0148-6071/06/3006-0474$03.00/0
JOURNAL OF PARENTERAL AND ENTERAL NUTRITION
Vol. 30, No. 6
Printed in U.S.A.

Original Communications

Impact of Caloric Intake on Parenteral Nutrition–Associated Intestinal Morphology and Mucosal Barrier Function

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ABSTRACT. *Background:* Parenteral nutrition (PN) is known to induce villus atrophy, epithelial cell (EC) apoptosis, and increase mucosal permeability. The study hypothesized that increasing amounts of energy delivery to mice would result in the best outcome, with the least effects on the mucosa. *Methods:* Mice were randomized to enteral controls (saline infusion with *ad libitum* enteral food) or to 1 of 3 PN groups (with no enteral nutrition): full (100% of daily average energy intake for the mouse), reduced (75% of energy intake) or very low (50% of energy intake). Mice received PN for 7 days. Mucosal morphology, EC apoptosis, and bacterial translocation were assessed. *Results:* Villus height decreased significantly with decreasing levels of caloric intake and was significantly lower in all PN groups compared with controls. Body weight loss was significantly greater in PN groups *vs* controls and was greatest in mice with the lowest caloric delivery. A consistent trend toward a higher EC apoptotic index with decreasing caloric intake was observed, and apoptosis in all PN groups exceeded controls (2-fold). All PN groups demonstrated greater bacterial translocation than controls. *Conclusions:* PN induces intestinal EC apoptosis and villus and crypt atrophy, even at 100% of predicted energy needs, and such changes increased with greater reduction of energy intake. This study supports a concept that lack of enteral nutrition, rather than absolute caloric levels, is responsible for many of the adverse effects of PN. The study also allows the investigators to better optimize a mouse model of PN delivery. (*Journal of Parenteral and Enteral Nutrition* **30:**474–479, 2006)

Parenteral nutrition (PN) is known to induce significant changes in mucosal structure and function. These changes include villus atrophy, epithelial cell (EC) apoptosis, and altered mucosal permeability. PN administration is associated not only with morphologic changes in the intestine but also with bacterial translocation and a loss of mucosal barrier function.[1–3] The mechanisms of PN-associated loss of epithelial integrity and morphologic changes have been studied extensively using a mouse model.[1–6] The use of a mouse model can offer great insights into these PN-associated effects on the gastrointestinal tract. Recent work by our group has helped to identify mechanisms that contribute to the occurrence of bacterial translocation and villus atrophy.[1–3,6] Unlike other animal models, the use of mice is relatively inexpensive, allows for an extensive study of the alterations in their immune system, and can be managed in a small laboratory setting. Delivery of PN to mice, however, is a challenge and has been associated with a moderate mortality.[7] To address this problem, this study was designed to determine the amount of energy delivery *via* PN using a mouse model, whereby the closest approximation to energy needs is met and with the fewest number of complications. We also sought to determine whether different levels of energy delivery, administered as PN, correlated with PN-associated intestinal changes. We further attempted to determine the most optimal amount of energy delivery while optimizing mouse survival and morbidity. We hypothesized that higher energy delivery *via* PN would result in fewer aberrations in intestinal morphology and improved survival.

Received for publication November 21, 2005.
Accepted for publication June 30, 2006.
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MATERIALS AND METHODS

Animals

C57BL/6J male specific-pathogen-free mice (8 weeks old) were obtained from Jackson Laboratory (Bar Harbor, ME) and were maintained under temperature-, humidity-, and light-controlled conditions. Mice were initially fed *ad libitum* with standard mouse chow and water and allowed to acclimate. During the administration of IV solutions, mice were housed in metabolic cages to prevent coprophagia. The studies reported here conformed to the guidelines for the care and use of laboratory animals established by the University Committee on Use and Care of Animals at the University of Michigan, and protocols were approved by that committee (No. 7703).

Operative Procedures and Study Groups

Mice were anesthetized with sodium pentobarbital (50 mg/kg/body weight, intraperitoneal). All surgical

TABLE I
Description of parenteral nutrition (PN) infusion

Groups	Caloric delivery (kcal/kg/d)*	Protein delivery (g/kg/d)	Fat delivery (g/kg/d)	% of normal enteral caloric intake	PN infusion rate (mL/h)
Full PN, n = 5	471	19.2	7.7	100	0.4
Reduced PN, n = 6	353	14.4	5.8	75	0.3
Very Low PN, n = 7	236	9.6	3.9	50	0.2
Control (enteral), n = 6*	486	30	6.6	100	0.2 (saline)

Mean caloric delivery was based on the initial average body weight (22 g). There was no difference between groups in initial body weight. PN groups did not receive any enteral intake.
*Estimate from Purina Mills Chow Inc., assuming 4-g consumption of food per day, a net metabolizable amount of nutrients (excludes nondigestible fibers), and a mouse weighing 22 g.

procedures were performed under magnification in a sterile fashion. The left jugular vein was exposed and cannulated with a silicone rubber catheter (0.012-inch ID, 0.025-inch OD; Dow Corning, Midland, MI). The distal end of the catheter was tunneled subcutaneously and exited between the scapulae. The catheter was attached to a swivel spring, which allowed the mice freedom of movement in their individual cages (Metamount System, Instech Corp, Plymouth Meeting, MA). Catheterized mice were immediately connected to an infusion pump (AIM pain provider pump, generously donated by Abbott Laboratories, Abbott Park, IL) and saline (dextrose 5% in 0.45 NS with 20 mEq KCl/L) was infused at an initial rate of 4.8 mL/d. After 24 hours, the animals were randomized to control or PN groups. Of note, the AIM pain provider pump is one of the most accurate available to date. Nevertheless, the delivery is cyclical and not continuous. Thus, as infusion rates increased, we found that some mice developed extravasation of IV PN. These mice were not included in the study results.

Average daily caloric intake was measured for mice provided enteral chow *ad libitum* (assessed *via* daily measurements of feed consumption with mice in metabolic chambers, data not shown) and was found to be 470 kcal/kg/d for 8-week-old mice of 22 g body weight, which closely agrees with known estimates of normal caloric consumption for mice,[6,7] as well as estimates provided by the Purina Mills Veterinary Service[8] (Table I).

Study Groups

Mice were then provided PN at 100%, 75%, or 50% of normal mouse energy intake (470 kcal/kg/d, Full group, n = 5; *vs* 350 kcal/kg/d, Reduced group, n = 6; and 235 kcal/kg/d, Very Low group, n = 7; respectively, Table I) and were not permitted any enteral nutrition but were allowed *ad libitum* water. PN was formulated by a clinical PN supplier (HomeMed of the University of Michigan Health System, Ann Arbor, MI) and contained a balanced mixture of amino acids, lipids, and dextrose, in addition to electrolytes and vitamins (Table II). All mice receiving PN were provided drinking water *ad libitum* but no enteral feedings during the 7 days receiving PN. The control group (n = 6) received IV physiologic saline at 0.2 mL/h, in addition to standard mouse chow and water *ad libitum*. All animals were euthanized with CO_2 at 7 days. Body weight was recorded both before the initiation of the study and after the mice were euthanized.

Bacteriological Cultures

Methods used were similar to those previously described.[9,10] After killing, and using sterile technique, samples of tissue from mesenteric lymph nodes, liver, and spleen were excised. Each tissue sample was incubated in thioglycolate broth for 24 h at 37°C and then subcultured onto both MacConkey media for isolation of Gram-negative bacteria, and colistin nalidixic acid media (Becton Dickinson, Cockeysville, MD) for isolation of Gram-positive bacteria in an aerobic environment. After an additional 24-hour incubation period, growth on each plate was recorded as either positive or negative. Positive cultures were considered indicative of bacterial translocation.[9,10]

Intestinal Morphology

Intestinal specimens were immediately fixed in 10% neutral buffered formalin after killing. Specimens were paraffin embedded and cut into 5-μm-thick tissue slices, parallel with the longitudinal axis of the bowel. Tissue slices were mounted and stained with hematoxylin and eosin for assessment of villus length and crypt

TABLE II
Parenteral nutrition (PN) formulation

	Amount per 50 mL formulated PN	Volume (mL) of component in 50 mL	kcal/mL of individual component
Aminosyn II 10%	2 g protein equivalent	20	0.40
Dextrose 70%	12.5 g dextrose	18	2.38
Liposyn II 20%	1 g fat	5	2.0
Electrolytes		6.07	0
Water		0.93	0
Total volume of final PN (mL)*		50	1.227kcal/mL

*All PN solutions were supplemented with multivitamins and a complete trace-elements package, as previously described.[22]

depth. Five different fields of each specimen were observed, and digital images were recorded with computer-aided video microscopy (Eclipse TS100, Nikon Inc, NY). Villus length and crypt depth for each specimen were measured and analyzed using commercially available quantitative digital image analysis software (Media Cybernetics Inc, MD). Each measurement consisted of the mean of 5 different fields. At least 20 villi were counted and averaged for each sample, and only villi with an intact central lymphatic channel were considered.

Assessment of Apoptosis

Tissue sections were stained with terminal-deoxynucleotidyl transferase nick-end labeling (TUNEL) for quantification of apoptosis *in situ*. The ApopTag InSitu Apoptosis Detection Kit (Serologic Corporation, Norcross, GA) was used according to the manufacturer's instructions. Apoptosis was defined by both the finding of apoptotic bodies with positive TUNEL staining as well as by morphologic criteria (pyknotic nuclei, condensed chromatin, and nuclear fragmentation).[11–13] Apoptosis was assessed by counting the number of apoptotic bodies identified within the crypts and villi. The apoptotic index was defined as the ratio of apoptotic cells to the total number within the crypt-villus axis per section. The mean value of 4 sections was calculated per sample.

Nutrition and Biochemical Parameters

Serum biochemical parameters, including total protein, albumin, gamma glutamyltransferase (GGT), and total bilirubin, were measured. Serum was drawn *via* inferior vena caval puncture immediately after killing. Tests were performed by the clinical laboratory in our hospital using standardized methods.

Data Analysis

Statistical analysis was carried out using the *t*-test for comparison of 2 means and a one-way ANOVA for comparison of multiple groups (with a Bonferroni *post hoc* analysis to assess statistical differences between groups). The χ^2 test was used for categorical data. Linear regression analysis was used to correlate the amount of energy delivered *via* PN to the several observed outcome changes, including survival and intestinal morphology. Prism software was used (GraphPad Software, Inc, San Diego, CA). Statistical significance was defined as $p < .05$. All data are expressed as means ± SD.

RESULTS

General Outcome

Survival progressively worsened in the PN mice as the rate of infusion increased (36%, 40%, and 58% for the Very Low, Reduced and Full PN groups) compared with 100% survival in the enteral control group. No correlation could be made, however, between the amount of energy delivery and survival ($p > .05$). The cause of death was consistently due to accumulation of a leakage of PN fluid in the neck of mice in the Full energy delivery group and due to a number of factors for mice in the lower rates, including PN extravasation, apparent sepsis, or line occlusion.

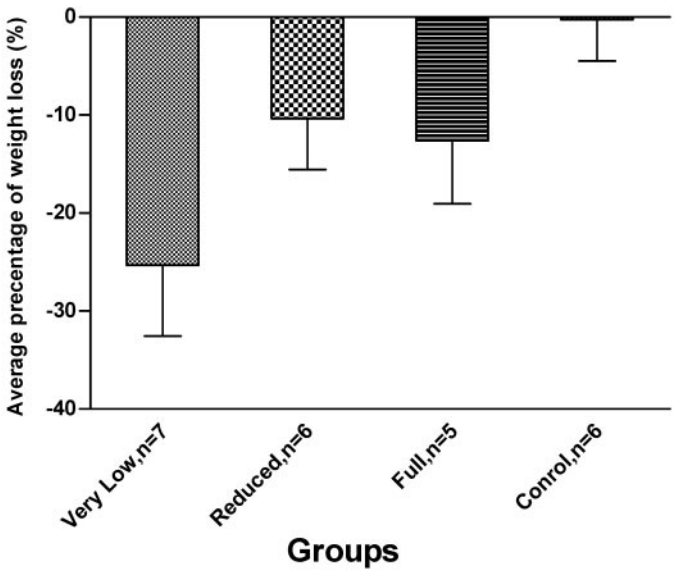

FIGURE 1. Body weight in all PN groups was significantly less compared with control after 7 days of infusion ($p < .01$). Body weight loss was greatest in the Very Low group. (Percentage weight loss calculated as: [Initial weight − Final weight]/[Initial weight] × 100).

Initial body weight of mice did not differ between PN and control groups ($p > .05$). After 7 days of PN, body weight in all 3 PN groups was significantly lower compared with controls ($p < .01$; Figure 1). Weight loss (% of initial weight) was greatest in the Very Low PN group, reaching 25.3% ± 7.2%, and was progressively less for the Reduced (10.35% ± 5.2%) and Full PN (12.63% ± 6.4%) groups; compared with them, only 0.24% ± 4.26% for control group. Weight losses between PN groups, however, were not significantly different ($p > .05$).

Intestinal Morphology

Villus lengths and crypt depths in the 3 PN groups were significantly lower compared with the control group ($p < .01$). In addition, villus length and crypt depth showed the greatest degree of atrophy in the Very Low PN group *vs* Reduced and Full groups ($p < .05$, Table III). Thus, progressively lower amounts of caloric delivery resulted in progressively greater degrees of mucosal atrophy (Figure 2); however, linear

TABLE III
Morphologic change correlates with caloric intake

Groups	Villus length (μm)	Crypt depth (μm)
Full PN	320.9 ± 63.6*†	82.6 ± 14.9*†
Reduced PN	308.4 ± 65.2*	76.1 ± 7.4*†
Very Low PN	259.5 ± 58.3*	56.6 ± 8.9*
Control	397.4 ± 49.5	121.4 ± 27.2

*Compared with control group: $p < .01$. †Compared with the Very Low PN group: $p < .05$.

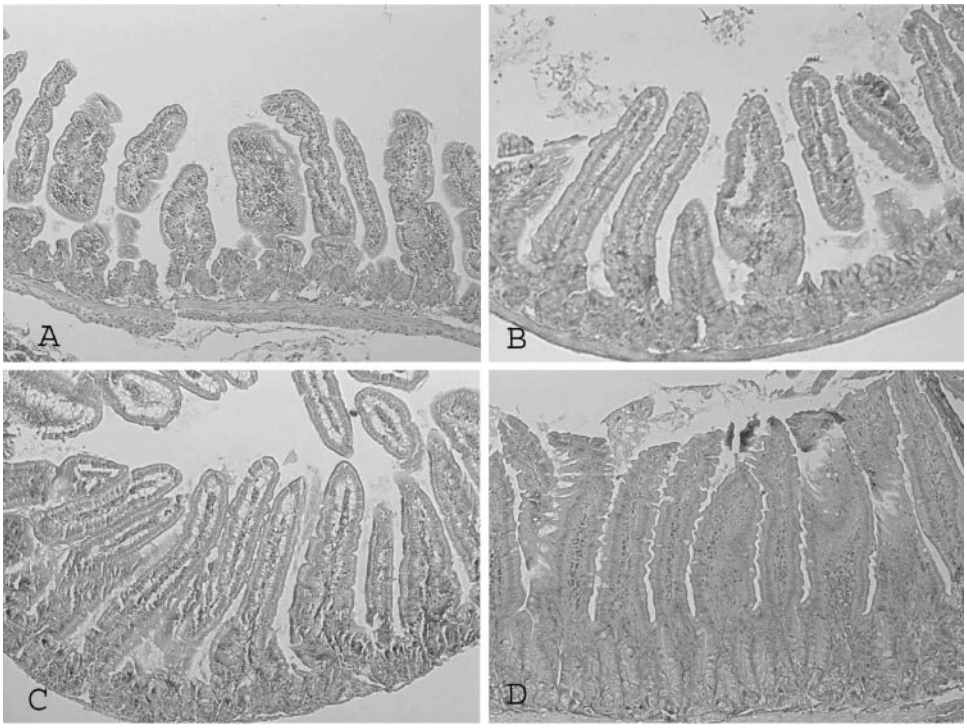

FIGURE 2. Progressively lower amounts of caloric delivery resulted in progressively greater degrees of mucosal atrophy. A, Very Low PN intestine villus; B, Reduced PN; C, Full PN; and D, Enteral control. Photomicrographs are taken at 40× magnification.

regression analysis did not demonstrate a statistically significant relation between the level of energy delivered and the degree of intestinal atrophy ($p > .05$).

Intestinal EC Apoptosis

The apoptotic index was significantly higher in all PN groups by >2-fold, in comparison to controls, both in the villi and in the crypts ($p < .01$). A consistent trend toward increasing apoptotic index was found with decreasing caloric intake, although the difference in apoptotic index between PN groups did not reach significance by ANOVA ($p > .05$, Table IV).

Biochemistry

After 7 days of PN, serum biochemical and nutrition parameters were similar to baseline (control) values (Table V), with the notable exception of alanine aminotransferase, which was markedly elevated in the Very Low group. Total bilirubin and GGT were elevated in the Full PN group, possibly suggestive of a cholestatic process. Other values (serum albumin and total protein) were not markedly affected by 7 days of PN either at Full, Reduced, or Very Low rates of caloric delivery. Blood glucose levels were somewhat elevated in all groups at the onset of the experimental week, including the control population, potentially due to the stress of the operative procedure (185, 224, 146, and 221 mg/Dl for Enteral control, Very Low, Reduced and Full PN groups, respectively, mean value from 3 mice). Levels decreased toward the latter half of the experiment, and none of the PN groups were felt to be excessively elevated (190, 140, 89, and 156 mg/dL for Enteral control, Very Low, Reduced, and Full PN groups, respectively; mean value from 3 mice).

Bacterial Translocation

Bacterial translocation results are shown in Table VI. In the PN groups, the percentage of bacterial translocation to mesenteric lymph nodes, liver, and spleen in Very Low, Reduced, and Full groups was 38.1%, 55.6%, and 33.3% for Gram-negative cultures and 52.4%, 55.6%, and 53.3% for Gram-positive cultures, respectively. In contrast, the control group demonstrated bacterial translocation in only 11.1% of Gram-positive and 0% of Gram-negative cultures ($p < .01$ *vs* all PN groups). No significance in bacterial translocation rates was found between PN groups.

TABLE IV
Intestinal epithelial cell apoptosis

	Very Low	Reduced	Full	Control
Apoptotic index/villus	0.16 ± 0.03*	0.17 ± 0.04*	0.14 ± 0.03*	0.06 ± 0.01
Apoptotic index/crypts	0.114 ± 0.03*	0.111 ± 0.02*	0.089 ± 0.03*	0.041 ± 0.01

Compared with control group: *$p < .01$.

TABLE V
Changes of biochemical parameters in each study group (mean value)

Groups	Total protein (g/dL)	Albumin (g/dL)	ALT (IU/L)	GGT (IU/L)	Total bilirubin (mg/dL)
Very Low, n = 7	5.6	2.7	177	17	1.7
Reduced, n = 6	5.6	2.5	62	7	1.5
Full, n = 5	6.3	4.3	56	51	2.7
Control, n = 6	5.6	2.7	44	10.3	1.6

Levels were analyzed using an Ektachem 750 system (Johnson & Johnson Inc, New Brunswick, NJ).
ALT, alanine aminotransferase; GGT, gamma glutamyltransferase.

DISCUSSION

This study has compared the effect of different levels of nutrition delivery on known PN-associated pathologic changes. Although PN is known to induce villus and crypt atrophy, epithelial apoptosis, and bacterial translocation in the mouse PN model, the effect of reducing nutrition delivery on these changes has not previously been well characterized. Our data indicate that although PN-associated epithelial derangements are significantly worsened by reducing the caloric PN delivery, even with full caloric delivery (100% of normal for the mouse), mucosal morphology and function are still significantly compromised in comparison to the control state (enteral nutrition). It is possible that the differences in this morphology may also be due to some differences in protein and fat delivery to PN mice compared with the enteral group. Even in the PN group receiving equivalent calories to the control group, fat delivery was slightly higher and protein delivery was lower (see Table I). These differences may account for some of these changes, although it was felt that the greatest difference was predominantly in the caloric delivery between the groups. Thus, PN-associated changes may be worsened by a reduction in caloric delivery but are not completely reversed by full caloric delivery in the absence of enteral nutrition. These findings are consistent with another study indicating that the absence of enteral nutrition, and not PN *per se*, is the most important reason for PN-associated intestinal pathology.[6] The current study, however, demonstrates a significant correlation between the degree of these adaptive changes and the amount of calories delivered *via* PN.

The significance of PN-associated villus atrophy has been debated. Villus atrophy may contribute to greater epithelial permeability and loss of barrier function, which may lead to bacterial translocation and subsequent sepsis.[14,15] The etiology of PN-associated villus atrophy is unknown, although numerous mechanisms responsible for this loss of villus length have been proposed.[16,17] Intestinal EC apoptosis is clearly elevated with PN.[2] It was also verified in this study that the apoptotic index was significantly higher in all PN groups by >2-fold, in comparison to controls, both in the villi and in the crypts. A consistent trend toward increasing apoptotic index was found with decreasing caloric intake. In addition, a loss of hormonal stimulation and of nutrition substrates may contribute to villus atrophy.[17–19] Several components of enteral nutrition, including dietary fiber and protein, have protective effects on the intestinal mucosa.[17–20] Furthermore, even when animals undergo minimal stress and gain sufficient body weight with PN, the absence of enteral nutrients results in a decrease in intestinal function and integrity.[20]

Biochemical aberrations included an increase in GGT and total bilirubin in the Full PN group and an increased alanine aminotransferase (ALT) in the Low PN group. Although the etiology of PN-related cholestasis is unknown, these laboratory changes suggest that PN may adversely affect the liver in different fashions, depending on the amount of delivered nutrients. Interestingly, the incidence of bacterial translocation was unaffected by the level of caloric PN delivery. In all PN groups, bacterial translocation was dramatically higher than in mice receiving enteral nutrition. The explanation for PN-associated bacterial translocation is incompletely understood, although it is clear that intraepithelial lymphocytes (IEL; closely associated with the intestinal epithelium) undergo phenotypic and function changes with PN administration, with a loss of thymic-derived $CD8\alpha\beta^+$ lymphocytes,[7] an upregulation of IEL-derived IFN-γ expression,[2] a decreased mucosal IgA level, and diminished T-cell and B-cell counts in Peyer's patches.[21] Thus, either epithelial apoptosis and increased permeability or associated mucosal immune changes may account for the observed elevation in bacterial translocation.

TABLE VI
Bacterial translocation

Groups	MacConkey agar (Gram-negative)			CAN (Gram-positive)		
	Positive	Negative	% Positive	Positive	Negative	% Positive
Very Low, n = 7	8*	13	38.1	11*	10	52.4
Reduced, n = 6	10*	8	55.6	10*	8	55.6
Full, n = 5	5*	10	33.3	8*	7	53.3
Control, n = 6	0	18	0	2	16	11.1

Compared with control group: *$p < .01$. No significance in bacterial translocation rates was found between PN groups.
CAN, colistin nalidixic acid media.

The current data show a clear correlation between level of caloric delivery and epithelial apoptosis. This suggests involvement of the starvation response in induction of intestinal epithelial apoptosis, although it is equally clear that absence of enteral nutrients serves as another key trigger for EC apoptosis, even when sufficient parenteral nutrients are provided. Local and systemic signals triggering intestinal EC apoptosis include interferon-γ and other cytokines elaborated by IEL, but control of EC apoptosis is not yet completely understood.[2,3]

The mouse PN model has been extensively used and is a key model for molecular studies in PN. As IV infusion studies are becoming increasingly common in mouse models, technical aspects of these models are of paramount importance. We therefore recorded technical complications of this model, which have not been described in detail previously. Increases in infusion rates are achieved by incrementally increasing the amount per bolus (and not by reducing the time interval between boluses).[22] Surprisingly, in our study, higher infusion rates were associated with a very high rate of technical failure (60%), usually attributable to extravasation of PN at the cannulation site. Mice with technical failure of IV infusion were not included in the study. If infusion rates could be increased by reducing the time interval between boluses (which is 20 seconds on our pumps) rather than by increasing the size of the individual bolus, we believe that this limitation in mice could be overcome. From an experimental viewpoint, the Reduced PN infusion group (0.3 mL/hour) appears to be the most practical for further work, as it optimized the balance between increased mortality associated with insufficient caloric intake *vs* technical failures (extravasation) associated with higher infusion rates. An adjustment in the micro-infusion pumps would probably resolve this difficulty. Although these technical points are irrelevant to PN-induced epithelial changes, we include this discussion because these points have not been adequately reported previously. These technical limitations should be considered by any investigator planning infusion studies in mice.

In summary, PN induces significant derangements in intestinal EC physiology, including higher apoptotic rates, villus atrophy, and greater degrees of mucosal permeability. The current study has shown that although a strong correlation exists between the level of caloric delivery and the severity of many of these derangements, the level of caloric delivery does not entirely account for these changes. To a substantial degree, villus atrophy, apoptosis, and mucosal permeability can only be corrected by provision of intraluminal nutrition.

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