¹³C]leucine (Fig. 2) and 12% (4–22%) for [¹³C]KIC (n = 11, number of measurements = 5, range 4–6). Linear regression analysis in every subject showed a nonsignificant correlation of plasma enrichment vs. time. Intravenous infusion allowed a steady state in ¹³CO₂ breath enrichment (CV = 5%; 1–9% range) to be reached as well.
Enrichments and Splanchnic Protein Metabolism in Gastric and Duodenal Experiments

Protocol A: ig administration of leucine tracer at 6 ml/h. There was no isotopic enrichment steady state for plasma L-[2H₃]leucine enrichment, because the mean CV was 43% (range 22–54%, n = 6, number of measurements = 5, range 4–6; Fig. 3). In subjects 1 and 4, linear regression analysis of plasma L-[2H₃]leucine enrichment vs. time showed a slope significantly different from zero. Enrichment variance at plateau was significantly greater after ig administration than with iv (statistic F = 48.5, P = 0.001) administration. Leucine splanchnic extraction was 28% yet varied over a wide range (from −26 to 49%; see Table 1).

Protocol B: ig and id administration of leucine tracer at 240 ml/h. For gastric infusion (n = 4) there was no steady state in plasma isotopic enrichment, as the mean CV (number of measurements = 7, range 7–8) was 33% (range 27–43%) for L-[1-13C]leucine (Fig. 4A) and 38% (range 24–55%) for KIC. In contrast, isotopic plateaus were observed in id infusion (n = 4) between 150 and 240 min, extending to 360 min (Fig. 4B), with a mean CV (number of measurements = 7, range 6–8) at 5% (range 3–9%) and 12% (range 9–19%) for L-[1-13C]leucine and [13C]KIC, respectively. The ratio of [13C]KIC to L-[1-13C]leucine enrichment in plasma was not significantly different between iv, ig, and id administration (Table 2). Linear regression analysis of plasma L-[1-13C]leucine enrichment vs. time showed that, in ig, subjects 9 and 11 had slopes significantly different from zero, with a 100% variation of enrichment, whereas this was not the case in any of the four id infusion experiments. Enrichment variance at plateau was significantly greater during ig administration than during iv (F = 41.9, P < 0.01) or id (F = 46.7, P < 0.01) administration. No difference was found between iv and id infusion (F = 2.5, P = 0.21). The mean values for f_{Leu} calculated for gastric (f_{iv/ig}) and duodenal infusion (f_{iv/id}; Table 1), were of the same order of magnitude, both being 22%. For splanchnic leucine extraction, there was a large variation be-
between subjects with ig vs. id infusion (21–54% vs. 14–26%). In contrast, both ig and id infusion allowed for a steady state in breath $^{13}$CO$_2$ enrichment with a CV of 11 and 8%, respectively. During ig infusion, $f_{ox}$ reached negative values in three out of four cases, whereas $f_{ox}$ was 2% after id infusion (range 23 to 6%), with a negative value in only one out of four subjects (Table 1).

### DISCUSSION

The present study demonstrates that, in the postabsorptive state, infusion of stable isotope-labeled leucine into the gastrointestinal tract yields a plateau of plasma isotopic enrichment (i.e., steady state), and a precise and reproducible determination of $f$ results when the tracer is infused via the duodenal route. In

<table>
<thead>
<tr>
<th>Route of Digestive Tracer</th>
<th>Rate of Tracer Delivery, ml/h</th>
<th>CV of Plasma Leucine Enrichment, %</th>
<th>Subjects with Slope Different from 0*</th>
<th>$f$, %</th>
<th>$f_{ox}$, %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Protocol A</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intragastric</td>
<td>6</td>
<td>43 ± 12 (22–54)</td>
<td>2/6</td>
<td>28 ± 28 (–26 to 49)</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Protocol B</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intragastric</td>
<td>240</td>
<td>33 ± 7 (27–43)</td>
<td>2/4</td>
<td>22 ± 29 (–21 to 54)</td>
<td>–1 ± 2 (–3 to 2)</td>
</tr>
<tr>
<td>Intraduodenal</td>
<td>240</td>
<td>5 ± 3 (3–9)</td>
<td>0/4</td>
<td>22 ± 5 (14–26)</td>
<td>2 ± 3 (–3 to 6)</td>
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</tbody>
</table>

Coefficient of variation (CV), splanchnic extraction coefficient ($f$) and leucine splanchnic oxidation ($f_{ox}$) values are means ± SD, and ranges are in parentheses. *Slope of plasma enteral leucine enrichment vs. time studied by linear regression analysis; with this technique, a slope significantly different from 0 indicates the unreliability of plateau. ND, not determined because the enterally infused tracer was deuterium labeled.

![Fig. 4. Plasma L-[1-$^{13}$C]leucine enrichment during L-[1-$^{13}$C]leucine gastric (A) and duodenal (B) administrations at 240 ml/h.](http://jap.physiology.org/)
Table 2. Tracer enrichments at plateau in plasma

<table>
<thead>
<tr>
<th>Route</th>
<th>n</th>
<th>Mole percent excess; KIC/Leu</th>
<th>iv</th>
<th>2.80 ± 0.40</th>
<th>2.24 ± 0.55</th>
<th>0.79 ± 0.10</th>
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<td>iv</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>ig</td>
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<td></td>
<td></td>
<td>1.94 ± 0.88</td>
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</tbody>
</table>

Protocol A

<table>
<thead>
<tr>
<th>Route</th>
<th>n</th>
<th>Mole percent excess; KIC/Leu</th>
<th>iv</th>
<th>3.74 ± 0.34</th>
<th>2.93 ± 0.53</th>
<th>0.78 ± 0.10</th>
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<tr>
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<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ig</td>
<td>4</td>
<td></td>
<td></td>
<td>4.11 ± 1.48</td>
<td>2.84 ± 1.41</td>
<td>0.69 ± 0.15</td>
</tr>
<tr>
<td>id</td>
<td>4</td>
<td></td>
<td></td>
<td>4.54 ± 0.34</td>
<td>3.45 ± 0.59</td>
<td>0.76 ± 0.10</td>
</tr>
</tbody>
</table>

Data are mean enrichments of each subject at the time of plateau of each period averaged together and presented as means ± SD. mpe, Mole percent excess; n, no. of subjects studied in protocol; iv, intravenous infusion; ig, intragastric infusion; id, intraduodenal infusion; KIC, α-ketoacid of leucine; Leu, leucine; KIC/Leu, ratio of KIC to Leu.

Table 3. Splanchnic l-[2-15N]glutamine metabolism in postabsorptive state in healthy adult men

<table>
<thead>
<tr>
<th>Route of Digestive Tracer</th>
<th>Rate of Tracer Delivery, ml/h</th>
<th>CV of Plasma l-[2-15N] glutamine Enrichment, %</th>
<th>Subjects with Slope Different from 0*</th>
<th>f, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protocol A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ig</td>
<td>6</td>
<td>53 ± 22 (31–76)</td>
<td>1/3</td>
<td>60 ± 22 (34–75)</td>
</tr>
<tr>
<td>Protocol B</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ig</td>
<td>240</td>
<td>49 ± 12 (36–59)</td>
<td>1/3</td>
<td>53 ± 6 (49–57)</td>
</tr>
<tr>
<td>id</td>
<td>240</td>
<td>6 ± 7 (2–15)</td>
<td>0/3</td>
<td>52 ± 2 (50–53)</td>
</tr>
</tbody>
</table>

For CV and f the values are means ± SD and ranges are in parentheses. *Slope of plasma enteral l-[2-15N]glutamine enrichment vs. time studied by linear regression analysis; with this technique, a slope significantly different from 0 indicates the unreliability of plateau.

contrast, the gastric route of infusion, regardless of the rate of infusion (6 or 240 ml/h), does not allow steady state to be reached.

The 240 ml/h (4 ml/min) rate was used because it is the known physiological rate of gastric emptying for nonnutritional solutions (13). Indeed, the kinetics of gastric emptying after administration of calorie-free liquids depend on volume and osmolarity. Our data suggest that the high variability of plasma enrichments with gastric infusion in the postabsorptive state is accounted for by known aspects of digestive physiology. The 6 ml/h rate of administration was probably too low to ensure a constant rate of gastric emptying for the tracer solution; therefore, we speculate that the variability of plasma enrichment in the postabsorptive state is likely due to changes in absorption kinetics related to changes in gastric emptying and digestive motility (26, 34). Although neither manometry of gastric tone nor assessment of small bowel motility was performed in the present study, the infusion of normal saline at a low, regular rate (5 ml/min) into the stomach is known to be associated with pulsatile gastric emptying in relation to antropyloric motility modifications in the pig (20). In humans, the gastric emptying of 50 or 200 ml of a calorie-free liquid is influenced by time of administration (26), because the cycle of interdigestive migrating myoelectric complexes ranges from 15 min to 3 h. Phase III of each migrating myoelectric complex increases gastric emptying during its average 8-min duration. During enteral feeding, the plasma isotopic enrichment of the ig-infused tracer had a CV of 15% (11). It can be postulated that this was due to regular gastric emptying, as observed during continuous enteral nutrition. The present study demonstrates that the digestive infusion of stable isotope-labeled leucine should not use the gastric route in the postabsorptive state, even under optimized conditions chosen to take gastrointestinal motility into account. The duodenal route of infusion was chosen because this route bypasses gastric emptying.

The duodenal tube was well tolerated without abdominal discomfort, vomiting, or diarrhea. We did not attempt to use a low rate of duodenal infusion because postabsorptive digestive motility would have compromised tracer absorption, as demonstrated by a variable oroacal transit time measured by Di Lorenzo et al. (12) with a hydrogen breath test after lactulose duodenal low-dose administration (i.e., 15 g in 10 ml of water). A plasmalike electrolyte solution was chosen because it mimics the composition of intestinal fluid at Treitz's flexure, prevents digestive secretion, and allows absorption of water and electrolytes from intestinal lumen into portal blood (13). The differences between the gastric and duodenal routes of tracer infusion are unlikely to reflect differences in absorption kinetics. If this were the case, results would differ between amino acids. In fact, preliminary experiments carried out in three subjects with labeled glutamine tracers (Table 3) revealed, compared with leucine, the same trend for glutamine, even though the absorption rate constants and transport mechanisms are different and rely on different transporters in the small intestine (28). That large and small CVs in plasma enrichment were observed after ig and id infusion, respectively, for both leucine and glutamine, suggests that our results are applicable to amino acids with different f values (22).

The present study confirms the previously documented f of 22% for l-[1-13C]leucine (15, 21). Nevertheless, splanchnic extraction seems to be variable in the literature, especially in the postabsorptive state between subjects (15), as well as between the postabsorptive state and the fed state (8, 10, 19) or as a function of age (4). In our study, digestive experiments were extended to a 6-h duration for two reasons. A delay in the rise in plasma tracer enrichment was expected because the enteraly infused tracer had to cross multiple membranes on its way through the splanchnic tissues. In fact, the time needed to reach plateau was the same between iv and id infusion. Although many of
the proteins synthesized in splanchnic tissues have a fast turnover (21), there was no evidence of leucine recycling during the course of a 6-h tracer infusion because there was no rise in enrichment by the end of the experiment in the studied subjects. Isotopic plateau in expired $^{13}$CO$_2$ breath air was comparable for all experiments. This was expected because of the slow kinetics of CO$_2$, a "terminal" metabolic product. With $f_s$, we were able to estimate the first pass $f_{ox}$, as described (22). Calculation of $f_{ox}$ yielded negative values with ig infusion, but, with id infusion, the mean $f_{ox}$ value was 2%, which was close to the 1.9% obtained by others in postabsorptive humans (21) and similar to the 3.5% determined by catheterization in the dog (34). This suggests that the splanchnic oxidation of leucine is low, consistent with the view that leucine is not a major fuel for the intestines and liver.

Based on 28 and 5% SDs for $f_{Leu}$ via the ig and id infusions, respectively, a tentative study designed to detect a 10% change in leucine splanchnic extraction with a 90% power would have to enroll 165 subjects with the gastrid route vs. only 6 subjects with the duodenal route of tracer delivery. Duodenal tracer infusion should therefore be suitable for in vivo exploration of splanchnic protein metabolism in humans in the postabsorptive state, even though calculation of splanchnic extraction is an indirect method for assessing splanchnic protein metabolism. The direct method for assessing in vivo protein intestinal metabolism requires biopsies to calculate fractional synthetic rate, i.e., the percentage of proteins synthesized over a given time period in a specific protein or tissue. For the small intestine, endoscopic biopsies can be performed after duodenal route of tracer delivery. The choice of tracer infusion route is important to consider because the major source of amino acids used for protein synthesis in enterocytes (1) is still controversial (7, 25).

In summary, our results suggest that the use of tracer infusion via the gastrointestinal tract to explore protein metabolism should ideally bypass the effect of gastric emptying in the postabsorptive state. To obtain a reliable steady state in amino acid enrichment, tracer infusion should be postpyloric. This technique would allow the effects of fasting and feeding on splanchnic protein metabolism to be reliably studied. This seems necessary for proper comparisons between healthy subjects and patients with pathological conditions, for comparisons between different nutritional regimens, and to study the fractional rate of protein synthesis in splanchnic tissues, such as small bowel mucosa.

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