Enteral nutrient intake level determines intestinal protein synthesis and accretion rates in neonatal pigs

BARBARA STOLL, XIAOYAN CHANG, MING Z. FAN, PETER J. REEDS, AND DOUGLAS G. BURRIN
United States Department of Agriculture/Agricultural Research Service, Children’s Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, Houston, Texas 77030

Received 19 October 1999; accepted in final form 18 February 2000

Stoll, Barbara, Xiaoyan Chang, Ming Z. Fan, Peter J. Reeds, and Douglas G. Burrin. Enteral nutrient intake level determines intestinal protein synthesis and accretion rates in neonatal pigs. Am J Physiol Gastrointest Liver Physiol 279: G288–G294, 2000.—Our objective was to determine the minimum enteral intake level necessary to increase the protein accretion rate (PAR) in the neonatal small intestine. Seven-day-old piglets received an equal total daily intake of an elemental diet, with different proportions given enterally (0, 10%, 20%, 40%, 60%, 80%, and 100%). After 7 days, piglets were infused intravenously with [2H3]leucine for 6 h, and the fractional protein synthesis rate (FSR) was measured in the proximal (PJ) and distal jejunum (DJ) and the proximal (PI) and distal ileum (DI). The jejunal FSR increased from 45%/day to 130%/day between 0 and 60% enteral intake, whereas the FSR in the ileum was less sensitive to enteral intake level. At 0% enteral intake, PAR was significantly negative in the PJ, DJ, and PI (range −70 to −43 mg/day) and positive in the DI (49 mg/day), whereas intestinal protein balance occurred at 20% enteral intake. At 100% enteral intake, the PAR was greatest in the DI, even though the rates of protein turnover were 50% lower than in the PJ. We conclude that there is net intestinal protein loss at 0% enteral intake, protein balance at 20% enteral intake, and maximal intestinal protein accretion at 60% enteral intake.

protein degradation; total parenteral nutrition; preterm infants; amino acid metabolism

AT BIRTH, THE ROUTE OF NUTRITION shifts from largely parenteral to enteral, so that intestinal absorptive function becomes a critical factor in the maintenance of physiological well-being. The shift in the mode of nutritional input stimulates rapid growth of neonatal intestine, and this is supported by a high rate of protein synthesis (5). Although total parenteral nutrition (TPN) is used to provide essential nutritional support for preterm infants who cannot tolerate enteral feeding, studies (11, 18, 21) with neonatal animals have shown that this mode of nutrition results in gut atrophy and presumably a net loss of mucosal protein. The effects of TPN on neonatal intestinal protein synthesis and degradation and whether it actually results in intestinal protein loss have not been established. Dudley et al. (9) found that jejunal mucosal protein synthesis was lower in parenterally vs. enterally fed piglets. Protein synthesis and degradation could be altered by both the lack of luminal substrate availability and the potential downregulation of trophic stimuli resulting from an absence of luminal nutrients (16). Clinical studies (3, 17) in preterm infants have found that providing small volumes of enteral feeding, referred to as minimal enteral feeding (MEF), enhances intestinal motility and other gastrointestinal functions, yet the effects on intestinal protein metabolism are unknown. Thus there is a physiological and clinical rationale to establish how the level of enteral nutrient intake affects the rates of synthesis, degradation, and balance of intestinal protein in the neonate.

Previous studies (23, 28) in enterally fed animals have shown that a substantial proportion (30–50%) of the dietary amino acids are utilized by the intestine and that the rates of utilization are higher in preterm infants than in adults (2, 13). Our primary objective in this study was to determine whether these estimates of splanchnic amino acid utilization represent the minimum level of nutrients that must be provided enterally to maintain intestinal metabolism and thereby stimulate protein accretion in the neonate. The secondary objective was to quantify the relative importance of protein synthesis vs. degradation. To accomplish these objectives, we used a stable isotopic tracer approach coupled with quantitative measurements of intestinal protein accretion to estimate the rates of both protein synthesis and degradation under conditions of variable levels of enteral nutrition using neonatal pigs as a model for the human neonate (19, 26).

MATERIALS AND METHODS

Animal care and study design. The protocol was approved by the Animal Care and Use Committee of Baylor College of Medicine and was conducted in accordance with the Guide for the Care and Use of Laboratory Animals [DHHS publication no. (NIH) 85–23, revised 1985, Office of Science and Health Reports, DRR/NIH, Bethesda, MD 20205]. Pregnant sows obtained from the Texas Department of Criminal Jus-
tice (Huntsville, Texas) were housed in the Children’s Nutrition Research Center (Houston, TX) at 74°F in free-standing farrowing crates and fed Purina porcine diet (St. Louis, MO) and water ad libitum. Sows farrowed naturally, and at 7 days of age, the piglets were removed from the sow and surgically implanted with a Tygon catheter (1.78 mm OD) in the carotid artery and a Silastic catheter (1.65 mm OD) in the external jugular vein and gastric fundus. The piglets were then randomly assigned to seven groups of equal mean body weight (3,112 ± 86 g). Each group received the same total nutrient intake, of which 0% (n = 7), 10% (n = 5), 20% (n = 5), 40% (n = 5), 60% (n = 5), 80% (n = 5), or 100% (n = 5) was given enterally via the intragastric catheter. The remainder of the nutrients were given parenterally, via the intravenous catheter. Thirty-seven pigs from five litters were studied and within each litter at least four treatment groups were replicated. In each litter, at least two pigs were given either 0% (negative control) or 100% enteral nutrition (positive control).

The elemental nutrient solution consisted of glucose (104 g/l), lipid (21 g/l), Intralipid, Baxter Healthcare, Deerfield, IL), a complete amino acid mixture (55 g/l; Ajinomoto, Tokyo, Japan), electrolytes, trace minerals, and vitamins sufficient to meet or exceed the requirements for neonatal pigs (20). The amino acid composition of the elemental solution was (in g/l) 2.70 alanine, 2.34 arginine, 4.15 aspartic acid, 1.20 cysteine HCl, 5.19 glutamic acid, 4.15 glutamine, 2.03 glycine, 1.35 histidine, 3.01 isoleucine, 5.35 leucine, 5.05 lysine HCl, 1.35 methionine, 2.75 phenylalanine, 3.90 proline, 2.91 serine, 3.26 threonine, 0.63 tryptophan, 0.63 tyrosine, and 3.28 valine. The nutrient solution was administered continuously, and after an initial 24-h postnatal period in which the animals received 50% of the total intake intravenously, the animals received 0% (n = 4), 25% (n = 4), 50% (n = 4), 75% (n = 3), and 100% (n = 3) according to their assigned treatment for 6 days.

Isotopic tracer protocol. On postoperative day 7, a baseline sample of arterial blood was obtained from each pig. Each animal then received a primed continuous intravenous infusion of L-[5,5,5-3H2]leucine (45 μmol/kg prime, infusion of 45 μmol · kg−1 · h−1; 83 mol% enrichment; Cambridge Isotopes, Woburn, MA) for 6 h. During the tracer infusion, arterial blood samples were taken at 4, 5.25, 5.5, 5.75, and 6 h. All blood samples were immediately transferred to Na2-EDTA tubes and centrifuged (1,200 g for 10 min at 4°C). The plasma was frozen in liquid nitrogen and then stored at −70°C until taken for analysis. After 6 h, the pigs were killed with an overdose of pentobarbital sodium (50 mg/kg; Sigma Chemical, St. Louis, MO). The abdomen was opened, and the entire small intestine distal to the ligament of Treitz was immediately flushed with ice-cold saline. After flushing, the small intestine was divided into two equal portions; the proximal half was designated the jejunum, and the distal, the ileum. These two segments also were divided in half, resulting in a total of four segments: proximal jejunum, distal jejunum, proximal ileum, and distal ileum. The four small intestinal segments were weighed, and then the tissue samples were snap-frozen in liquid nitrogen and stored at −70°C until analysis for tracer enrichment, protein, and DNA as described previously (6).

Sample analysis. Plasma samples for amino acid concentration measurements were mixed with an equal volume of methionine sulfone (4 mM) as internal standard and centrifuged at 10,000 g for 30–60 min at 4°C through a 10-kDa cutoff filter. The filtrate was dried, and the amino acids were analyzed by reverse-phase HPLC of their phenyl isothiocyanate derivatives (PicoTag, Waters, Woburn, MA). For isotopic analysis, 0.1 ml plasma was mixed with 0.25 ml 1 M HCl. The solution was then applied to a 2-ml bed volume of Dowex 50Wx8 (H+ form; BioRad, Richmond, CA). The columns were rinsed four times with 1 ml of 0.01 M HCl, and the flow-through was discarded. The amino acids were eluted with four 1-ml aliquots of 5 M NH4OH and dried under vacuum.

Samples (~1 g) of small intestinal tissue were pulverized in liquid nitrogen. Subsamples of ~200 mg were homogenized in 4 ml deionized water (Ultra Turrax, Tekmar, Germany) and kept in an ice bath. Aliquots were removed quickly for analysis of protein and DNA as previously described (6). The homogenate was then treated with 1 ml 2 M perchloric acid (PCA) and centrifuged (1,200 g for 20 min at 4°C). The supernatant was separated from the precipitate and brought to pH 7 with 4 M KOH. After removal of the potassium perchlorate precipitate (1,200 g for 20 min at 4°C), the supernatant was dried under vacuum. The protein precipitate was washed two times by resuspension in 5 ml 0.2 M PCA. After the second wash, the precipitate was hydrolyzed and 2 ml 6 M HCl at 110°C for 18–24 h. The protein hydrolysate was dried, dissolved in water, and redried. The dried supernatant and the dried protein hydrolysate were resuspended each in 1 ml 0.1 M HCl, centrifuged, and separated from insoluble residue. Next, 250 μl of the supernatant were mixed with 250 μl 1 M HCl, and tissue-free amino acids (soluble in PCA) were isolated by cation-exchange chromatography as were the plasma amino acids (see above). A total of 50 μl of the dissolved hydrolysate were treated equally.

Mass spectrometry analysis of leucine from plasma, the small intestinal tissue-free pool, and protein hydrolysate was conducted with the n-propyl ester heptfluorobutyramide derivative using methane-negative chemical ionization as previously described (23). The analyses were performed with a 5890 series II gas chromatograph linked to a model 5989B quadrupole mass spectrometer (Hewlett-Packard, Palo Alto, CA). The isotopic enrichment of leucine was determined by monitoring ions at a mass-to-charge ratio of 349 to 352.

Calculations. Whole body leucine flux (Q) and fractional protein synthesis rate (FSR) were calculated from the equations described previously (22). Whole body leucine flux (Q) in μmol · h−1 · kg−1 was calculated as follows:

\[
Q = R \times \left( \frac{\text{IE}_{\text{infectus}}}{\text{IE}_{\text{plasma}}} - 1 \right)
\]

where R is the tracer infusion rate (in μmol · h−1 · kg−1) and IEinfectus and IEplasma are the isotopic enrichments (i.e., tracer-to-tracee ratio expressed as mol%) of the infused tracer and plasma, respectively. The rate of endogenous leucine appearance (R") was calculated according to the following equation:

\[
R" = Q - I
\]

where Q is whole body leucine flux and I is leucine intake. Protein synthesis was calculated as a fractional rate (FSR; in %/day):

\[
\text{FSR} = \frac{\text{IE}_{\text{bound}}}{\text{IE}_{\text{free}}} \times 24/t \times 100
\]

where IEbound and IEfree are the isotopic enrichments (in mol%) of [3H]leucine of the PCA-insoluble (protein bound) and PCA-soluble (tissue free) leucine pool; t is time of labeling in hours, and 24 is the number of hours per day.

The absolute protein accretion rates, DNA accretion rates, and the absolute and fractional protein synthesis and degradation rates were calculated as described previously (8). The absolute protein accretion rate (AAR; in mg/day) of the four small intestinal segments was calculated from the difference.
in tissue protein mass measured at the end of the 7-day treatment period (TPm) and that estimated at day 0 (TPp):

\[ \text{AAR} = \frac{(\text{TP}_m - \text{TP}_p)}{7} \]

The small intestinal protein mass of four control pigs at postnatal day 7 (i.e., day 0 of the treatment period) was measured to obtain the average intestinal protein mass per kilogram of body weight. The intestinal protein mass of all pigs at day 0 (TPp) was estimated by multiplying their respective body weight (measured on day 0) by the average tissue protein mass of each segment per unit of body weight measured directly in the four control pigs that were killed on day 0 of the treatment period (data not shown). The absolute rates of DNA accretion were calculated similarly. The absolute protein synthesis rate (ASR; in mg/day) was calculated as the FSR measured at the end of the 7-day treatment period multiplied by the average tissue protein mass (TPavg).

\[ \text{ASR} = \text{FSR} \times \text{TP}_{\text{avg}} \]

The average tissue protein mass (TPavg; in mg) in pigs during the 7-day treatment period was calculated from the TPp and TPm.

\[ \text{TP}_{\text{avg}} = \frac{(\text{TP}_p + \text{TP}_m)}{2} \]

The absolute protein degradation rate (ADR; in mg/day) was calculated as the difference between the absolute protein synthesis and accretion rate.

\[ \text{ADR} = \text{ASR} - \text{AAR} \]

The fractional protein degradation rate (FDR; in %/day) was calculated as the ADR divided by the average tissue protein mass.

\[ \text{FDR} = \frac{(\text{ADR/TP}_{\text{avg}})}{100} \]

Statistics. Data were subjected to one-way ANOVA to detect differences associated with the level of enteral nutrient intake. When intake level was statistically significant, differences between specific intake levels were determined using the Fisher’s protected multiple comparison test. Values of intestinal protein and DNA accretion were tested for difference from 0 using a one-sample t-test. For all other variables, the lowest enteral intake level that was statistically different from the negative control (i.e., no enteral intake) was designated as the minimum stimulatory level. Means were also tested for statistical difference from the positive control (i.e., 100% enteral intake). Statistical significance was assigned at \( P < 0.05 \).

RESULTS

\(^{2}\text{H}_{3}\text{leucine enrichments and whole body flux.} \) The initial and final body weights (3.11 ± 0.09 and 4.74 ± 0.48 kg, respectively), and hence weight gain, were not significantly different in all seven treatment groups. The mean rates of daily weight gain (in g/kg) were 58, 57, 63, 61, 63, 61, and 53 (pooled SD = 8, \( P \) value = .539) in the 0, 10%, 20%, 40%, 60%, 80%, and 100% enteral intake groups, respectively. Table 1 shows the plasma enrichment (tracer-to-tracee ratio) between 4 and 6 h of \(^{2}\text{H}_{3}\text{leucine infusion and whole body leucine flux.} \) In each treatment group, regression analysis indicated that there were no significant differences in the plasma leucine enrichment between 240 and 360 min. The mean leucine enrichments between 4 and 6 h were not different in groups receiving 0–60% enteral intake. However, in groups receiving 80% and 100% enteral intake, the mean leucine enrichments were significantly higher than in the 0% enteral intake group. These differences in mean plasma leucine enrichments translated into similar differences in the rate of whole body proteolysis as defined by the endogenous leucine appearance (\( R_e \)). Consequently, the rate of whole body proteolysis did not change from 0 to 60% enteral intake but showed a significant decrease at 80% and 100%. There were no differences in the plasma leucine concentrations among the seven treatment groups.

![Table 1. Plasma and intestinal mucosal tracer enrichments](http://ajpgi.physiology.org/)
The intestinal tissue-free $[^2\text{H}_3]$leucine enrichment after 6 h of tracer infusion in each of the four intestinal segments was expressed as a percentage of the plasma enrichment (Table 1). At 0 and 10% enteral intake, the values (74–81%) were similar among the four intestinal segments. In the remaining treatment groups, the tissue leucine enrichments decreased progressively with increasing enteral intake, but the response varied depending on the intestinal segment. Compared with the 0% enteral intake group, the tissue leucine enrichments were significantly lower above 20% enteral intake in the proximal jejunum, above 40% in the distal jejunum, above 60% in the proximal ileum, and above 80% in the distal ileum. At 100% enteral intake, the tissue $[^2\text{H}_3]$leucine enrichment of the proximal jejunum was only 24% of the plasma leucine isotopic enrichment, whereas that of the distal ileum was 60%.

**Protein and DNA accretion rates.** Figure 1 shows the relationships between the accretion rates (mg/day) of protein and DNA in all four segments and the level of enteral intake. With the exception of the distal ileum, the protein and DNA accretion rates were negative ($P < 0.05$ vs. 0) at 0% enteral intake and both increased significantly between 0% and 100% enteral intake. Strikingly, in the distal ileum, both protein and DNA accretion were positive at 0% enteral intake but decreased significantly between 0% and 20% enteral intake and then increased between 20% and 100%. These changes in tissue protein and DNA accretion were also evident in villus morphology; the villus heights were (in μm) 570, 482, 430, 801, 1,019, 1,068, and 933 (pooled SD = 180) in the groups receiving 0%, 10%, 20%, 40%, 60%, 80%, and 100% enteral intake, respectively. Villus height decreased slightly by 25% between the 0% and 25% levels before increasing significantly ($P < 0.05$) at the enteral intake level of 40% and higher. The approximate enteral intakes at which positive protein balance occurred were 20%, 20%, 40%, and 20% enteral intake for the proximal jejunum, distal jejunum, proximal ileum, and distal ileum, respectively. When accretion rates in all four segments were averaged, intestinal protein balance occurred at 20% enteral intake. In all four segments, both protein and DNA accretion were significantly positive ($P < 0.05$ vs. 0%) between 60% and 100% enteral intake.

**Rates of protein synthesis and degradation.** Table 2 shows the changes of fractional (%/day) and absolute (mg/day) rates of protein synthesis and degradation in all four segments in each enteral intake group. In the jejunum, none of the four parameters increased significantly until 60% of enteral intake was achieved, with the exception of the fractional synthesis rate in the proximal jejunum at 40%. At 60% enteral intake, all four parameters were significantly increased and normalized (i.e., not significantly different from 100% enteral intake) in the proximal and distal jejunum. In contrast, in the ileum, there were no significant differences in the fractional rates (synthesis or degradation). Even so, the proximal segment showed a significant increase in the absolute rate of synthesis and degradation at 60% enteral intake and 60% to 80%, respectively, but in general the distal ileum did not show such an increase.
DISCUSSION

The primary objective of our study was to determine the optimal level of enteral intake to achieve intestinal protein balance in TPN-fed neonatal pigs. Our results indicate that TPN resulted in net intestinal protein loss in neonatal pigs. On average, intestinal protein balance occurred at 20% enteral intake and protein accretion was stimulated by 60–100% enteral intake. However, there were differences among the four intestinal segments. At 0% enteral intake, the jejunum and proximal ileum were in negative protein balance, whereas balance in the distal ileum was positive. Regional differences in the intestinal response to TPN have not been reported in previous studies, although there is evidence that ileal mass is relatively well preserved during starvation (14). Because the input of luminal nutrients becomes progressively lower in the distal small intestine, even at 100% enteral intake, it is conceivable that this region is less dependent on enteral intake for maintenance of protein balance.

The differences in protein balance in response to enteral intake level were generally reflected in the relative rates of protein synthesis and degradation; however, the response of the jejunum and ileum differed significantly from one another. Although the protein synthesis and degradation rates in all four regions were similar at 0% enteral intake, at the 100% level, the rates were approximately twice as high in the jejunum than in the ileum. This proximal-to-distal decrease in the rates of protein synthesis along the intestine is consistent with previous findings under fed conditions (1, 8, 12). In the jejunum, the minimal enteral intake necessary to significantly increase the protein synthesis and degradation rates was ~40%, and interestingly, this was the level of enteral intake at which protein synthesis and degradation rates were normalized, i.e., not significantly different from 100%. In contrast, there were no statistically significant differences in fractional protein synthesis and degradation rates in the ileum between 0% and 100% enteral intake. This may seem paradoxical given that the rates of protein accretion increased significantly with enteral intake level. However, the explanation for this observation lies in the fact that, at 100% enteral intake, the difference between the fractional synthesis and degradation rates in the proximal jejunum and distal ileum was 6.2%/day and 7.0%/day, respectively, even though the value for the fractional synthesis rate was twofold higher in the proximal jejunum (124%/day) than in the distal ileum (59%/day). These results highlight the fact that the differences between protein synthesis and degradation, albeit relatively small and apparently insignificant, can lead to markedly different rates of protein accretion.

The differences in fractional protein synthesis rate between the proximal and distal intestine were largely due to differences in the [2H3]leucine enrichment in the mucosal-free pool rather than in the protein-bound fraction. In general, the mucosal-free leucine enrichment progressively decreased at 0% and 100% enteral intake, yet the differences were approximately twice as large in the jejunum as in the ileum. Although the dilution of the mucosal-free leucine enrichment with increasing enteral intake was predictable on the basis of past studies (9), the difference in tracer dilution along the length of the small intestine is a novel observation. Once again, this result suggests that luminal availability and mucosal uptake of unlabeled...

### Table 2. Fractional and absolute rates of protein synthesis and degradation

<table>
<thead>
<tr>
<th>Enteral Intake</th>
<th>Pooled SD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0% 10% 20% 40% 60% 80% 100%</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Fractional synthesis rate, %/day</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proximal jejunum</td>
<td>45.4</td>
<td>67.6</td>
</tr>
<tr>
<td>Distal jejunum</td>
<td>45.9</td>
<td>47.4</td>
</tr>
<tr>
<td>Proximal ileum</td>
<td>48.3</td>
<td>44.2</td>
</tr>
<tr>
<td>Distal ileum</td>
<td>50.9</td>
<td>46.2</td>
</tr>
<tr>
<td><strong>Absolute synthesis rate, mg/day</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proximal jejunum</td>
<td>837</td>
<td>1,186</td>
</tr>
<tr>
<td>Distal jejunum</td>
<td>849</td>
<td>857</td>
</tr>
<tr>
<td>Proximal ileum</td>
<td>997</td>
<td>921</td>
</tr>
<tr>
<td>Distal ileum</td>
<td>1,071</td>
<td>908</td>
</tr>
<tr>
<td><strong>Fractional degradation rate, %/day</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proximal jejunum</td>
<td>49.4</td>
<td>70.4</td>
</tr>
<tr>
<td>Distal jejunum</td>
<td>48.3</td>
<td>48.4</td>
</tr>
<tr>
<td>Proximal ileum</td>
<td>51.4</td>
<td>47.2</td>
</tr>
<tr>
<td>Distal ileum</td>
<td>48.9</td>
<td>47.0</td>
</tr>
<tr>
<td><strong>Absolute degradation rate, mg/day</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proximal jejunum</td>
<td>907</td>
<td>1,235</td>
</tr>
<tr>
<td>Distal jejunum</td>
<td>891</td>
<td>869</td>
</tr>
<tr>
<td>Proximal ileum</td>
<td>1,064</td>
<td>974</td>
</tr>
<tr>
<td>Distal ileum</td>
<td>1,023</td>
<td>898</td>
</tr>
</tbody>
</table>

Values are means from 5–7 pigs/group with pooled SD. *Significantly different from 0% enteral intake.
enteral leucine was substantial in the proximal jejunum, but progressively diminished along the length of the small intestine. This seems logical given that the function of the small intestine is to efficiently absorb as much of the dietary nutrient input as possible. However, because there is considerable protein accretion in the distal intestine, despite this apparently reduced luminal amino acid availability, it raises the intriguing possibility that the distal intestine derives a larger proportion of its amino acids needed for protein synthesis from the circulation than from the diet. If indeed this is the case, it could explain why the fractional rate of protein turnover is lower in the distal than in the proximal intestine. Furthermore, the fact that protein accretion in the distal ileum still increased substantially with enteral intake level suggests that growth in this region of the gut may be influenced to a greater degree by humoral signals released in response to enteral intake, rather than by the local stimulus of luminal nutrients.

In addition to our observations of small intestinal protein metabolism, we found that the level of enteral intake significantly altered whole body protein turnover. The total nutrient intake, including leucine, was similar in all seven treatment groups, although the 60% and 80% groups were slightly (~5–7%), but significantly lower than the average intake. However, the endogenous rate of leucine appearance trended downward with increasing enteral intake, being statistically significant at the 80% and 100% levels. According to the principles of the single-pool model (25), this suggests that whole body protein turnover was apparently suppressed, especially above the 60% enteral intake level; a finding consistent with that reported in low-birthweight infants (2) and human adults (4). Because we did not measure the leucine oxidation rate, it is unknown whether whole body protein synthesis was also increased at these enteral intakes. It is conceivable that these relatively high enteral intakes trigger the release of hormones that suppress protein turnover. Interestingly, we found that the circulating concentration of insulin-like growth factor-I (IGF-I), but not insulin, also increased abruptly (by approximately twofold) between 60% and 100% enteral intake (7). Given the well-known protein-anabolic actions of IGF-I (10, 15, 24), the increased circulating IGF-I may have suppressed whole body protein turnover.

However, an alternative interpretation is that the first-pass splanchnic utilization of enteral leucine was directly proportional to small intestinal protein mass, and because this increased significantly above 60% enteral intake, there was a progressive increase in the first-pass intestinal utilization of enteral leucine. We have found the first-pass intestinal utilization of enterally fed [2H3]leucine in young pigs can be as much as 35–40% of the dietary intake (23). A consequence of this would be that the dilution of the intravenous [15N]leucine tracer enrichment in the plasma by enterally derived leucine was progressively diminished with increasing enteral intake and hence the estimate of endogenous leucine Ra would decrease.

**Perspectives**

Our results indicate that in neonatal pigs TPN resulted in intestinal protein loss; protein balance occurred at 20% enteral intake, and accretion was stimulated at 60% to 100% enteral intake. The fractional rates of protein synthesis and degradation in the jejunum were significantly increased in response to increasing enteral nutrient intake, however, the rates of protein turnover in the ileum were less responsive to the level of enteral intake. Tracer kinetics indicated that mucosal uptake of luminal amino acid is substantially diminished despite high rates of protein accretion in the ileum, suggesting that perhaps it derives a larger proportion of amino acids to support protein accretion from the circulation than from the diet. From a clinical perspective, it is conceivable that the formulas and breast milk usually given as minimal enteral nutrition to preterm infants could have a greater intestinal trophic effect than the elemental nutrient solution used in the present study. Furthermore, we should mention that the pigs we used were not premature and were allowed to suckle normally before starting the study, both of which are different from the typical nutritional support of preterm infants. However, despite this, given that MEF levels in preterm infants commonly start at <5% of total intake, it would appear that this level of enteral intake is insufficient to maintain intestinal protein balance. This implies that the beneficial functional outcomes associated with the levels of MEF previously documented in clinical studies are probably independent of significant changes in intestinal protein balance. From a nutritional perspective, the current results support the idea that enteral nutrition is required for the maintenance and stimulation of intestine protein balance, but there may be a substantial metabolic cost of providing amino acids via the enteral route in the form of reduced systemic availability for whole body protein deposition.

We are sincerely grateful to Vincent Chan and Kiersten Week for technical assistance. We also thank Jane Schoppe and Adam Gillum for assistance in preparation of the manuscript and Leslie Lodelke for editorial assistance.

This work is a publication of the United States Department of Agriculture/Agricultural Research Service (USDA/ARS) Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine and Texas Children’s Hospital, Houston, TX.

This work was supported in part by National Institutes of Health Grants R01-HD33920 (D. G. Burris) and R01-HD35679 (P. J. Reeds) and by federal funds from the USDA/ARS under Cooperative Agreement 58-6250-6-001. The contents of this publication do not necessarily reflect the views or policies of the USDA, nor does mention of trade names, commercial products, or organizations imply endorsement by the United States Government.

A portion of this study has been published previously (7).

**REFERENCES**
