Gut mucosal protein synthesis in fed and fasted humans

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Bouteloup-Demange, Cormine, Yves Boirie, Pierre Déchelotte, Pierre Gachon, and Bernard Beaufre (1). Gut mucosal protein synthesis in fed and fasted humans. Am. J. Physiol. 274 (Endocrinol. Metab. 37): E541–E546, 1998.—Fractional protein synthesis rate (FSR) of duodenal mucosa was measured in two groups of six healthy young men, either in the fed state at the end of a 10-day standardized diet or after a 36-h fast. Protein synthesis rate was measured during a 9-h intravenous infusion of [13C]leucine and [3H2]phenylalanine. The fed group also received an intragastric tracer, [13C]leucine, mixed with the liquid diet. At the end of the tracer infusion, an endoscopy was performed to take duodenal mucosal biopsies. The major results were that 1) duodenal mucosal protein synthesis was high, 48.0 ± 8.5% (SE)/day by use of intravenous leucine tracer and intracellular leucine enrichment; 2) it was not affected by feeding whatever the tracer or the precursor pool used for the calculations; 3) the two intravenous tracers gave different FSR values; and 4) with the intragastric tracer, FSR was 25–220% of the rate calculated with the intravenous tracer, depending on the precursor pool used for the calculation. Thus absolute values of FSR should be taken with caution, because they depend on the precursor pool chosen, the route of tracer administration, and the tracer itself. However, gut mucosal protein synthesis as assessed by an intravenous tracer is not affected by feeding in humans.

protein turnover; duodenum; stable isotopes; leucine; phenylalanine

MODIFICATIONS OF PROTEIN TURNOVER induced by feeding have been widely investigated at the whole body level in humans (see review in Ref. 18). By contrast, there are fewer data regarding the response to feeding in specific human body tissues. In muscle, which is the most abundant deposit of protein, the effects of feeding on fractional protein synthesis rate (FSR) are controversial; some studies have shown a stimulation (27, 31), whereas another has shown no significant change (17). In liver and the main hepatic exported protein, albumin, studies are in agreement and show an increase in protein synthesis with feeding (9, 12). The third tissue of major quantitative importance in whole body protein turnover is the gut. In humans, gut mucosal protein synthesis has been measured only in the postabsorptive state, either in normal subjects (21) or in subjects with intestinal diseases (20, 25). Studies in growing animals have shown an increase of intestinal protein synthesis during feeding (8, 19), but the only study in adult animals has shown no modification (29).

The primary aim of this study was to measure the fractional rate of gut mucosal protein synthesis in healthy humans in the fed and fasted states. Amino acids used for gut protein synthesis, however, may be derived from either the luminal or vascular side; thus the secondary aim was to assess the route of administration, i.e., intragastric or intravenous, of the amino acid tracers ([13C]leucine and [3H2]phenylalanine) used to measure gut protein synthesis. These studies were conducted in the fed state. The rate of protein synthesis may also depend on the choice of amino acid used for the tracer (24); thus a tertiary objective was to determine whether similar results are obtained using two intravenous amino acid tracers ([13C]leucine and [3H2]phenylalanine). Also, when a deuterated tracer is used, tracer incorporation can be measured by gas chromatography-mass spectrometry (GC-MS), a method which is simpler than isotopic ratio mass spectrometry (IRMS), which is needed for detecting low amounts of [13C]leucine in gut mucosal protein.

MATERIALS AND METHODS

Subjects, diet, and experimental design. Seven healthy men participated in the study. They were 22.6 (18–25) yr old and had a body mass index of 19.9 ± 1.7 (SE) kg/m2. The study was approved by the local ethical committee, and the subjects gave their written informed consent. All subjects were in good general health, without any hepatic, renal, digestive, or cardiac dysfunction, and none was taking medication on a regular basis. At the beginning, the resting energetic expenditure (REE) of each subject was measured by indirect calorimetry (Deltatrac MBM 100, Metabolic Monitor, Datex Instrumentation, Helsinki, Finland), and an activity and dietary questionnaire was done. Two groups of six volunteers were studied either in the fed state at the end of a 10-day standardized diet (fed group) or after a 36-h fast (fasted group). Five volunteers participated in the two studies; the order of the studies was randomized, and they were separated by ≥1 wk. Two more volunteers each participated in a single study. The 10-day controlled diet provided 1.4 g protein·kg−1·day−1 and 1.5–1.7 × REE, depending on the usual activity level. The fat content was 1.5 g·kg−1·day−1 (30–35% of total energy intake). During the last three days, foods known to have a high natural 13C abundance were excluded from the diet in all volunteers. All meals were prepared and consumed in the laboratory during the feeding experiment. During the 36-h fast, subjects remained under medical supervision in the clinical study unit.

Measurement of protein synthesis. The isotope study was carried out between 0800 and 1700, after a 13-h overnight fast in the fed group or after a 36-h fast. After collection of baseline blood samples, a primed (equivalent to 1 h of infusion) continuous intravenous infusion of tracer was started at rates of 0.06 (fast) or 0.10 (fed) µmol·kg−1·min−1 for L-[1-13C]leucine [98.8 mole percent enrichment (MPE), Euriso-top, Saint-Aubin, France], and 0.05 (fast) or 0.08 (fed) µmol·kg−1·min−1 for L-[ring-2H5]phenylalanine (97.4 MPE, Tracer Technologies, Sommerville, MA). The tracers were tested for sterility and a pyrogenicity. In the fed group, the subjects drank 80 ml of a liquid diet every 30 min during the

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first 8 h of tracer infusion; this provided 30 ± 2 kcal/kg and 1.00 ± 0.02 g protein/kg, which was equivalent to two-thirds of the daily food intake of the ten previous days. The diet was made of protein (Protiphar (80% casein, 20% whey), Nutricia, Rueil Malmaison, France), oil (ISIO 4, Lesueur, Boulougne-Billancourt, France), and potato dextrin (Avebe France, Corbey-Essones, France). L-[5,5,5-2H]leucine (97 MPE, Eurisotop, Saint-Aubin, France) was mixed with the liquid diet, and then 6 µmol/kg were given every 30 min. The rate of administration of the oral tracer was twice as high as that for the intravenous tracer to facilitate measurements of the enrichments in mucosal proteins. Feeding and the intragastric tracer were stopped 1 h before the end of the intravenous tracer infusions to perform the upper endoscopy under safe conditions.

Blood was sampled from a vein in the contralateral hand after "arterialization" (the hand was placed in a heating box) before infusion (baseline sample) and at regular intervals during the infusion. Breath samples were also collected in a Douglas bag and then transferred into Vacutainers (Becton-Dickinson, Grenoble, France) before the tracer infusion and at regular intervals during the last 90 min of infusion. Carbon dioxide production was measured by indirect calorimetry during four 20-min periods in the last 90 min of tracer infusion. At the end of stable isotope infusion (i.e., time 9 h), an endoscopy was performed (Olympus XQ10, Japan). In the fed group, the absence of significant gastric residue was checked. No aspiration was necessary. Distal duodenal mucosal biopsies (in the second duodenum, after the papilla) were taken. Five or six separate samples were necessary to obtain 20–30 mg of tissue. The mucosal tissue samples were frozen in liquid nitrogen immediately after removal and stored at −80°C until analyzed.

Sample analysis. The enrichments of plasma free amino acids and metabolites ([13C]leucine, [13C]ketoisocaproate ([13C]KIC), [13H3]leucine, [13H3]KIC, and [13H3]phenylalanine were measured by means of standard GC-MS (MSD 5971, Hewlett-Packard, Palo Alto, CA) with tert-butylidimethylsilyl (t-BDMS) derivatives, as previously described (7). Calculations of the [13H3]KIC enrichment were corrected for the participation of [13C]KIC enrichment (4). 13C enrichments of CO2 in breath samples were measured by IRMS (VG Isotech, Fisons Instruments, Middlewich, UK). Mucosal tissue samples (20 mg) were processed as described by Preedy and Garlick (26). Briefly, mucosal tissue samples were carefully rinsed with ice-cold 0.9% NaCl and ground; proteins were precipitated with 10% trichloroacetic acid (TCA). The protein pellet was rinsed three times with 10% TCA. Free amino acids were isolated from the pooled supernatants, and their enrichment was measured by GC-MS. The pellet was dissolved in 1 M NaOH. One aliquot of this was used for the measurement of the deuterated tracers by GC-MS and the other was used for the measurement of the [13C]leucine enrichment by GC-combustion-IRMS. The former was hydrolyzed in 6 M HCl at 110°C for 18 h (preliminary experiments showed that hydrolysis is complete by 18 h for leucine and phenylalanine and that further hydrolysis results in a loss of deuteria on the phenylalanine ring; data are not shown). For the latter, a 24-h hydrolysis was used under the same conditions. Enrichments were determined using the t-BDMS derivatives for GC-MS and N-acetyl-propyl ester derivatives for IRMS, as previously described (6). Appropriate standard curves (0.1 to 1.2 MPE for [13H3]leucine, 0.5 to 1.1 MPE for [13H3]phenylalanine, and 0.2 to 0.4 MPE for [13C]leucine) were run simultaneously.

Calculations. For whole body protein metabolism, all kinetic data were determined at steady state with [13C]leucine and [13H3]phenylalanine. For leucine, the reciprocal pool model was used for calculations (30). Total leucine flux, an index of whole body protein turnover, was calculated as the ratio of whole body lipid turnover (corrected for isotopic purity) divided by the plasma [13C]KIC enrichment. This value includes the tracer administration. Endogenous leucine flux, an index of whole body protein synthesis, was equal to total leucine flux minus the tracers and minus the oral leucine intake. For oxidation calculations, recovery factors of 0.7 and 0.82 were used in the fasted and fed states, respectively (11). Nonoxidative leucine disposal (NOLD), an index of whole body protein synthesis, was the difference between total flux and oxidation. For phenylalanine, total phenylalanine flux and endogenous phenylalanine flux were calculated with the same equations, but with plasma [13H5]phenylalanine enrichment as the precursor pool. The fractional synthesis rate of duodenal mucosal protein (FSR, in %/day) was calculated as follows (21, 25)

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FSR (%/day) = \frac{E(t) - E(0)}{t} \times \frac{1}{Ep} \times 60 \times 24 \times 100
\]

where \(Ep\) is the enrichment of the precursor pool at plateau in %, \(t\) is the duration of the infusion of tracer in min, \(E(t)\) is the enrichment, in %, in the intestinal mucosal proteins at time \(t\), and \(E(0)\) is the natural abundance of the labeled amino acid in mucosal proteins, in percent. The precursor pool used was either plasma free amino acid or ketoisocaproate, or the intracellular free amino acid pool. Because it was difficult to perform a gastroscopy at baseline in the participating subjects, the baseline isotopic enrichment was determined in normal duodenal biopsies from 12 ambulatory patients undergoing upper endoscopy for medical reasons. There was little interindividual variation of natural abundance (0.000 ± 0.002 MPE for [13H3]phenylalanine, −0.030 ± 0.025 MPE for [13H5]leucine, and −0.038 ± 0.003 MPE for [13C]leucine).

Statistical analysis. All results are expressed as means ± SE. To evaluate the effects of the precursor pool, the tracer, and feeding or fasting state on whole body protein kinetics and gut FSR, statistical differences were assessed using a repeated-measures analysis of variance followed by Scheffé's test. In the fed group, to compare the gut FSR calculated with the intravenous and intragastric tracer, the statistical differences were assessed using a paired Student's t-test.

RESULTS

In all studies, steady state for concentrations and enrichments of leucine and phenylalanine and for CO2-13C enrichments were obtained 2 and 4 h after the beginning of the isotope study for the intravenous and intragastric tracers, respectively (data not shown).

Whole body protein metabolism. Total leucine flux and oxidation increased during feeding (2.10 ± 0.08 vs. 2.65 ± 0.12 µmol·kg⁻¹·min⁻¹, \(P < 0.01\), and 0.57 ± 0.05 vs. 0.90 ± 0.04 µmol·kg⁻¹·min⁻¹, \(P < 0.01\), respectively; Fig. 1). Endogenous leucine flux decreased with feeding (2.03 ± 0.08 vs. 0.76 ± 0.11 µmol·kg⁻¹·min⁻¹, \(P < 0.01\)), whereas NOLD was unaffected (1.54 ± 0.07 vs. 1.76 ± 0.09 µmol·kg⁻¹·min⁻¹, \(P > 0.05\)). Similar qualitative results were obtained when [13C]leucine was used instead of [13C]KIC enrichment as precursor pool (data not shown). Total phenylalanine flux increased (0.70 ± 0.04 vs. 0.90 ± 0.05 µmol·kg⁻¹·min⁻¹, \(P < 0.05\)) and endogenous phenylalanine flux decreased with feeding (0.64 ± 0.04 vs. 0.17 ± 0.05 µmol·kg⁻¹·min⁻¹, \(P < 0.01\)).
Duodenal mucosal protein synthesis. For the intravenous tracers, the enrichments in the different precursor pools are reported in Table 1. During feeding or fasting, intracellular free leucine enrichments were 45 and 59%, respectively, and intracellular phenylalanine enrichments were 29 and 34%, respectively, of the plasma enrichments. KIC enrichments were 81 and 89% of plasma leucine enrichments during feeding and fasting, respectively. The FSR values calculated with the different precursor pools are reported in Table 2. When leucine was used as the tracer, FSR calculated using the intracellular enrichments was twofold higher than that using the plasma amino acid or plasma KIC enrichments. When phenylalanine was used as the tracer, FSR calculated using the intracellular enrichments was threefold higher than that using the plasma enrichments. When intracellular enrichment was used as the precursor pool, FSR was twofold higher when phenylalanine compared with leucine was used as the intravenous tracer (P < 0.001). Whatever the tracer or the precursor pool used for the calculation, FSR of mucosal proteins was not affected by feeding.

For the intragastric tracer ([2H3]leucine) (Table 1), which was given only during the feeding experiment, the plasma leucine enrichment (when normalized for the tracer infusion rate) was 26% lower than that of the intravenous tracer; this corresponds to the first-pass splanchnic extraction of the oral leucine. Tracer incorporation into proteins was higher than with the intravenous tracer, even after normalization for the different tracer infusion rate, 0.46 vs. 0.27 MPE. Therefore, FSR was higher when measured with the intragastric than with the intravenous tracer when the plasma leucine pool was used as precursor. The intracellular enrichments of [2H3]leucine were averaging 36 MPE, resulting in a low FSR calculated with this precursor pool (Table 2).

**DISCUSSION**

Calculation of an FSR depends on the choice of the precursor amino acid pool. This problem is solved partly by use of the flooding dose method. However, this method is controversial, because it could induce by itself an increase in protein synthesis (28). Also, it would be impossible to do a flooding dose with an intragastric tracer. Aminoacyl-tRNA is the true precursors.
ror of protein synthesis, but its labeling is technically difficult to measure, and such a determination was impossible on small gut biopsies. Various surrogates can be used, which in the case of leucine are plasma leucine, plasma KIC, or intracellular free leucine. For the intravenous tracer, intracellular free leucine enrichment was quite low in our experiment, representing only 45–60% of the plasma leucine enrichment; this is consistent with previous reports on gut in humans (21) or other tissues in animals (3, 24). This is probably due to a high dilution of the label by a large amount of unlabeled amino acids derived from rapid proteolysis in gut. However, labeling of aminoacyl-tRNA probably derives from both the intracellular free amino acid pools (1, 16), and therefore it is likely that the true FSR of duodenal mucusosal protein lies between the values calculated with plasma and intracellular leucine, i.e., 20 and 40%/day. The values that we obtained during short-term fasting are similar to the few data available in humans in the postabsorptive state by use of an intravenous leucine tracer (21, 25). Nakshabendi et al. (21) found an intestinal mucosal FSR of 62%/day (with intracellular leucine as the precursor), and O’Keefe et al. (25) found a value of 39%/day (with plasma KIC as the precursor). Thus we confirm that gut mucosa has a high rate of protein synthesis.

The problems arising from the choice of a precursor pool affect the assessment of protein synthesis in all tissues. In the gut, there is another inherent problem: do the amino acids utilized for mucosal protein synthesis originate from the luminal or basolateral (i.e., vascular) side? In fed or fasted rats (2, 10), the luminal pool appears to be predominant. On the contrary, in fed lambs (14), a greater contribution comes from the vascular pool. In fasted humans studied by means of a simultaneous intragastric and intravenous tracer infusion, Nakshabendi et al. (21) recently suggested that both pools participate in gut protein synthesis. We administered an oral tracer only during feeding, because we failed to obtain an appropriate plasma steady state during fasting. Indeed, in preliminary experiments, subjects received labeled leucine given either as small repeated doses (every 20 min) or continuously through a nasogastric tube over 8 h in the postabsorptive state. Over this period, plasma enrichments varied widely between two consecutive sampling times, sometimes by a factor of six, which we attributed to sudden gastric emptying of small amounts of tracer accumulating in the stomach. An apparent steady state is obtained by averaging the values of all the subjects; however, this situation remains inappropriate for FSR calculation because a steady state should be obtained in each individual. By contrast, an adequate steady state was obtained when the tracer was given together with enteral feeding, which induces a more continuous gastric emptying. The resulting enrichment in the free intracellular leucine pool was very high, averaging 35%. Leucine enrichment in the diet was 12%; the actual enrichment at the site of absorption was probably lower because of the dilution by unlabeled secreted proteins, such as mucus or enzymes. It is theoretically impossible to obtain a free intracellular enrichment higher than that of the diet. The explanation is probably the following: dietary proteins were probably not yet fully hydrolyzed in the second duodenum. Therefore, in this part of gut, where the biopsies were performed, the dietary unlabeled amino acids were not absorbed in totality (15, 23), whereas the free labeled amino acid (i.e., the tracer) was. This resulted in an artificially high tracer-to-tracee ratio, and therefore enrichment. This problem could be overcome during feeding by using a diet made of an intrinsically labeled protein, which was not available at the time of the experiment. Nevertheless, a significant contribution of the luminal amino acid pool to gut protein synthesis is highly likely, because the enrichment into gut protein is 1.7 times higher with the intragastric tracer than with the intravenous tracer after normalization for the tracer infusion rate. This same ratio was found to be 2.5 by Nakshabendi et al. in fasted humans, suggesting that luminal contribution does not increase with feeding. Another problem with the intragastric tracer method during feeding is that we had to stop feeding 1 h before performing the gut biopsies; we cannot exclude that the intracellular enrichment of the intragastric tracer was not stable over the last hour. However, we believe that there was a continuing release of dietary amino acids, because the plasma appearance of the intragastric tracer was stable in each subject over the last hour. Furthermore, there was no increase of the intravenous tracer plasma enrichment over the last hour, suggesting that there was still a dilution of these tracers by dietary amino acids.

We also assessed amino acid incorporation into mucosal protein with labeled phenylalanine. Theoretically, two different amino acids should give the same FSR for a given protein. Indeed, identical gut FSR were reported with leucine and valine, i.e., two branched-chain amino acids (21). We did find similar FSR with leucine and phenylalanine when using plasma amino acid enrichments as the precursor pool. However, the intracellular-to-plasma enrichment ratio was much lower for phenylalanine than for leucine, resulting in a higher FSR when intracellular amino acid enrichment was used as the precursor pool. We have no clear explanation for this fact. A lower enrichment could be due to a higher dilution by unlabeled phenylalanine derived from proteolysis, but phenylalanine content in the mucosal protein was not particularly high (4.5 vs. 4% into the whole body). We may speculate that a particular phenylalanine-rich protein could turn over very rapidly and thus could induce such a high dilution. Such discrepancies between FSR measured with various amino acids have already been reported (24) and again emphasize the fact that absolute values of FSR should be taken with caution.

The pattern of whole body kinetics in the fed and fasted states is consistent with the literature (18). With feeding, whole body protein breakdown decreases, whereas whole body protein synthesis increases moderately or not at all (at least for a protein intake of ~0.1
g·kg⁻¹·h⁻¹), such as in our study. The absence of whole body protein synthesis modification does not mean that tissue protein synthesis does not change. For example, it is well demonstrated that albumin synthesis increases with a meal, even with a moderate protein intake (9, 12). However, in our study, duodenal mucosal protein synthesis was not modified, suggesting that, in humans, intestinal protein synthesis is little sensitive to short-term variations in the nutritional state. These results are consistent with data from animal studies. In young rats or pigs (5, 8, 19), feeding increases intestinal protein synthesis. In lambs, intestinal protein synthesis is stimulated only by a very high protein energy intake compared with a normal intake (13, 22). By contrast, in adult rats intestinal protein synthesis is not modified by a 24-h fast and decreased by 20% only after 5 days of starvation (29). Therefore, it appears that the modification of intestinal protein synthesis with the nutritional state depends on the stage of development of animals (5, 8). Also, as in adult animals (29), we could possibly have observed a decrease of intestinal mucosal protein synthesis with a more prolonged fast than in our experiment. Other possible reasons explaining the absence of variation of the FSR between the fasted and the fed states in our experiment could be the following. 1) The proximal gut, which is the only available mucosal tissue in the human volunteer, is less sensitive than the distal gut to a variation of nutritional state, as was shown in animals (10). 2) It is possible that FSR was underestimated because of the loss of labeled mucosal protein in the lumen, and it is likely that this loss was more important during feeding.

In summary, our data confirm that duodenal mucosal protein turnover is high and that amino acids of two origins, i.e., luminal and vascular, contribute to protein synthesis during feeding. They also emphasize the fact that absolute values of FSR should be taken with caution, because they depend on the precursor pool chosen, the route of administration of the tracer, and the tracer itself. Duodenal mucosal protein synthesis is not different during feeding and fasting, at least in our experimental conditions. Finally, it is possible to measure intestinal protein FSR in humans by GC-MS and a deuterated tracer.

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