Minimal enteral nutrient requirements for intestinal growth in neonatal piglets: how much is enough?1–4

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ABSTRACT

Background: Parenterally nourished preterm infants commonly receive minimal enteral feedings, the aim being to enhance intestinal function. Whether this regimen increases intestinal growth has not been established.

Objective: Our objective was to determine the minimal enteral nutrient intakes necessary to stimulate and to normalize neonatal intestinal growth.

Methods: Intestinal growth and cell proliferation were quantified in neonatal pigs given equal amounts of an elemental nutrient solution for 7 d. Different groups (n = 5–7 per group) received 0%, 10%, 20%, 40%, 60%, 80%, or 100% of total nutrient intake enterally, with the remainder given parenterally.

Results: In the jejunum, wet weight, protein mass, and villus height were significantly greater at enteral intakes > 40%. Stimulation of ileal protein mass required a higher enteral intake (60%). In both segments, abrupt increases in DNA mass, crypt depth, ornithine decarboxylase activity, and crypt cells in S-phase occurred between enteral intakes of 40% and 60%. Circulating concentrations of glucagon-like peptide-2 and peptide YY, but not gastrin, increased significantly between enteral intakes of 40% and 60% and closely paralleled indexes of cell proliferation.

Conclusions: The minimal enteral nutrient intake necessary to increase mucosal mass was 40% of total nutrient intake, whereas 60% enteral nutrition was necessary to sustain normal mucosal proliferation and growth. Our results imply that providing < 40% of the total nutrient intake enterally does not have significant intestinal trophic effects. Am J Clin Nutr 2000;71:1603–10.

KEY WORDS Cell proliferation, glucagon-like peptide-2, GLP-2, PYY, peptide YY, total parenteral nutrition, enteral nutrition, minimal enteral feeding, preterm infants, premature infants, low-birth-weight infants, neonatal nutrition, neonatology

INTRODUCTION

Total parenteral nutrition (TPN) is used commonly in the clinical management of infants who are unable to tolerate full enteral feedings (1). However, a lack of enteral nutrient support may lead to reduced growth and functional development of the intestinal mucosa (2, 3). In infants with short-bowel syndrome, enteral nutrient support is critical for intestinal adaptation but is often restricted because of concerns regarding malabsorption. To circumvent these concerns, the practice of giving TPN-fed preterm infants small volumes of enteral nutrition, referred to as minimal enteral feeding, has been adopted. Minimal enteral feeding is a strategy for accelerating the maturation of gastrointestinal motility and the tolerance of full enteral feedings, and hence for reducing the duration of hospitalization (4).

It has been postulated that the beneficial effects of minimal enteral feeding may result from the intestinal trophic effect of enteral nutrients. Indeed, there is evidence that luminal nutrients stimulate intestinal growth and maintain mucosal integrity in adult and neonatal animals (5–13). However, the optimal amounts and composition of nutrients necessary for normal gastrointestinal growth and function in neonates are unknown. Gastrointestinal tissues represent only ≈ 5% of body weight, but because of their inherently high rates of metabolism, they represent a disproportionate fraction (≈ 15–35%) of whole-body oxygen consumption and protein turnover (14–16). Moreover, we have shown that the intestinal mucosa utilize substantial amounts (40–60%) of dietary amino acids and lesser amounts (5%) of glucose (17, 18).

The relative significance of cellular and physiologic signals that mediate the intestinal trophic effects of enteral nutrients have not been clearly established. Studies with cultured intestinal cells indicated that specific nutrients can directly activate
signaling pathways that lead to increased cell proliferation, protein synthesis, and apoptosis (19–21). However, enterally administered nutrients also stimulate the secretion of several peptide hormones that have intestinal trophic effects (22–25).

The aim of this study was to quantify the amounts of enteral nutrition necessary to minimally stimulate and to normalize small intestinal growth in neonates. We used neonatal piglets because of their well-established homology to preterm infants in terms of gastrointestinal development and function, body composition, and metabolism (26–30). On the basis of previous results (17, 18), we hypothesized that an enteral intake between 40% and 60% of total nutrient intake would maintain intestinal growth in neonatal pigs. We measured endpoints of intestinal growth and cell proliferation in groups of neonatal pigs that received different proportions of their total nutrient intake via the enteral route, with the remainder given parenterally. To identify potential humoral signals linking enteral nutrients and intestinal growth, we also determined the relations between circulating concentrations of intestinal trophic hormones and intestinal growth.

MATERIALS AND METHODS

Materials

Pigs were purchased from the Texas Department of Criminal Justice (Huntsville). The sows were fed a commercial, nonpurified diet (Purina Mill Inc, St Louis). Amino acids were obtained from Ajinomoto Inc (Tokyo). Intralipid was obtained from Baxter Healthcare Corp (Deerfield, IL). Dextrose, bromodeoxyuridine, and other chemicals were obtained from Sigma-Aldrich (St Louis) unless otherwise indicated.

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 National Research Council guidelines. Pregnant sows were housed in the Children’s Nutrition Research Center (Houston) at 74°F in free-standing farrowing crates and were given food and water ad libitum. The sows farrowed naturally; after 7 d, the piglets were removed from the sows and were surgically implanted with a polyvinyl chloride catheter (1.78 mm outer diameter) in the carotid artery and a silastic catheter (1.65 mm outer diameter) in the external jugular vein and gastric fundus. They were then randomly assigned to 7 groups of equal mean body weight (3112 ± 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 total nutrient intake was given parenterally via the intravenous catheter. We studied a total of 37 pigs from 5 litters; within each litter, ≥6 treatment groups were replicated. In each litter, ≥2 pigs were given either 0% (negative control) or 100% (positive control) enteral nutrition.

The elemental nutrient solution consisted of glucose (104 g/L), a complete amino acid mixture (55 g/L), lipid (Intralipid; 21 g/L), electrolytes, trace minerals, and vitamins. The nutrient solution was administered continuously. After an initial 24-h period in which the animals received 50% of the total intake, they received 900 kJ·kg⁻¹·d⁻¹, 13 g amino acids·kg⁻¹·d⁻¹, and a fluid intake of 240 mL·kg⁻¹·d⁻¹ for 6 d.

Tissue collection and analysis

On postoperative day 7, each animal received an intravenous injection of 5-bromodeoxyuridine (BrdU; 50 mg/kg) to measure the in vivo crypt cell proliferation index. Four hours after the injection of BrdU, the pigs were killed with an overdose of sodium pentobarbital (50 mg/kg). The abdomen was opened and the entire small intestine distal to the ligament of Treitz was immediately flushed with ice-cold saline. The segment of small intestine proximal to the ligament of Treitz, designated as the duodenum, and the stomach were removed, flushed with ice-cold saline, weighed, and snap-frozen in liquid nitrogen. After flushing, the remainder of the small intestine was divided into 2 equal portions; the proximal half was designated the jejunum and the distal half, the ileum. These segments also were divided in half. The 4 small-intestinal segments were weighed, an aliquot was removed for determination of ornithine decarboxylase activity, and a section of each was placed in 10% buffered formalin for subsequent histologic evaluation. The remaining tissue sample was snap-frozen in liquid nitrogen and stored at −80°C until analyzed for protein and DNA as described previously (31). Tissue samples (100–200 mg) were homogenized in water and aliquots were removed for analysis of protein content and lactase activity (32).

BrdU crypt-labeling and morphometry

Formalin-fixed, paraffin-embedded sections (5 μm) of each segment were mounted on slides at 60°C for 30 min. The slides were incubated at 70°C for 10 min, rehydrated with distilled deionized water, rinsed with phosphate-buffered saline (PBS), and incubated in target unmasking fluid (Boehringer Mannheim, Mannheim, Germany) at 90°C for 10 min. The slides were incubated with 1% sheep serum (The Binding Site, San Diego) at 40°C for 15 min. The serum was removed and the slides were incubated with a mouse anti-BrdU/nuclease reagent (Amersham Pharmacia Biotech Inc, Piscataway, NJ) at 40°C for 45 min. The slides were rinsed with PBS to remove excess primary antibody and incubated with biotinylated universal second antibody (anti-mouse IgG2a; The Binding Site) at 40°C for 30 min after the incubation with PBS containing 0.3% H2O2 and 0.1% NaN3 for 10 min at room temperature. Staining of BrdU-labeled cells was achieved by using an ABC Reagents Kit and a DAB (3,3′-diaminobenzidine) Substrate Kit for peroxidase (Vector Labs, Burlingame, CA). The slides were incubated in avidin–biotin complex followed by a solution containing 0.3% H2O2, DAB, and nickel. The slides were counter-stained with 0.1% hematoxylin solution for 45 s and dehydrated through an increased series of ethanol and xylene solutions; cover slips were attached. As crypt cells undergo S-phase of the cell cycle, they incorporate BrdU into their nuclei. The proportion of proliferating crypt cells was quantified by counting the number of BrdU-labeled nuclei in 15 vertically well-oriented crypts and expressing this as a percentage of total nuclei per crypt.

For morphometry analysis, formalin-fixed intestinal samples were embedded in paraffin, sliced to ≈5 μm, and stained with eosin and hematoxylin. Villus height, crypt depth, and muscularis thickness were measured by using an Axioskop microscope (Carl Zeiss Inc, Werk Göttingen, Germany) and NIH IMAGE software, version 1.60 (National Institutes of Health, Bethesda, MD) in 15 vertically well-oriented crypt–villus units.

Ornithine decarboxylase assay

One gram of fresh tissue was immediately homogenized in 19 volumes of 10 mmol tris-HCl buffer/L, pH 7.2 (1:20 wt:vol)
containing 5 mmol dithiothreitol/L, 1 mmol EDTA/L, and 50 μmol pyridoxal phosphate/L (33). The homogenate was centrifuged (3000 × g for 25 min at 4°C), frozen in liquid nitrogen, and stored at −70°C. To quantify enzyme activity, the frozen supernate was quickly thawed, and in a stoppered Erlenmeyer flask, 900-μL aliquots were incubated with 900 μL buffer (freshly prepared, same as above) and 50 μL buffer containing 5 μL (4.5 nmol) l-[1-14C]ornithine (1.7 Bq/μmol; Amersham Pharmacia Biotech Inc) for 60 min at 37°C in a shaking bath. The reaction was stopped by injecting 500 μL 10% trichloroacetic acid through the rubber stopper into the reaction mixture. The 14CO2 that was liberated was trapped by incubating for an additional 30 min at 37°C with 50 μL 2 mol NaOH/L suspended in a center well inside the Erlenmeyer flask. The 14CO2 was measured by using liquid scintillation spectrometry. Aliquots of the 30000 × g supernate were analyzed for protein as described previously (31). Results are expressed as pmol 14CO2·mg protein−1·h−1. To measure nonspecific decarboxylation, incubation mixtures similar to those described above (900 μL supernate, 900 μL buffer, 50 μL l-[1-14C]ornithine) were incubated and treated similarly in the presence of 5 mmol α-difluoromethylornithine/L (Ekkehard Böhme, Hoechst Marion Roussel Research Institute, Hoechst Marion Roussel Research Institute, Hoechst Marion Roussel Inc, Cincinnati), a suicide inhibitor of ornithine decarboxylase.

Peptide hormone radioimmunoassays

Blood samples were drawn into tubes coated with EDTA (4.5 mg), were mixed gently, and were immediately centrifuged (2000 × g for 5 min at 4°C) to obtain plasma. The chilled plasma samples were quickly frozen in liquid nitrogen and stored at −70°C until analyzed. All samples assayed for a given hormone were run in one assay. Plasma glucagon-like peptide 2 (GLP-2) concentrations were quantified as described previously (34). Plasma samples were extracted with 3 volumes of 70% ethanol and centrifuged (3000 × g for 30 min at 4°C). The supernate was decanted, lyophilized, and reconstituted to the original plasma volume in assay buffer (0.1 mmol tris buffer/L, pH 8.5, containing 2.9 g NaCl/L, 2 g human serum albumin/L, 20 mmol EDTA/L, and 400000 Kallikrein inhibitor units aprotinin/L). Approximately 300 μL extracted sample and human GLP-2 (1–33) standards were incubated with 100 μL rabbit GLP-2 antiserum (final dilution 1:10000) raised against human GLP-2; this antiserum recognizes both the human and porcine full-length GLP-2 (1–33) peptide. The sensitivity of this assay is ≈100 pg/tube and the intraassay CV was 10%.

Plasma peptide YY (PYY) concentrations were quantified as described previously (35). Plasma samples (100 μL) and porcine PYY standards were incubated with rabbit PYY antiserum (final dilution 1:100000) raised against the full-length porcine PYY (1–36) peptide. The sensitivity of this assay is ≈5 pg/tube and the intraassay CV was 15%. Gastrin and gastric-inhibitory polypeptide (GIP) concentrations were measured by using commercially available radioimmunoassay kits (Peninsula Laboratories Inc, San Carlos, CA). Plasma samples (100 μL) and human gastrin-17 standards were incubated with rabbit gastrin antiserum raised against human gastrin-17; the antiserum recognizes both gastrin-17 and gastrin-34. The sensitivity of the assay was 5 pg/tube and the intraassay CV was 11%. GIP was measured in plasma samples (100 μL) incubated with rabbit antiserum raised against porcine GIP and compared with porcine GIP standards. The sensitivity of the assay was 25 pg/tube and the intraassay CV was 7%. Plasma insulin and insulin-like growth factor (IGF) were quantified as described previously (36). The intraassay CVs for insulin and IGF-I were 12% and 10%, respectively.

Statistics

Data were subjected to one-way analysis of variance (ANOVA) to detect differences associated with the amount of enteral nutrient intake. When the one-way ANOVA for amount of enteral intake was statistically significant, differences between specific intake amounts were determined with Tukey’s multiple-comparison test. The lowest enteral intake amount that was significantly different from the negative control (ie, 0% enteral intake) was designated as the minimum stimulatory amount, whereas the highest amount that was not significantly different from the positive control (ie, 100% enteral intake) was designated as the enteral intake necessary to normalize intestinal growth. Normal growth was defined as that observed with 100% enteral nutrient intake. Significance was assigned at P < 0.05. All data are expressed as means ± SEMs.

RESULTS

Daily weight gain (59.3 ± 1.4 g·kg−1·d−1) did not differ significantly among the 7 treatment groups (group means not shown). In the small intestine, a progressive, proximal-to-distal change occurred in the magnitude of the response to enteral feeding; the proximal segments were more sensitive to the amount of enteral intake than were the distal segments. To illustrate the main points and minimize the quantity of data presented, we show results only for the proximal jejunum and proximal ileum. In Figure 1, we show the changes in the composition of intestinal growth associated with the different amounts of enteral intake. The increase in intestinal mass was first manifested in wet tissue weight and was subsequently evident in protein content and DNA content. In the jejunum, the minimal enteral intake necessary to increase wet weight and protein content was 40% of total nutrient intake, whereas in the ileum, none of the variables increased significantly until an enteral intake of 60% of total nutrient intake was provided. In both segments, the enteral intake required to normalize wet weight and protein content was 80% of total nutrient intake. The DNA content was less sensitive to enteral intake, and in neither segment was there a significant increase until the animal received 60% of its intake enterally.

In the proximal jejunum, the protein-to-DNA ratio, an index of cell size, was 13.9, 14.6, 15.9, 16.9, 18.3, 17.8, and 17.9 in pigs given 0%, 10%, 20%, 40%, 60%, 80%, and 100% enteral intake, respectively. In both the proximal jejunum and proximal ileum, the protein-to-DNA ratio was significantly (P < 0.05) greater at enteral intakes ≥40% (data for proximal ileum not shown). However, DNA content in both segments was normalized at an enteral intake of 80%. Despite the relatively large changes in small intestinal growth, there were no relations between amount of enteral intake and gastric weight, protein content, or DNA content (data not shown).

In Figure 2, we show the morphologic changes in the mucosa. Irrespective of the amount of enteral intake, villus height was lower and crypt depth was greater in the jejunum than in the ileum. Villus height tended to parallel the changes in tissue wet weight and protein, increasing significantly above the negative control at a 40% enteral intake in both the jejunum and ileum. Villus height was normalized at a 60% enteral intake in the jejunum and ileum. Crypt depth tended to parallel changes in DNA content contained in DNA.
There were no significant differences in the circulating concentrations of either gastrin ($\bar{X}$: 125 ± 4 pmol/L) or insulin ($\bar{X}$: 16.7 ± 1.3 mU/L) among the 7 enteral-intake groups. In contrast, as shown in Figure 4, there were clear relations between the amount of enteral intake and circulating concentrations of GLP-2, PYY, GIP, and IGF-I. Both GIP and GLP-2 showed evidence of increases at a 40% enteral intake, although in the case of GLP-2, significant changes did not occur until 60% of the nutrients were given enterally. It is important to note that the GLP-2 assay we used recognizes the N-terminal region of the peptide and thus measures the biologically active form. PYY concentrations showed an abrupt 4-fold increase between enteral intakes of 40% and 60%, whereas the mean circulating concentration of IGF-I did not increase significantly until the animals received between 80% and 100% of total nutrients enterally. An enteral intake ≥60% was required to normalize the circulating concentrations of GIP, GLP-2, and PYY, whereas IGF-I concentrations were normalized at an 80% enteral intake.

On the basis of regression analysis, circulating concentrations of GIP, GLP-2, and PYY were significantly ($P < 0.001$) correlated with several of the endpoints of intestinal growth. In general, circulating concentrations of GIP, GLP-2, and PYY were more highly correlated with growth in the ileum than in the jejunum. When GIP, PYY, and GLP-2 were correlated with endpoints of growth in the ileum, they each accounted for a substantial percentage of the variation in protein content and increased significantly only at or above a 60% enteral intake. Moreover, crypt depth was also normalized in both segments at a 60% enteral intake. There were no significant differences among groups in the muscularis mucosa layer (data not shown).

In Figure 3, we show the relation between the amount of enteral intake and 2 indexes of cell proliferation, ornithine decarboxylase activity and percentage of S-phase crypt cells (i.e., BrdU labeling). The pattern of response in both of these measurements was strikingly similar to that of crypt depth and increased significantly at a 60% enteral intake. Both of these cell-proliferation indexes were normalized at a 60% enteral intake in both segments.
It is important to note that these values represent the product of lactase-specific activity (μmol·min⁻¹·g protein⁻¹) and protein mass (g/kg body weight) and thus represent the lactase digestive capacity of each intestinal segment. In both segments, lactase activity increased significantly (P < 0.001) as amount of enteral intake increased. However, lactase activity did not increase significantly until the enteral intake reached 80% and 100% in the proximal jejunum and proximal ileum, respectively.

**DISCUSSION**

The clinical practice of providing a small proportion (≤10%) of total nutrient intake enterally, often referred to as minimal enteral feeding, is being used with increasing frequency in preterm infants. The aim is to accelerate the maturation of gastrointestinal function and hence the tolerance of full enteral feeding (4). Despite encouraging results showing the beneficial effects of this regimen on the development of intestinal motility and the prevention of cholestatic jaundice, the effect of minimal enteral feeding on neonatal gastrointestinal growth has not been explored. Although it is well established that some amount of enteral nutrition is critical for the stimulation and maintenance of intestinal growth (7, 8), the minimum amount that achieves these effects is not known. Therefore, our primary aim in this study was to quantify both the minimum amount necessary to provide some stimulus and the amount necessary to normalize intestinal growth.

Our results indicate that the minimal enteral nutrient intake necessary for stimulation varied with the endpoint chosen as the measure of growth and the region of the intestine. However, irrespective of the region studied, the amount of enteral nutrition required to increase mass and protein content (40% of total nutrient intake) was consistently less than that required to stimulate proliferative activity (60% of total nutrient intake), at least as inferred from measurements of DNA content, crypt depth, and BrdU incorporation. Similarly, the protein mass of the proximal region of the intestine was clearly more responsive than was that of the distal region. In contrast, the amount of enteral nutrition needed to increase cell proliferation showed much less regional variation.

The biochemical and structural changes, observed mainly in the proximal intestine, suggest that relatively low amounts of enteral intake (20–40% of total intake) were sufficient to increase cell hydration and size (protein-to-DNA ratio), especially in villus cells, because mucosal DNA content was unchanged. The increase in protein content that paralleled the increased tissue hydration may have resulted from the activation of cell-volume-dependent processes and hence possibly reflected direct stimulation by enteral nutrients (20, 37, 38). The fact that the jejunum was more sensitive to enteral intake than was the ileum in these respects may simply reflect proximal absorption and the amount necessary to normalize intestinal growth.

We also examined the extent to which the amount of enteral intake affected intestinal function by measuring the changes in intestinal lactase activity; lactase is the enzyme responsible for hydrolysis of the major milk carbohydrate, lactose. In both segments, relatively high enteral intakes (i.e., ≥80%) were needed to stimulate lactase activity. Thus, the increases in intestinal protein mass observed at lower enteral intakes (i.e., 40%) in the proximal intestine were not paralleled by increased carbohydrate digestive function. It is conceivable that this functional response to minimal enteral nutrition might be evident with formula or breast milk, rather than an elemental diet, and indeed this was reported recently in TPN-fed piglets given 20% enteral nutrition (39).

An especially intriguing finding of our study was the consistent stimulation of the indexes of cell proliferation between enteral intakes of 40% and 60%. This was particularly striking in the proximal jejunum, in which both wet weight and protein content increased linearly up to a 40% enteral intake, whereas under these conditions the indexes of crypt cell proliferation were unchanged. Given that evidence obtained with cell culture indicates that cell proliferation is directly responsive to extracellular nutrients (19, 20), at face value the results suggest that proliferative responses are less sensitive to the amount of...
enteral nutrient input than processes controlling cell volume and protein metabolism. However, we suggest that the difference is more likely a reflection of humoral regulation of proliferation and that the relatively high amounts of enteral nutrients required to activate proliferation do so by stimulating the secretion of key humoral factors into the circulation.

There is considerable evidence to support the concept of humoral growth regulation in the small intestinal mucosa (22, 40). However, a variety of hormones and growth factors have been postulated as mediators of the intestinal growth; these include gastrin, PYY, IGF-I, and most recently, GLP-2, yet there is little consensus as to the relative importance and physiologic significance of these factors (23–25). The criteria we considered essential for candidate humoral signals that mediate the trophic effects of enteral nutrients are as follows: 1) the hormone must be correlated with both the amount of enteral nutrient intake and intestinal growth, and 2) the hormone should stimulate intestinal growth when given systemically at either physiologic or pharmacologic doses. According to the first criterion, our results suggest that neither circulating gastrin nor insulin is an important humoral growth signal under the conditions of our experiment. This is contrary to previous reports stating that gastrin concentrations are suppressed during TPN and increased with feeding. However, apart from the species and developmental differences between the present study and those that implicated gastrin (22, 41), it may be important that the present study was designed such that the animal growth rate and the circulating amino acid and glucose concentrations were maintained at high and constant values across all the groups.

On the other hand, circulating PYY, GLP-2, IGF-I, and GIP concentrations were significantly correlated with both enteral intake and intestinal growth such that, according to our first criterion, all are good candidates for intestinal growth regulators. Moreover, there is good evidence that GLP-2 is an intestinal trophic peptide (23), but the evidence for PYY- and IGF-I-mediated effects is more equivocal because PYY and IGF-I produced intestinal trophic effects in many (24, 25, 42), but not all (43–45), instances. We are not aware of any studies showing an intestinal trophic effect of GIP. However, evidence suggests that GIP functions as a proximal endocrine signal released in response to

![FIGURE 4. Mean (±SEM) changes in circulating plasma concentrations of glucagon-like peptide 2 (GLP-2), peptide YY (PYY), gastric-inhibitory polypeptide (GIP), and insulin-like growth factor I (IGF-I) in neonatal pigs given 0–100% of their total nutrient intake enterally for 7 d (n = 5–7 per enteral-intake group). *Significantly different from respective 0%-enteral-intake group, P < 0.05.](image)

![FIGURE 5. Mean (±SEM) changes in intestinal lactase activity in the proximal jejunum (●) and proximal ileum (○) of neonatal pigs given 0–100% of their total nutrient intake enterally for 7 d (n = 5–7 per enteral-intake group). *Significantly different from respective 0%-enteral-intake group, P < 0.05.](image)
duodenal nutrient input which, in conjunction with neural signals, stimulates the secretion of proglucagon-derived peptides and PYY from the L-cells located in the distal intestine (35, 46). Thus, it is conceivable that the close correlation between circulating GIP concentrations and intestinal growth is a reflection of the increased secretion of GLP-2 and PYY in response to GIP.

In summary, our results indicate that the minimal amount of enteral nutrients necessary to stimulate intestinal growth is 40% of total nutrient intake, whereas an enteral intake of ≥60% is needed to normalize intestinal growth. When compared with results of our previous studies (17, 18), the present results suggest that the total amount of enteral nutrients metabolized in first-pass metabolism by the small intestine is similar to that necessary to maintain growth. We have also shown that some gut-related peptides, namely GLP-2 and PYY as well as IGF-I, may be important humoral signals mediating the trophic effects of enteral nutrients. However, the direct physiologic relevance of these peptides, as well as the trophic potential of GIP, awaits further study. 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 did the elemental nutrient solution used in the present study. We should note 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 usual conditions under which nutritional support is given to preterm infants. Furthermore, attempting to provide amounts of enteral feeding (ie, 40%) sufficient to stimulate intestinal growth in preterm infants can lead to feeding intolerance and necrotizing enterocolitis if the enteral nutrition is delivered too rapidly. Despite these caveats, however, it appears that minimal enteral feeding at <5–10% of total intake may have minimal effects on intestinal growth. This implies that the beneficial functional outcomes associated with minimal enteral feeding that were previously documented in clinical studies were probably independent of significant changes in intestinal mass.

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REFERENCES

26. Shulman RJ. The piglet can be used to study the effects of parenteral and enteral nutrition on body composition. J Nutr 1993;123:395–8.