TRANSLATIONAL PHYSIOLOGY

Trypsin and splanchnic protein turnover during feeding and fasting in human subjects

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1Division of Gastroenterology, University of Pittsburgh Medical School, Pittsburgh, Pennsylvania; 2Virginia Commonwealth University Health Systems, Richmond, Virginia; and 3United States Department of Agriculture/Agricultural Research Service’s Children’s Nutrition Research Center, Baylor College of Medicine, Houston, Texas

O’Keefe, Stephen J. D., Ronzo B. Lee, Jing Li, Wen Zhou, Barbara Stoll, and Qianyu Dang. Trypsin and splanchnic protein turnover during feeding and fasting in human subjects. Am J Physiol Gastrointest Liver Physiol 290: G213–G221, 2006. First published August 25, 2005; doi:10.1152/ajpgi.00170.2005.—Knowledge of the stimulatory effects of enteral and parenteral (intravenous) feeding on the synthesis and turnover of trypsin would help in the management of acute pancreatitis, because the disease is caused by the premature activation of trypsin. To investigate this, we labeled intravenous infusions with [1-13C]leucine and enteral with [2H]leucine and measured isotope enrichment of plasma, secreted trypsin, and duodenal mucosal proteins over 6 h by duodenal perfusion/aspiration and endoscopic biopsy. Thirty healthy volunteers were studied during fasting (n = 7), intravenous feeding (n = 6), or postpyloric enteral feeding [duodenal polymeric (n = 6), elemental duodenal (n = 6), and jejunal elemental (n = 5)]. All diets provided 1.5 g·kg–1·day–1 protein and 40 kcal·kg–1·day–1 energy. Results demonstrated that compared with fasting, enteral feeding increased the rate of appearance (71 ± 4 vs. 91 ± 5 min, P = 0.01) and secretion (546 ± 80 vs. 219 ± 37 U/h, P = 0.01) of newly labeled trypsin and expanded zymogen stores (1,660 ± 237 vs. 749 ± 133 units, P = 0.03). These differences persisted whether the feedings were polymeric or elemental, duodenal, or jejunal. In contrast, intravenous feeding had no effect on basal rates. Differential labeling of the plasma amino acid pool by enteral and intravenous isotope infusions suggested that 35% of absorbed amino acids were retained within the splanchnic bed during enteral feeding and that mucosal protein turnover increased from a fasting rate of 34 ± 6 to 108 ± 8%/day (P < 0.05) compared with no change after intravenous feeding. In conclusion, all common forms of enteral feeding stimulate the synthesis and secretion of pancreatic trypsin, and only parenteral nutrition avoids it.

pancreatic enzymes; humans; trypsin synthesis; enteral parenteral nutrition

The introduction of intravenous feeding [total parenteral nutrition (TPN)] in the 1960s heralded a major breakthrough in the management of patients with intestinal failure. Even patients with total loss of the intestine could be rehabilitated to a relatively normal lifestyle. However, TPN is unphysiological and thus associated with a number of serious side effects (17).

Since that time, further advances have been made in restoring enteral function in patients with less severe forms of intestinal failure by specialized forms of enteral tube feeding. Comparative studies between TPN and enteral tube feeding have universally shown that enteral feeding is superior in the management of hospitalized patients because it reduces septic and metabolic complication rates and is cheaper (10).

TPN, however, continues to be used in the management of acute pancreatitis in many centers because of the fear that enteral feeding may exacerbate the disease process, despite the fact that several clinical trials have again shown that outcome is better with jejunal feeding (1, 12). To better understand this, we recently conducted a series of studies in healthy volunteers where we measured the pancreatic secretory responses to common forms of enteral and parenteral feeding (21). We concluded that the stimulatory effect of oral feeding could be reduced by 50% if the diet was delivered directly into the intestine by jejunal tube feeding if it was given in elemental form (i.e., “predigested”). However, only intravenous feeding reduced secretion to rates measured during fasting. What remains unknown, however, is whether these changes can be accounted for by changes in the rates of synthesis of new enzymes or whether it simply represents a reduction in the rate of release of previously synthesized enzymes from zymogen stores contained within pancreatic acinar cells. For example, is the suppression of luminal secretion observed during intravenous feeding due to synthesis arrest or due to slowed release of zymogen stores? This question is important to investigate because a block in secretion with continued synthesis would lead to expansion of intracellular zymogen stores that might “feed fuel to the fire” in patients with acute pancreatitis, because the disease is caused by intracellular trypsin activation.

During our secretion studies, we coadministered stable isotope-labeled amino acids with our enteral and parenteral dietary infusions and thus were able to investigate our hypothesis that the reduced enzyme secretion during parenteral feeding was due to synthesis arrest rather than reduced release of stores. Our isotope infusions also allowed the acquisition of secondary objectives of measuring the relative utilization of enteral and parenteral amino acids for the synthesis of splanchnic proteins, such as mucosal proteins, to better understand the superiority of enteral feeding over parenteral feeding in the support of hospitalized patients.

METHODS

Experimental Procedure

Thirty healthy adult volunteers were recruited to compare the relative effects of various types of commonly used enteral and parenteral feeding on the synthesis and secretion of trypsin in human subjects. Ten healthy adult subjects were recruited to compare the relative effects of various types of commonly used enteral and parenteral feeding on the synthesis and secretion of trypsin in human subjects. All subjects were entered into the University of Pittsburgh’s Institutional Review Board-approved human study protocol. All were free of acute and chronic diseases and were fasting for at least 12 h before the initiation of an isotope infusion. All subjects participated in five separate experiments with no more than 1 week between them. All experiments were performed at the University of Pittsburgh’s Research Service’s Children’s Nutrition Research Center, Baylor College of Medicine, Houston, Texas.

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parenteral feeding techniques on pancreatic enzyme synthesis. All subjects were admitted to the General Clinical Research Center (GCRC) the evening before the pancreatic study and fasted from midnight. The effects of fasting and feeding on pancreatic enzyme and splanchnic protein turnover were then measured over a 6-h period. One group was not fed (fasting group, n = 7) and served as the control. The remaining three groups were all given diets providing 1.5 g protein and 40 kcal energy·kg ideal body wt·day⁻¹. However, the mode of delivery of the diet and the composition varied as follows. The enteral group (n = 17) was given one of three common forms of nasoenteral tube feeding: 1) continuous duodenal infusions of a polymeric formula (Ensure; Ross Abbott Laboratories, Chicago, IL; polymeric duodenal subgroup, n = 6); 2) continuous duodenal infusions of an elemental formula (Vivonex; Novartis, Minneapolis, MN; duodenal elemental subgroup, n = 6); or 3) continuous proximal jejunal infusions of the same elemental diet (jejunal elemental subgroup, n = 5). The parenteral group (n = 6) received a continuous intravenous infusion of a sterile formulation of the same elemental formula synthesized in our pharmacy given into an antecubital vein (i.e., a “3-in-1” mixture of amino acids, glucose, and fat). The composition and demographic details of each subgroup are summarized on Table 1 and showed no significant baseline differences. Whereas the different diets were matched for protein and caloric content, the proportion of calories accounted for by fat was lower in the intravenous and elemental groups (i.e., 6% calories from fat) than in the polymeric group (22% calories from fat). The protein source in the elemental diet was free amino acids and the sources for carbohydrate and fat were maltodextrin, modified starch, and soybean oil, whereas the polymeric diet contained sodium and calcium caseinates, soy proteins, corn syrup, maltodextrin, sucrose, safflower oil, canola oil, and soy lecithin.

Informed, signed consent was obtained from all volunteers after reviewing the protocol and approval by the GCRC and institution review board.

**Nutritional Assessment**

Body mass index was calculated in kilograms per square meter from weight and height measurements.

**Usual Dietary Intake**

Three-day dietary recall was documented on admission to the GCRC by the dietitian associated with the study. Average daily macronutrient intakes were then calculated with the help of standardized software as previously described (21).

**Metabolic Expenditure**

Basal and resting metabolic rates were measured before and during the dietary infusion studies by indirect calorimetry (model 2900 metabolic cart; SensorMedics) under standard (resting, supine in a quiet room) conditions. Recordings were made after a steady state (at rest or a minimum of 10 min with a variance in oxygen consumption of <10% and in resting energy expenditure of <5%) had been achieved.

**Measurement of Trypsin Secretion and Turnover**

The method followed that previously described where pancreatic enzymes are labeled with stable isotopes and collected from the duodenum by perfusion/aspiration (16, 19, 23). As enteral and parenteral diets were studied, the isotope-labeled amino acids were given by gut and by vein. For intravenous delivery, a primed (1 mg/kg ideal body wt), 6-h continuous (1 mg·kg ideal body wt·h⁻¹) infusion of [t-[^13]C]leucine ([t-[^13]C]leucine, chemical and isotopic purity of >99%, MassTrace, Woburn, MA) was used, and, for enteral labeling, a continuous infusion of [t-[^5,5,5-²H₃]leucine (D3 leucine, chemical and isotopic purity >99%, MassTrace) was given at a rate of 2 mg·kg ideal body wt·h⁻¹ over the same period. [t-[^13]C]leucine was given combined with normal saline or the intravenous diet via a peripheral antecubital vein, whereas D3 leucine was confined with the duodenal perfusate that contained the enteral diet in subjects belonging to the enteral groups. In four subjects, two from the polymeric diet and two from the fasting group, the route of isotope infusion was reversed, such that the [t-[^13]C]leucine was given enterally rather than intravenously, to verify that the two isotope labels were metabolized in a similar manner. Venous blood samples were taken from the contralateral antecubital vein at 0, 120, 240, and 360 min for the measurement of isotope enrichment. Resting metabolic rate and total carbon dioxide production were measured between 2 and 3 h by indirect calorimetry as described previously.

Pancreatic secretions released into the duodenum were recovered by a double-lumen nasoduodenal tube placed manually with fluoroscopic guidance or by transnasal endoscopy (18), such that the proximal perfusion port was adjacent to the papilla of Vater and the aspiration port was located 20 cm distally. Throughout the 6-h period, the duodenal segment was perfused with a mixture of the enteral feed and normal saline at 300 ml/h, whereas mild suction (80 mmHg) was applied to the distal port. To measure the completeness of enzyme recoveries in aspirates and thus calculate secretion rates, the perfusate contained a marker [polyethylene glycol (PEG), molecular mass 3,500 Da, 5 g/l, Sigma, St. Louis, MO). Aspirations were collected continuously on ice and separated into 30-min subsamples before transport to the laboratory for immediate measurement of pH and trypsin concentration. Duplicate samples were stored at −80°C for extraction of trypsin for the measurement of isotopic enrichment by gas chromatography-linked mass spectrometry (GCMS; see Isotopic enrichment measurements).

**Sample Analysis**

Pancreatic enzymes. Trypsin concentrations were measured within 2 h of aspiration of duodenal juice by measuring hydrogen ion liberation after incubation with p-toluenesulfonyl-L-arginine methyl ester as previously described (21). Results were expressed in national formulary units. Duplicate samples were used to measure percentage

**Table 1. Patient demographics and diet**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Age, yr</th>
<th>Sex, (male/female)</th>
<th>BMI, kg/m²</th>
<th>BMR, kcal/day</th>
<th>Usual Daily Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enteral group</td>
<td>17</td>
<td>33 (3)</td>
<td>10/7</td>
<td>25 (1)</td>
<td>1,535 (90)</td>
<td></td>
</tr>
<tr>
<td>Duo-poly</td>
<td>36</td>
<td>35 (5)</td>
<td>3/3</td>
<td>25 (3)</td>
<td>1,364 (96)</td>
<td></td>
</tr>
<tr>
<td>Duo-elem</td>
<td>29</td>
<td>32 (3)</td>
<td>4/2</td>
<td>26 (2)</td>
<td>1,693 (171)</td>
<td></td>
</tr>
<tr>
<td>Jejunal-elem</td>
<td>34</td>
<td>37 (4)</td>
<td>3/2</td>
<td>24 (4)</td>
<td>1,502 (131)</td>
<td></td>
</tr>
<tr>
<td>Parenteral group</td>
<td>6</td>
<td>31 (1)</td>
<td>4/3</td>
<td>25 (0.9)</td>
<td>1,660 (104)</td>
<td></td>
</tr>
<tr>
<td>Fasting group</td>
<td>7</td>
<td>34 (4)</td>
<td>4/3</td>
<td>26 (1.4)</td>
<td>1,594 (74)</td>
<td></td>
</tr>
</tbody>
</table>

Values are group means (SE); n, no. of subjects. BMI, body mass index; BMR, basal metabolic rate; CHO, carbohydrate; duo-poly, duodenal infusion of a polymeric formula; duo-elem, duodenal infusion of an elemental formula; jejunal-elem, jejunal infusion of the elemental formula.
of duodenal marker (PEG) concentration by turbidity/spectrophotometry (7).

Trypsin extraction and purification. Affinity chromatography was used to extract pure trypsin from duodenal juice (14). Briefly, a commercially available trypsin inhibitor-agarose medium (Sigma) was held in an affinity column perfused by a low-pressure chromatography system (Biologic LP System, Bio-Rad, Chicago, IL). Five-milliliter juice samples were loaded onto the column. The nontrypsin proteins were washed off with 0.05 M Tris-HCl and 0.5 M NaCl buffer (pH 8.0). Trypsin was released from the antibody by subsequent perfusion with 0.025 M citrate and 0.025 M CaCl2 buffer (pH 2.5). The eluted fraction containing the trypsin peak was then desalted by gel filtration (HiPrep 26/10, Amersham Pharmacia Biotech, Piscataway, NJ). Subsequently, the protein was dried by rotary evaporation and further processed so that the isotopic enrichment of leucine with 13C and deuterium could be measured.

Isotopic enrichment measurements. The 13C and 2H1 isotopic enrichments of free leucine, derived from duodenal juice trypsin, and α-ketoisocaproic acid (KIC), the oxidative product of intracellular leucine released into the plasma, were measured by gas chromatograph-mass spectroscopy (GC-MS). The oxime-tert-butyldimethylsilyl derivative of KIC and the heptfluorobutyric anhydride (HFBA) derivative of leucine were prepared as previously described (27, 31). Briefly, acidified plasma (50–100 μl) was applied to a 1-ml bed volume Dowex 50X-8 cation exchange column (100–200 mesh; Bio-Rad Laboratories, Hercules, CA) and KIC was eluted with 0.01 M HCl. The KIC eluate was made alkaline with 10 M NaOH. After freshly prepared 0.36 M hydroxylamine hydrochloride was added (Fisher Scientific, Fair Lawn, NJ), the mixture was sonicated for 1 min, heated at 60°C for 30 min, and finally cooled in an ice bath. Subsequently, the samples were acidified with 6 M HCl (pH < 2) and the oxime derivative was extracted with ethyl ether. The extracts were dried under nitrogen, 50 μl N-methyl-N-(t-butyldimethylsilyl)trifluoroacetamide +1% tert-butyldimethylchlorosilane (Regis, Morton Grove, IL) were added, and the samples were kept tightly capped in a desiccator at room temperature for 24 h.

Purified trypsin was hydrolyzed by incubation with 6 M HCl at 110°C for 24 h to release free amino acids. The hydrolysate was dried, reconstituted in 1 M acetic acid, and applied to a 1-ml bed volume Dowex 50X-8 cation exchange column. The resin was rinsed with 0.01 M HCl before amino acids were eluted with 5 M NH4OH. The leucine-containing eluate was then dried under vacuum, and the leucine HFBA derivative was prepared.

The [13C]KIC and [1H2]KIC enrichments were analyzed by selected ion monitoring (SIM) GC-MS (HP 5890/5898A, Hewlett-Packard, Palo Alto, CA) using the electron impact mode. With the same instrument, [13C]leucine and [1H]leucine enrichments were determined by SIM GC-MS using positive chemical ionization with methane as the reagent gas. For KIC, mass-to-ion ratios (m/z) 316, 317, and 319 were monitored to represent unlabeled KIC, [13C]KIC, and [1H2]KIC, and, for leucine, m/z 349, 350, and 352 were monitored to represent unlabeled leucine, [13C]leucine, and [1H]leucine.

Enrichment of carbon dioxide with 13C was measured by isotope ratio mass spectrometry (courtesy of Dr. D. Matthews, University of Vermont) (13).

Calculations

Pancreatic enzyme secretion. Measurement of enzyme (en) and PEG marker concentrations in duodenal juice samples (PEGout) allowed calculation of enzyme secretion rates from the following relationship (21): Enzyme secretion (U/h) = (en)(PEGin/PEGout) (PEG infusion rate), where PEGin is the PEG concentration in the perfusate.

Trypsin turnover. The isotopic enrichment curve over 6 h for trypsin protein was modeled mathematically as before (16) to obtain objective measurements of 1) the time it takes for newly synthesized enzyme to first appear in secreted juice and 2) the slope of the isotopic enrichment curve. Primary measurements were based on the primed continuous intravenous infusions of [1-13C]leucine. A simple, nonlinear exponential model with a delay parameter provided the best fit for the complete data. In fitting the isotopic data for each subject to this model, the following definitions were made. First, the enrichment of leucine in the precursor amino acid pool for protein synthesis will be equal to the estimated plateau value (MX) for enzyme leucine isotopic enrichment (ESA) from the precursor-product relationship. Second, the slope of the curve is the slope at the point at which the ESA has achieved 50% of MX (i.e., time = half time, in min) and is given by d(ESA)/dτ = A × MX/2, where A is the estimated rate parameter from the exponential model. Finally, the intracellular (zymogen) enzyme pool turnover rate (EPTR; %/h) is then calculated from EPTR = 100 × (A/2) × 60.4. Intracellular (zymogen) enzyme pool size equals enzyme secretion divided by EPTR. Curve fitting was accomplished by using the SAS statistical package procedure NLIN (SAS, Cary, NC). The final analysis only included those subjects whose enrichments fitted the model with an r2 value of >0.8.

Systemic and splanchnic amino acid turnover. Earlier studies have shown that the rate of whole body flux of leucine (Q) can be measured during the primed continuous intravenous infusion of isotopically labeled amino acid from the relationship between the rate of isotopic infusion and the plateau value of plasma KIC enrichment in the plasma achieved, assuming the precursor pool for whole body protein synthesis is the intracellular free leucine pool (3, 5, 13, 16, 25).

\[
Q(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}) = \left( \frac{E_{in}}{E_{KIC}} - 1 \right) \times F
\]

where E in is the enrichment of the infusate (i.e., isotopic purity) in atoms percent excess and F is the rate of infusion in micromoles per kilogram per minute. Similarly, the rate of oxidation of leucine can be measured from the relationship between the rate of excretion of 13C in breath as 13CO2 (ECO2) and the plateau value of plasma KIC enrichment

\[
ECO2(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}) = VCO2 \times \left( \frac{1}{E_{KIC}} - 1 \right) \times 100
\]

where VCO2 is carbon dioxide production. However, there is substantial evidence that the systemic administration of isotopic label will underestimate the contribution of splanchnic proteins to whole body turnover, particularly in the fed state, because a significant proportion of absorbed amino acids is removed on “first pass” for the synthesis of rapidly turning over splanchnic proteins such as enzymes and mucosa (30, 32). Consequently, we infused two different isotopically labeled amino acids, one by vein (e.g., [13C]leucine) and one by gut (e.g., D3 leucine) to independently label the systemic and splanchnic amino acid pools. The difference between the two calculations provided a measure of splanchnic amino acid turnover (Qs).

Mucosal protein turnover rates. Early studies (3, 5, 19, 22, 25) have shown that during isotopic steady-state labeling conditions achieved by constant intravenous isotope infusion, the uptake of labeled amino acid into specific body protein compartments, such as the mucosa, is linear and that the fractional turnover of the protein [mucosal protein turnover (MPTO) rate] can then be calculated from

\[
\text{MPTO (%/day)} = \frac{E_{prec} \times E_{post} - E_{post} \times 24 \times 100}{E_{prec}} \times \frac{6}{24 \times 100}
\]

where Eprec is the enrichment of the precursor amino acid pool for protein synthesis and Epost is the enrichment of leucine bound to the specific protein being sampled at the commencement of the isotopic infusion and is the enrichment at the end. With regard to the measurement of mucosal turnover, Eprec is the enrichment of proteins.
contained in the endoscopic biopsies at 6 h. Because biopsies will not be taken from patients before isotope infusion, background enrichment (i.e., $E_{\text{preinf}}$) is assumed to be the same as that measured in enzyme protein aspirated before isotope infusion. As previously, we also assumed that the isotope enrichment of plasma ketoisocaproic acid provides a measure of the enrichment of the precursor pool for mucosal protein synthesis. (3, 5, 19, 22, 25). Second, because we are investigating the effects of the diet on mucosal turnover, our measurements are based on the uptake of the isotope given with the diet.

Statistical evaluation. Results are presented throughout as group means ± SE. The primary objective of our statistical analysis was to evaluate the validity of our hypothesis: that the reduction in trypsin secretion during intravenous feeding was due to suppression of enzyme synthesis. To first determine whether there were any overall differences in enzyme kinetic rates among the enteral, parental, and fasting groups, ANOVA was employed to test the null hypothesis. Each outcome variable was modeled separately. The variable zymogen turnover was log transformed because it was not normally distributed. Values obtained during the fasting state were used as "controls" to determine whether intravenous or enteral feeding stimulated or suppressed trypsin synthesis and turnover rates, employing ANOVA. $P$ values of <0.05 were accepted as significant.

RESULTS

Table 1 summarizes the demographic details of the groups and subgroups studied and shows that there were no significant baseline differences in age, sex distribution, nutritional, metabolic, or dietary parameters.

Dietary Intakes

The hourly infusion rates of energy, protein, carbohydrate, and fat actually received by each subgroup are shown in Table 2. Intakes with respect to energy and protein were similar in enterally fed and parenterally fed subjects, but fat intake was higher in the enteral group as a whole ($P = 0.02$). However, in the subgroup given the enteral elemental diet, the fat intake was the same as the parenteral group.

Plasma Amino Acids

Total amino acid concentrations were significantly higher in the parenteral group than the enteral group ($3,258 \pm 186$ vs. $2,320 \pm 193 \mu$mol/ml, $P < 0.01$) and the fasting group ($2,621 \pm 225 \mu$mol/ml, $P < 0.05$). Consistent with this were the significantly higher concentrations of serine, glycine, histidine, alanine, arginine, methionine, and phenylalanine (all $P < 0.0001$) in parenterally fed patients compared with those enterally fed.

Trypsin Secretion

Overall analysis by ANOVA showed that there were significant differences ($P = 0.006$) among the fasting, enteral, and parenteral groups, rejecting the null hypothesis. Compared with fasting (placebo saline infusion), mean hourly secretion rates were significantly higher in enterally fed subjects ($546 \pm 80$ vs. $219 \pm 37$ U/h, $P = 0.01$) but not in parenterally fed subjects ($279 \pm 78$ U/h). Subgroup analysis in Table 3 shows that all three forms of enteral feeding stimulated trypsin secretion, but the stimulation was highest with the duodenal polynmeric infusion ($P = 0.0002$). Fluid secretion rates were $8.1 \pm 0.6$ ml/min during fasting, $11.4 \pm 1.5$ ml/min during intravenous feeding, and $12.3 \pm 1.2$ ml/min during enteral feeding. Because there was concern that the secretion rates may have been underestimated during fasting and intravenous feeding because of defective conversion of trypsinogen to trypsin, selected samples from one subject from the placebo group and one from the intravenous group were reanalyzed following the addition of excess enterokinase ($0.2$ mg added or 0.28 U enterokinase/300 $\mu$l sample, giving a concentration of 667 mg/l). Table 4 shows that the trypsin activities were not increased, making it unlikely that the lower activities in these two groups were due to incomplete conversion of trypsinogen. The slightly lower activities after the addition of enterokinase were noted by ourselves in a similar previous study (23) and may be explained by secondary trypsin inactivation as reported by Kunitz (11).

Isope Studies

Trypsin turnover. A summary of the differences in uptake of enterally and parenterally administered isotopically labeled leucine into trypsin secreted into the duodenum is illustrated in Figs. 1 and 2.

Figures 1 and 2 outline the relative differences in uptake of isotopically labeled leucine when it was infused intravenously (Fig. 1) and into the duodenum (Fig. 2). The curves show that the uptake of intravenous infused tracer into secreted trypsin commenced between 60 and 90 min, whereas the uptake of enteral tracer was delayed to between 90 and 120 min. The uptake of intravenous tracer then followed a curvilinear course, plateauing by 300 min, whereas plateau enrichment may not have been achieved with the enteral isotope infusion by 360 min.

The isotope incorporation pattern into trypsin was similar in the four subjects where the route of isotope infusion was switched from enteral D3 to $^{13}$C as shown in Fig. 3.

The intravenous isotope incorporation data for all but one subject, from the placebo group, fitted well with our model, with correlation coefficients of $>0.80$ (Fig. 4), allowing calculation of the values shown on Table 3. Curve fitting was, however, less good with the enteral isotope infusions, and the data could not be used from three subjects from the duodenal elemental and one from the jejunal elemental group. Exploratory analysis by ANOVA detected significant differences among parenteral and parenterally fed groups for the time taken for labeled trypsin to first appear in the duodenum (i.e., synthesis time) and for the zymogen pool size ($P = 0.01$) measured by intravenous isotope infusion and for pool size measured by enteral labeling ($P = 0.02$). Subgroup analysis showed that trypsin synthesis time was significantly shorter ($P = 0.01$) and the zymogen pool size was larger ($P = 0.03$) during enteral feeding compared with the fasting. In contrast, intravenous feeding had no significant effect on either parameter, thus rejecting our hypothesis that intravenous feeding...
Table 3. Effects of fasting and feeding on trypsin secretion, synthesis, and turnover measured by IV and enteral infusions of isotope-labeled leucine

<table>
<thead>
<tr>
<th></th>
<th>Secretion, U/h</th>
<th>Synthesis, min</th>
<th>Zymogen Turnover</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>%/h</td>
</tr>
<tr>
<td>IV isotope incorporation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td>6</td>
<td>219 (37)</td>
<td>91 (5)</td>
</tr>
<tr>
<td>Enteral (total)</td>
<td>17</td>
<td>546 (80)*</td>
<td>71 (4)*</td>
</tr>
<tr>
<td>Duo-poly</td>
<td>6</td>
<td>770 (160)†‡</td>
<td>77 (2)</td>
</tr>
<tr>
<td>Duo-elem</td>
<td>6</td>
<td>423 (81)*</td>
<td>73 (7)</td>
</tr>
<tr>
<td>Jejunal-elem</td>
<td>5</td>
<td>416 (87)</td>
<td>51 (6)*</td>
</tr>
<tr>
<td>Parenteral</td>
<td>6</td>
<td>279 (77)</td>
<td>89 (10)</td>
</tr>
<tr>
<td>Enteral isotope incorporation</td>
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<tr>
<td>Fasting</td>
<td>7</td>
<td>102 (11)</td>
<td>18 (1)</td>
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<td>Enteral (total)</td>
<td>13</td>
<td>95 (9)</td>
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<tr>
<td>Duo-poly</td>
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<td>20 (3)</td>
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<tr>
<td>Duo-elem</td>
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<tr>
<td>Jejunal-elem</td>
<td>4</td>
<td>79 (9)</td>
<td>26 (2)</td>
</tr>
<tr>
<td>Parenteral</td>
<td>6</td>
<td>125 (16)</td>
<td>15 (3)</td>
</tr>
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</table>

Values are group means (SE); n, no. of subjects. IV, intravenous. *P < 0.05, †P < 0.01, ‡P < 0.001 compared with fasting.

Table 4. Effect of enterokinase addition to trypsin activity in nonenterally fed subjects

<table>
<thead>
<tr>
<th>Sample</th>
<th>Diet group</th>
<th>pH</th>
<th>Before EK</th>
<th>After EK</th>
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</thead>
<tbody>
<tr>
<td>V13-0’</td>
<td>Fasting</td>
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<td>2,420</td>
<td>2,095</td>
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<tr>
<td>V13-150’</td>
<td>Fasting</td>
<td>5.0</td>
<td>1,035</td>
<td>975</td>
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<tr>
<td>V13-240’</td>
<td>Fasting</td>
<td>5–6</td>
<td>548</td>
<td>460</td>
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<tr>
<td>V6-0’</td>
<td>IV</td>
<td>7.0</td>
<td>1,620</td>
<td>1,310</td>
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<td>V6-150’</td>
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<td>5.5</td>
<td>222</td>
<td>100</td>
</tr>
<tr>
<td>V6-240’</td>
<td>IV</td>
<td>6.5</td>
<td>794</td>
<td>618</td>
</tr>
</tbody>
</table>

BAEE, Nα-benzoyl-L-arginine ethyl ester; EK, enterokinase.

suppressed synthesis; rather, the difference between enteral and intravenous feeding could be accounted for by a stimulatory effect of enteral feeding. Similar directional changes were observed with the incorporation of enterally administered isotope-labeled amino acid, but only the increase in zymogen pool achieved significance (P = 0.03).

Subgroup analysis showed that the trypsin synthesis time by intravenous isotope administration was fastest in response to the jejunal elemental feeding (54 ± 6 compared with 91 ± 5 min for fasting, P = 0.005). Zymogen pool size was, however, was highest in the duodenal polymeric group (2,291 ± 559, P = 0.001). The analysis from the uptake of enteral isotope label showed that the increase in pool size with enteral feeding was again predominantly due to a significant increase in the group given the polymeric diet (4,130 ± 838, P = 0.0001, vs. 1,213 ± 163 by fasting).

Whole body leucine turnover. The infusion of enteral and parenteral isotope-labeled amino acids allowed the calculation of two sets of whole body amino acid turnover rates. Because of compartmentation between systemic and splanchnic amino acid pools and because the common metabolic pool is taken to be the plasma, the calculations based on enteral infusions differ from those based on the intravenous infusions by the fraction of total amino acid metabolism contributed by first-pass splanchnic metabolism. Overall analysis by ANOVA detected significant group differences in leucine flux rates measured by both isotope infusions (13C, P = 0.01; D3, P = 0.02) and in leucine oxidation (P = 0.0007). The results are summarized in Table 5 and show that turnover measured by enteral isotope infusion was 17% higher than turnover measured by intravenous isotope infusion during fasting, 35% higher during enteral feeding, and 22% higher during parenteral feeding. Whole body leucine turnover rates measured by intravenous isotope infusion were significantly higher during both enteral (P = 0.004) and parenteral feeding (P = 0.04) compared with fasting rates. Similar directional changes were observed with the calculations based on the enteral isotope infusions (P < 0.003). Leucine oxidation rates were also significantly higher during enteral (P = 0.01) but not parenteral feeding (P = 0.07). Despite this, the uptake of leucine into whole body protein was only significantly higher during enteral feeding (P = 0.05). Splanchnic flux rates were also higher during enteral feeding, but not significantly so. In the four subjects in which the route of administration of the [13C]leucine was switched from intravenous to enteral, the enrichment of 13C02 was significantly higher (32.8 ± 8.8 vs. 19.7 ± 1.3 mol%...
excess, \( P = 0.0004 \), as was the calculated leucine oxidation rate (0.85 ± 0.4 vs. 0.52 ± 0.26 \( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \), \( P = 0.016 \)).

Subgroup analysis of the enterally fed groups showed that the highest flux rates measured by intravenous isotope labeling were associated with the elemental formulae, whether it was given duodenally (3.2 ± 0.3, \( P < 0.0001 \) vs. fasting) or jejunally (2.5 ± 0.1, \( P = 0.005 \)). This was accompanied by higher oxidative clearance rates, but the fraction incorporated into whole body protein was also highest in the duodenal elemental group (2.5 ± 0.3, \( P = 0.001 \)). Splanchnic flux was also highest in the duodenal elemental group (1.4 ± 0.4 vs. fasting).

Mucosal protein turnover. Mucosal turnover was higher during enteral feeding (108 ± 8%/day) compared with intravenous feeding (38 ± 7%/day, \( P < 0.0001 \)). During fasting, calculations based on intravenous amino acid labeling gave turnover rates of 34 ± 6%/day, whereas calculations based on enteral amino acid labeling gave considerably higher rates of 137 ± 22%/day.

**DISCUSSION**

It is well recognized that pancreatic secretion continues at a basal rate during fasting, with fluctuations coincident with migrating motor complexes, thus contributing to what has been termed “intestinal housekeeping” (28). Our investigations suggest that basal secretion is not simply maintained by slow release of zymogen stores but that enzymes continue to be synthesized to replace secretions and maintain the zymogen pool at a contracted size. Second, our studies in fed patients indicate that the increase in enzyme secretion results from an acceleration of the rate of secretion of newly synthesized enzymes and an expansion of the zymogen pool. Differential effects were noted between enteral infusions of elemental and polymeric formulae, with the balanced polymeric diet having the greatest effect on pool expansion and secretion and the low-fat elemental having the greatest acceleration on new enzyme release. In contrast, parenteral delivery of nutrients had no appreciable influence on enzyme synthesis, turnover, or secretion. Plasma amino acid concentrations only increased significantly in intravenous-fed subjects, signifying the lower metabolic utilization of dietary amino acids for the synthesis of body proteins. Thus it appears that all major nutrients are less effectively metabolized if infused intravenously, because our earlier studies showed defective plasma clearance of glucose and fatty acids (21), explaining some of the metabolic complications associated with TPN. Our simultaneous measurements of splanchnic, pancreatic enzyme, and mucosal protein turnover.
It is unlikely that our measurements of lower trypsin secretion in fasted and intravenous fed subjects could be explained by incomplete conversion of secreted trypsinojen to trypsin within the duodenal lumen, because the in vitro addition of the activating enzyme, enterokinase, did not increase the measured trypsin activity in duplicate samples. Our recent publication in patients with acute pancreatitis also found that the low trypsin activity could not be explained by “enterokinase deficiency” (23). This is perhaps not surprising because activated trypsin also activates trypsinojen, and we have yet to identify healthy volunteers, or patients with pancreatitis, with immeasurable duodenal trypsin concentrations. Furthermore, in the present study, we were able to extract sufficient activated trypsin protein with our affinity column containing soybean trypsin inhibitor to measure trypsin’s enrichment with isotope-labeled leucine. Whereas the isotope enrichment curve corresponded closely to that predicted by our model for human trypsin synthesis and turnover (16) in most subjects, we experienced irregular labeling with the intravenous isotope in one subject and in four with the enteral isotope administration. Irregular labeling can occur either by variable absorption, which is only a problem with enteral administration, or by collection problems associated with duodenal aspiration, which can occur with both intravenous and enteral administration. Consequently, it is reasonable to assume that our measurements of trypsin activity are directly related to the total mass of trypsinojen released by the pancreas. Therefore, our combination of measurements of trypsin enrichment and trypsin activity to calculated pancreatic zymogen stores should be legitimate.

Despite an extensive search of the published literature, we were unable to find attempts by others to measure the effect of feeding and fasting on the production and turnover of pancreatic enzymes in humans. This is surprising, because the pancreas is an ideal model for the study of protein turnover, because it literally churns out large quantities of export protein that can easily be sampled by duodenal aspiration. Direct measurement of enzyme synthesis is, however, clouded by the presence of a large pool of stored enzymes within the acinar cells, termed zymogens. Thus the rate of incorporation of labeled amino acids in enzyme secretions does not reflect enzyme synthesis, as was previously proposed (26), but rather the rate of labeling and turnover of the zymogen pool (16). Analysis of the isotope incorporation curve allows us to calculate the rate of turnover of zymogen stores and, because we also measure enzyme secretion, we can also calculate the size of the stores. Our measurements suggest that the rate of turnover of zymogens remains fairly constant during feeding and fasting but that the size increases during feeding. Should synthesis fail, our calculations suggest that there would be sufficient stored enzyme to cover digestive needs for 3.5–4 h. This reserve capacity enabled humans, in nature, to survive on infrequent meals eaten irregularly when food became available. For example, African Bushmen are able to gorge over 3 kg of meat in one sitting (personal observations), enabling them to survive in a “feast or famine” environment (20). The considerable depth of zymogen stores, plus the known excess of secretions produced in response to feeding, helps explain why such extreme intakes can be tolerated.

There is strong evidence that enteral diets are more efficiently used than parenteral infusions for the synthesis of splanchnic proteins and, in particular, mucosal proteins (30, 32). Consequently, we might have expected greater enrichment of enzyme proteins with enteral isotope amino acid labeling. Interestingly, allowing for the fact that more isotope label was infused enteraally, the reverse was found, and although the pattern of isotope incorporation was the same, there was a slower rate of appearance of newly labeled trypsin and the delayed plateau enrichment. However, it is important to stress that the modes of enteral and parenteral isotope infusions were different, i.e., the enteral isotope tracer was given simply as a constant infusion with the diet, whereas the intravenous isotope infusion was delivered as a primed-continuous infusion, and therefore, the data are not strictly comparable. The reason we chose not to prime the enteral infusion was that a priming dose injected into the duodenum might have triggered CCK release and thus stimulated the pancreas, which would have made our fasting studies difficult to interpret. In contrast, there is no evidence that intravenous leucine stimulates the pancreas. Indeed, our earlier studies of pancreatic enzyme synthesis and secretion found that intravenous amino acids, if anything, inhibited CCK-stimulated secretion, suggesting negative feedback control (24). To answer this question, we will need to perform further studies in enteraally fed patients in which the enteraally administered isotope will also be given as a primed-continuous infusion. In this situation, any potential stimulation of secretion by the priming dose will be minimized by the stimulatory effect of the diet itself.

There are divergent views on the effect of fasting and feeding on the trafficking of newly synthesized enzymes within the acinar cell. The traditional interpretation is based on Jamieson and Palade’s studies of preparations of slices of guinea pig pancreases using pulse-chase radiolabeling techniques (8). They described four steps where, first, amino acids are taken up into the cell and incorporated into enzyme protein on poly-somes; second, proenzymes move through the Golgi for modification; third, new enzymes are condensed into vacuoles; and, finally, the vacuoles coalesce with previously synthesized en-zymes in a common zymogen pool from which secretion occurs. Consequently, fasting would slow the delivery of new enzymes to the gut, whereas feeding would speed it up, which fits with what we observed. An alternative view is based on Arvan and Castle’s conclusion that trafficking can be divided into “constitutive,” i.e., hormone independent, and “regulated,” i.e., hormone-sensitive pathways (2, 6). They based their conclusions on published evidence, again from animal studies, that during fasting unstimulated conditions, newly synthesized en-zymes are diverted at the level of the trans-Golgi network from the regulated pathway described above into the constitutive pathway and secreted directly without prior equilibration within the zymogen pool. Thus, although there will be quantitatively less enzyme secreted, there would be a more rapid appearance of highly isotope-enriched enzyme in digestive juice, which is the reverse of what we found. Many such differences have been documented between human and animal pancreatic physiology (29), cautioning the extrapolation of findings from one mammal to another.
Our secondary objective was to measure the effects of enteral and parenteral feeding on whole body, splanchnic, and mucosal protein turnover. Our use of dual-isotope label infusions, one by an enteral route and the other by a parenteral route, allowed us to investigate the mixing of amino acid fluxes from the splanchnic and systemic circulations during the 6-h study period. Our results showed that in all conditions, namely, fasting, enteral feeding, and parenteral feeding, a “common metabolic pool” of amino acids does not exist because the amino acid flux rates calculated by enteral and intravenous isotope-labeled amino acid infusion gave different results, despite the achievement of reasonable isotopic steady states in the plasma. On average, calculations based on enteral isotope infusion were 17% higher during fasting, 35% during enteral feeding, and 25% during intravenous feeding. It is unlikely that the differences could be accounted for by delayed absorption of the triple-labeled deuterated leucine because when we switched isotopes, the enrichment of trypsin with [13C]leucine was very similar to the enrichment with D-3-leucine (Fig. 3). The observation can be explained by internal recycling of amino acids from rapidly turning over proteins within the splanchnic pool, which do not equilibrate with the systemic circulation. It is known that the gut has a high demand for amino acids for the synthesis of proteins with short half-lives, such as enzymes and mucosal proteins (32), and that luminal amino acids may be used directly for the synthesis of mucosal proteins (30). With the use of in vivo multiple tracer-labeled amino acid studies in pigs, where catheters and flow recorders were placed in the portal, venous, and arterial bloodstreams for sampling, Stoll et al. (30) have performed a series of elegant studies on the first-pass uptake of dietary amino acids by the gut and splanchnic bed. Their measurements showed that roughly 60% of dietary amino acids were retained during feeding but that there was considerable variation between amino acids such that nearly all aspartate, glutamine, and glutamate was consumed by the gut, whereas there was net splanchnic production of arginine and tyrosine (32). Investigating disposal, they demonstrated that only 20% was incorporated into mucosal protein and concluded that the bulk was oxidized for energy purposes (30). This may account for our finding that 13CO2 generation from [13C]leucine was higher if the isotope was delivered enterally. Biolo et al. (4) performed similar studies to ours in human volunteers but used a mixture of four different radioactive and stable isotopes of leucine and phenylalanine. They calculated that 58% of dietary phenylalanine and 25% of leucine, very similar to our calculation of 17–35%, never appeared in the systemic circulation, implying direct splanchnic consumption. The converse is also true, that because of compartmentalization between the splanchnic and systemic circulations, parenteral feeding will be less effective in supplying substrate amino acids to the splanchnic bed for the synthesis of mucosal, pancreatic, and liver proteins, thus accounting for the gut mucosal atrophy and pancreatic hyosecretion associated with TPN and bowel rest. Our calculations of mucosal protein turnover support this view as protein turnover was threefold higher when nutrition plus isotope label was delivered enterally compared with parenterally.

In summary, our results provide strong evidence that all forms of commonly used enteral feeding techniques, including jejunal elemental, stimulate pancreatic trypsin synthesis and secretion and that the only way of currently feeding without stimulating (i.e., “pancreatic rest”) is to use TPN. Consequently, the superiority of enteral over parenteral feeding in the clinical management of acute pancreatitis is unlikely to be due to any positive influence of enteral feeding on the injured pancreas, as the disease itself is triggered by trypsin (23). Our secondary measurements of splanchnic and mucosal protein turnover suggest that the beneficial effects are more likely to be a consequence of the greater ability of enteral feeding to support splanchnic protein synthesis that is needed to maintain mucosal integrity and repair the pancreatic damage. Finally, the lower metabolic clearance of nutrients infused by vein help explain the metabolic complications of TPN. Distal jejunal feeding may prove to be the best solution to feeding problems in acute pancreatitis, because exciting preliminary studies of ours (9) support suggestions by others (33) that this technique may provide the benefits of enteral feeding without stimulating the pancreas.

GRANTS

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