Original Communication

Journal of Parenteral and Enteral Nutrition
Volume 34 Number 5
September 2010 503-512

10.1177/0148607110362586
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Parenteral but Not Enteral Omega-3 Fatty Acids (Omegaven) Modulate Intestinal Regrowth After Massive Small Bowel Resection in Rats

Igor Sukhotnik, MD[1,2]; Annette Shany, MD[1]; Yulia Bashenko, MSc[1]; Lili Hayari, MD[1]; Elena Chemodanov, MD[4]; Jorge Mogilner, MD[2]; Arnold G. Coran[5]; and Ron Shaoul, MD[3]

Financial disclosure: This work was supported by an Israel Science Foundation (ISF) research grant.

Background: The purpose of the present study was to evaluate the effects of ω-3 fatty acids (Omegaven) on early intestinal adaptation in rats with short bowel syndrome (SBS). *Methods:* Male Sprague-Dawley rats were randomly assigned to 1 of 4 groups: sham rats underwent bowel transection; SBS rats underwent 75% bowel resection; SBS-O ω-3 rats underwent bowel resection and were treated with oral Omegaven given by gavage; and SBS-I ω-3 rats underwent bowel resection and were treated with Omegaven given intraperitoneally. Rats were killed on day 14. Parameters of intestinal adaptation (bowel and mucosal weight, mucosal DNA and protein, villus height and crypt depths, cell proliferation and apoptosis) were determined at time of death. Real-time polymerase chain reaction was used to determine the level of Bax and Bcl-2 messenger RNA (mRNA). Statistical analysis was performed using Kruskal-Wallis test followed by post hoc test, with $P < .05$ considered statistically significant. *Results:* Oral ω-3 supplementation did not significantly change intestinal regrowth. In contrast, parenteral ω-3 in rats that underwent resection resulted in higher bowel and mucosal weights, mucosal DNA and protein in ileum, villus height in ileum, crypt depth in jejunum and ileum, and greater rates of cell proliferation in jejunum and ileum compared with SBS animals. The initial decreased levels of apoptosis corresponded with the early decrease in Bax and increase in Bcl-2 mRNA levels. *Conclusions:* Parenteral but not enteral Omegaven augments and accelerates structural bowel adaptation in a rat model of SBS. Increased cell proliferation and decreased apoptosis reflect increased cell turnover in Omegaven-treated animals. (*JPEN J Parenter Enteral Nutr.* 2010;34:503-512)

Keywords: short bowel syndrome; intestinal adaptation; fatty acids, omega-3; proliferation; apoptosis

Short bowel syndrome (SBS) is defined as an intestinal failure following a loss of intestinal length that causes marked maldigestion and malabsorption of dietary nutrients and induces major fecal loss of energy, nitrogen, and fat.[1] SBS is a common problem in pediatric surgery and occurs in newborns and infants suffering from necrotizing enterocolitis, intestinal atresia, and volvulus that require massive intestinal resection.[2] Despite advances in resuscitation, the availability of parenteral nutrition (PN) and potent antibiotics, and modern techniques of organ support, SBS remains a significant cause of infant morbidity and mortality.[2]

The survival of patients after massive small bowel resection depends on the ability of the residual bowel to adapt. *Adaptation* is the term applied to progressive recovery from intestinal failure following a loss of intestinal length.[3,4] The remaining intestine dilates, elongates, and thickens; villous height and crypt depth increase, leading to mucosal hyperplasia and increasing the absorptive surface area (structural adaptation). In addition, increased nutrient absorption by isolated enterocytes occurs (functional absorption). Over the past decades, much research has focused on determining which factors promote intestinal regrowth.

From the Bruce Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, [1]Laboratory of Intestinal Adaptation and Recovery, [2]Department of Pediatric Surgery, [3]Department of Pediatrics, and [4]Department of Pathology, Bnai Zion Medical Center, Haifa, Israel; and [5]Section of Pediatric Surgery, C.S. Mott Children's Hospital and University of Michigan Medical School, Ann Arbor, Michigan.

Received for publication December 5, 2008; accepted for publication November 19, 2009.

Address correspondence to: Igor Sukhotnik, Department of Pediatric Surgery B, Bnai Zion Medical Center, 47 Golomb St, POB 4940, Haifa, 31048, Israel; e-mail: igor-dr@internet-zahav.net.

Enteral feeding is one of the major trophic factors in stimulating intestinal adaptation.[5] However, not all nutrients have equal stimulating trophic effects. Although traditional dietary management advocates a restricted-fat diet containing 50% to 75% medium-chain triglycerides as a source of fat, there are still questions concerning the relative value of high-fat vs high-carbohydrate diets for these patients. Among the factors promoting postresection intestinal adaptation, long-chain fatty acids (FAs) are considered the most effective.[1,5] Several experimental studies have demonstrated that bowel regrowth can be altered by changing dietary ratios of different FAs. It has been reported that animals with SBS receiving a diet deficient in essential FAs have significantly impaired intestinal mucosal hyperplasia.[6] The FA arachidonic acid appears to be a potent stimulator of intestinal adaptation, more than its precursor, linoleic acid.[7] However, dietary linoleic acid has higher stimulating effects on postresection mucosal hypertrophy compared with palmitic acid.[8] We have recently shown that restriction of palmitic acid in the diet inhibits intestinal adaptation and that a diet high in palmitic acid has a mildly stimulating effect on intestinal regrowth.[9]

Over the past years, several preliminary clinical trials have described the positive effects of ω-3 FA fish oil–based lipid (Omegaven; Fresenius Kabi, Bad Homburg, Germany) in prevention of PN-associated liver disease (PNALD) in children with SBS. In a recent review, Diamond et al[10] discussed the rationale for the use of ω-3 FAs. The authors noted that the beneficial effects of substituting ω-6 FAs with ω-3 FAs are likely related to 3 major mechanisms: (1) the addition of ω-3 FAs improves bile flow; (2) ω-3 FAs decrease steatosis; and (3) the addition of ω-3 FAs shifts the eicosanoid profile toward a less inflammatory state. Together, these actions result in decreased cholestasis, hepatitis, and subsequent fibrosis.[10] Because liver disease may hinder intestinal adaptation,[11] ω-3 FAs may ultimately facilitate adaptation in these children by treating their liver disease.

The purpose of the present study was to evaluate the effects of Omegaven on structural intestinal adaptation in a rat model of SBS.

Methods

Animals

The Rappaport Faculty of Medicine (Technion, Haifa, Israel) Institutional Animal Care and Use Committee approved the animal facilities and protocols. Briefly, male rats weighing between 240 and 260 g were kept in individual stainless steel cages at constant temperature and humidity, and a 12-hour light–dark cycle was maintained. Rats were fasted 12 hours before the experiment with free access to water.

Experimental Design

Because intraperitoneal injection of FAs may cause an inflammatory response, a pilot study was performed in normal (nonoperated) rats that were given either intraperitoneal or oral Omegaven. After 7 days of treatment, the rats were killed. Blood samples (1 mL) were obtained from the left ventricle (peripheral blood). Cholesterol and triglyceride levels in serum were measured at time of death using standard enzymatic methods (Cobas Integra 700 Analyzer; Roche, Holliston, MA). The peritoneum and intestinal walls were examined for macroscopic and microscopic signs of inflammation.

Forty rats were randomly assigned to 1 of 4 groups. In group A (n = 10), sham rats underwent bowel transection; in group B, the SBS group (n = 10), animals underwent bowel resection; in group C, the SBS-O ω-3 group (n = 10), rats underwent bowel resection and were treated with 1 mL/d oral Omegaven given by gavage in group D, the SBS-I ω-3 group (n = 10), rats underwent bowel resection and were treated with 1 mL/d intraperitoneal Omegaven. The dose of Omegaven was chosen in accordance with previously described studies.[12] The use of intraperitoneal PN in animal models is well-known and was summarized by LeLeiko,[13] who concluded that intraperitoneal PN provides a relatively simple and efficient method of studying PN in laboratory animals.

Rats were pair-fed and followed for 14 days with monitoring of food and fluid intake and weight measurement.

Surgical Procedure

After a 12-hour fast, animals were anesthetized with intraperitoneal ketamine (90 mg/kg) and xylasine (10 mg/kg). The abdomen was opened using a midline incision with sterile techniques. A sham operation was performed by simple transection of the ileum 15 cm proximal to the ileocecal junction and reanastomosis. Resected animals underwent 75% small bowel resection from 5 cm distal to the ligament of Treitz to 10 cm proximal to the ileocecal valve. Mesenteric vessels were ligated with 5-0 silk sutures, and bowel continuity was restored by end-to-end anastomosis using 5-0 Dexon (Davis & Geck, NY) interrupted sutures. For all operations, the abdominal cavity was closed in 2 layers with a running suture of 3-0 Dexon (Davis & Geck). Before closure of the abdomen, the rats were resuscitated with a 3-mL intraperitoneal injection of warm normal saline. Rats were fasted for 24 hours but were allowed free access to water.

Parameters of Intestinal Adaptation

On postoperative day 15, the animals were killed after intraperitoneal injection of pentobarbital (75 mg/kg). The

small intestine from the pylorus to the ileocecal valve was removed and divided into 2 segments: the jejunum proximal to anastomosis and the terminal ileum. Equivalent lengths of intestine from sham-operated animals were obtained from the corresponding areas. Portions of intestine located 1 cm to either side of anastomosis were discarded. Each segment was weighed and cut longitudinally. Mucosa was scraped using a spatula, collected, and weighed. Bowel and mucosal weights were calculated per cm of bowel length per 100 g of body weight, as described previously.[14,15] DNA and protein were extracted using TRIzol Reagent (Invitrogen) as described by Chomczynski.[16] The DNA concentrations were recorded spectrophotometrically and calculated per cm of bowel length per 100 g of body weight. Final protein concentration was measured spectrophotometrically using a commercially available kit (Bio-Rad Protein Assay, Bio-Rad Laboratories Ltd, Rishon Le Zion, Israel) and was calculated per cm of bowel length per 100 g of body weight.

Histological Examination

Intestinal samples from the proximal jejunum and distal ileum were fixed in 10% formalin, dehydrated in progressive concentrations of ethanol, cleared in xylene, and embedded in paraffin wax. Deparaffinized 5-mcm sections were stained with hematoxylin and eosin. These sections were studied microscopically using a micrometer eyepiece. Histologic images were loaded on a 760×570-pixel resolution buffer using a computerized image analysis system composed of a trichip RGB video camera (Sony, Japan) installed on a light microscope (Zeiss, Germany) and attached to an IBM-compatible personal computer equipped with a frame grabber. Images were captured, digitized, and displayed on a 17-inch, high-resolution color monitor. The villus height and crypt depth were measured using Image Pro Plus 4 image analysis software (Media Cybernetics, Baltimore, MD). Ten villi and crypts in each section were measured, and the mean reading was recorded in mcm.

Enterocyte proliferation and apoptosis. Standard 5-bromodeoxyuridine (5-BrdU) labeling reagent (Zymed Laboratories, San Francisco, CA) was injected intraperitoneally at a concentration of 1 mL per 100 g of body weight 2 hours before animals were killed. Crypt cell proliferation was assessed using a biotinylated monoclonal anti-BrdU antibody system provided in a kit (Zymed Laboratories). Briefly, 5-mcm sections were cut from paraffin-embedded blocks. For antigen retrieval, sections were microwave treated in preheated 0.01 molar citrate buffer (pH 6.0) for 10 minutes. Sections were then pretreated with denaturing solution for 20 minutes and blocked with horse serum for 10 minutes to block nonspecific binding before incubation with the primary antibody. The slides were stained with a biotinylated monoclonal anti-BrdU antibody for 60 minutes at room temperature. Then the slides were treated with streptavidin peroxidase for 10 minutes. BrdU-positive color development was obtained by incubating the sections with diaminobenzidine (DAB) mixture for 3 to 5 minutes. An index of proliferation was determined as the ratio of crypt cells staining positively for BrdU per 10 crypts.

Additional 5-mcm thick sections were prepared to establish the degree of enterocyte apoptosis. Immunohistochemistry for caspase 3 was performed to identify apoptotic cells using a combination of streptavidin–biotin–peroxidase method and microwave antigen retrieval on formalin-fixed, paraffin-embedded tissues according to the manufacturers' protocols. Briefly, the sections were deparaffinized, rehydrated in graded alcohol, and microwave pretreated in ethylenediaminetetraacetic acid buffer for 10 minutes. Then the specimens were incubated in peroxidase quenching solution (3% H_2O_2 in methanol) for 10 minutes and blocked with serum-blocking solution for 10 minutes. Thereafter, samples were stained with primary cleaved caspase-3 polyclonal antibodies (diluted 1:100; Biocare Medical, Walnut Creek, CA) for 60 minutes in a moist chamber at room temperature. After the primary antibody was washed off with phosphate-buffered saline, slides were incubated with a secondary human-absorbed, biotinylated, affinity-purified antibody. Enhanced horseradish peroxidase–conjugated streptavidin was subsequently applied at room temperature for 10 minutes before the sections were visualized with DAB to create an intense brown deposit around the antigen–antibody–enzyme complex in the sample. The apoptotic index was defined as the number of apoptotic cells per 10 villi. Assessment was performed in a blinded manner by a qualified pathologist.

Real-time PCR. Expression of Bax and Bcl-2 levels was determined by quantitative real-time polymerase chain reaction (PCR) (7500 Real-Time PCR System–Applied Biosystems, Foster City, CA) on complementary DNA (cDNA) samples using SYBR Green Master Mix (Rovalab, Teltow, Germany) with the exception of template and primers. Primers for Rattus norvegicus Bax and Bcl-2 were synthesized by Syntezza Bioscience (Jerusalem, Israel), and 18s ribosomal RNA with a 260:280 nm ratio of absorbance was purchased as a control kit from Eurogentec, EGT Group (Israel). Total RNA was isolated using TRIzol according to the manufacturer's instructions. The amount of total RNA was measured by spectrophotometry (Thermo Scientific NanoDrop 1000). Two mcg RNA were reverse-transcribed to cDNA at 37°C using a reverse transcription kit (AffinityScript; Stratagene, Ornat, Israel). The following thermal cycler (T3 Thermocycler; Biometra, Tamar, Israel) settings were used: 94°C for 5 minutes followed by 30 cycles of 94°C × 15 seconds, 55°C × 15 seconds, and 72°C × 30 seconds, followed by a 7-minute extension at 72°C. Thermal cycler settings were optimized to ensure

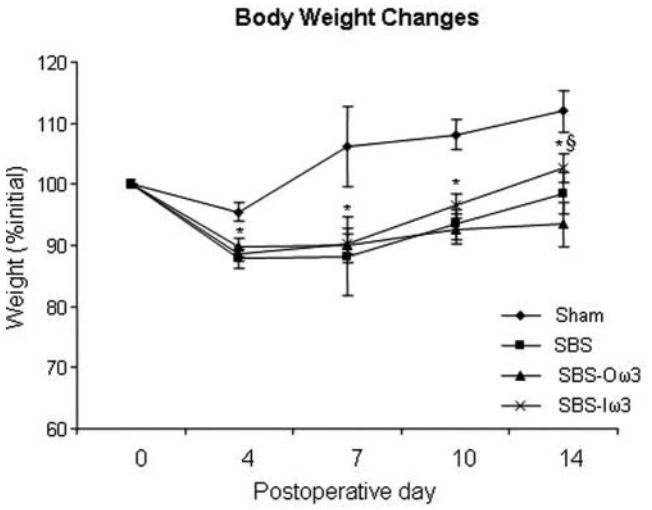

Figure 1. Effect of bowel resection and Omegaven on the body weight changes. Values are mean ± standard error of the mean. SBS, short bowel syndrome; Oω3, oral ω-3; Iω3, intraperitoneal ω-3. Administration of enteral ω-3 did not significantly change body weight gain compared with SBS untreated animals. Treatment with parenteral Omegaven resulted in greater weight gain compared with the other 2 resected groups. *$P < .05$ SBS vs sham rats; §$P < .05$ SBS-Oω3 vs SBS-Iω3 rats.

that products were in the linear phase of production. Amplification was followed by a melting curve analysis to confirm PCR product. No signals were detected in no-template controls. The experimental threshold was calculated using the algorithm enhancements provided by 7500 System SDS version 1.2.3 software (Applied Biosystems). All samples were run in duplicate, and the mean value of each duplicate was used for all further calculations.

Statistical Analysis

The data are expressed as the mean ± standard error of the mean. Statistical analysis of parameters of adaptation, enterocyte proliferation, and apoptosis was performed using Kruskal-Wallis test followed by post hoc test for multiple comparisons. A P value < .05 was considered statistically significant.

Results

Effects of Omegaven on Abdominal Cavity and Plasma Lipids in Normal Rat

In normal, nonoperated rats, neither oral nor intraperitoneal Omegaven caused an inflammatory response (macroscopic and microscopic) within the peritoneal cavity and intestinal wall. Treatment with intraperitoneal Omegaven did not change cholesterol levels but significantly increased triglyceride levels (67 ± 2 vs 42 ± 4 mg/dL, $P <$ .05). Administration of oral Omegaven resulted in a lower cholesterol level (91 ± 4 vs 108 ± 7 mg/dL, $P < .05$) but did not change triglyceride levels compared with non-treated animals.

Body Weight

The body weight of sham-operated control rats remained unchanged during the first 4 days, followed by a gradual increase in weight throughout the next 10-day observation period (Figure 1). The bowel resection group (group B) experienced a significant reduction in weight during the first 4 days, followed by a gradual increase in weight during the next 10 days. However, final body weight was significantly lower in SBS rats compared with sham animals. Administration of enteral ω-3 did not significantly change weight gain compared with SBS untreated animals. Nevertheless, treatment with parenteral Omegaven resulted in a greater weight gain compared with the 2 other groups that underwent resection.

Intestinal Mucosal Parameters

Compared with sham animals (group A), a 75% proximal small bowel resection (group B) resulted in a significant increase in bowel weight in the jejunum (5-fold increase, $P < .05$) and ileum (2-fold increase, $P < .05$); mucosal weight in the jejunum (5-fold increase, $P < .05$) and ileum (2-fold increase, $P < .05$) (Figure 2); mucosal DNA in the jejunum (3-fold increase, $P < .05$); and ileum (3-fold increase, $P < .05$); and mucosal protein in the jejunum (8-fold increase, $P < .05$) and ileum (3-fold increase, $P < .05$) (Figure 3). Resected animals receiving enteral ω-3 had similar bowel and mucosal weight in jejunum and ileum (Figure 3), mucosal DNA in jejunum and ileum, and mucosal protein in jejunum compared with SBS counterparts (group B, Figure 3). Only ileal protein content was increased in this group compared with SBS animals. Compared with SBS animals (group B), treatment with parenteral ω-3 (group D) significantly increased jejunal (24%, $P < .05$) and ileal (35%, $P < .05$) bowel weight; ileal (39%, $P < .05$) mucosal weight; ileal (50%, $P < .05$) mucosal DNA content; and jejunal (49%, $P < .05$) and ileal mucosal protein content (3-fold increase, $P < .05$).

Histological Findings

Data for histological changes in remaining bowel are shown in Figure 4. As expected, small bowel resection (group B) markedly increased villus height in the jejunum (33%, $P < .05$) and ileum (34%, $P < .05$) and crypt depth in the jejunum (31%, $P < .05$) compared with sham animals (group A). Treatment with enteral ω-3 (group C) did not significantly change villus height and crypt depth compared with SBS untreated animals (group B). Treatment with parenteral Omegaven led to a significant

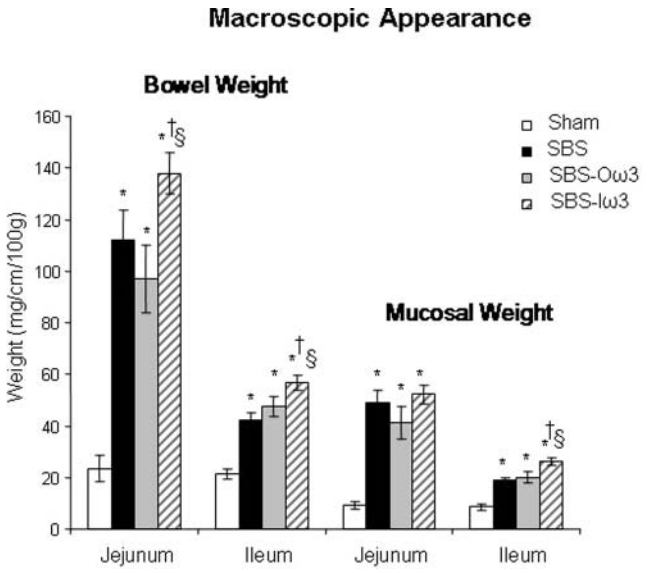

Figure 2. Effect of bowel resection and ω-3 fatty acid supplementation on the macroscopic appearance of the remaining small intestine. SBS, short bowel syndrome; Oω3, oral ω-3; Iω3, intraperitoneal ω-3. Massive small bowel resection significantly increased bowel and mucosal weight in jejunum and ileum compared with sham animals that did not undergo resection. Resected animals receiving enteral ω-3 had similar bowel and mucosal weight in jejunum and ileum compared with SBS animals. Treatment with parenteral ω-3 (group D) significantly increased jejunal and ileal bowel weight as well as ileal mucosal weight compared with SBS animals. Values are mean ± standard error of the mean. *$P < .05$ SBS vs sham rats; †$P < .05$ SBS-Oω3 and SBS-Iω3 vs SBS rats; §$P < .05$ SBS-Oω3 vs SBS-Iω3 rats.

increase in villus height in the ileum (30%, $P < .05$) and crypt depth in jejunum (21%, $P < .05$) and ileum (24%, $P < .05$) compared with SBS animals (group A).

Cell Proliferation and Apoptosis

A significant increase in cell proliferation was seen in rats that underwent resection (group B) compared with sham animals (group A) in both jejunum (264 ± 17 vs 211 ± 17 BrdU-positive cells per 10 crypts, $P < .05$) and ileum (261 ± 15 vs 192 ± 19 BrdU-positive cells per 10 crypts, $P < .05$) (Figure 5). Following oral Omegaven supplementation, SBS rats (group C) demonstrated a mild to significant decrease in jejunal (232 ± 8 vs 264 ± 17 BrdU-positive cells per 10 crypts, $P < .05$) proliferation rates compared with SBS untreated animals (group B) and a trend toward decreased cell proliferation in the ileum; however, this trend was not statistically significant. In contrast, treatment with parenteral ω-3 (group D) significantly increased jejunal (304 ± 11 BrdU-positive cells per 10 crypts, $P < .05$) and ileal (307 ± 17 BrdU-positive cells per 10 crypts, $P < .05$) proliferation rates compared with the other 3 groups.

SBS rats (group B) demonstrated a significant increase in cell apoptosis in jejunum (2.0 ± 0.06 vs 0.60 ± 0.21 apoptotic cells per 5 villi, $P < .05$) and ileum (3.66 ± 0.80 vs 1.31 ± 0.50 apoptotic cells per 5 villi, $P < .05$) compared with sham animals (Figure 6). Following enteral and parenteral ω-3 supplementation, SBS rats (groups C and D) showed a significant decrease in cell apoptosis in jejunum (0.75 ± 0.24 and 1.08 ± 0.32 vs 2.0 ± 0.06 apoptotic cells per 5 villi, $P < .05$, respectively) and ileum (1.55 ± 0.40 and 2.07 ± 0.28 vs 3.66 ± 0.80 apoptotic cells per 5 villi, $P < .05$, respectively) compared with SBS untreated animals.

Expression of Bax and Bcl-2 Genes

An elevated rate of apoptosis in SBS rats (group B) was accompanied by a 3-fold increase in Bax messenger RNA (mRNA) expression ($P < .05$) and no change in Bcl-2 mRNA expression compared with control animals (group A, Figure 7). Treatment with oral ω-3 (group C) attenuated the proapoptotic effects of bowel resection. SBS-O ω-3 rats showed a significant decrease in Bax expression (4-fold decrease, $P < .05$) and a 3-fold increase in a Bcl-2 mRNA expression compared with SBS animals. Parenteral administration of ω-3 (group D) resulted in a more significant decrease in Bax mRNA expression (12-fold decrease, $P < .05$) and a similar oral ω-3 increase in Bcl-2 mRNA levels (3-fold increase, $P < .05$) compared with SBS animals.

Discussion

The ability to absorb many nutrients is decreased following massive bowel resection, and lipid absorption is generally considered the most vulnerable function.[17,18] The combined loss of absorptive surface area and compromised enterohepatic circulation,[19] decreased bile acid pool,[20,21] and decreased pancreatic lipase secretion[22] result in steatorrhea and inefficient fat absorption. Therefore, patients with SBS are often given elemental diets containing medium-chain lipids during the early stages of enteral feeding. However, increasing evidence suggests that long-chain fats are more effective stimulators of intestinal adaptation than either medium-chain fats or carbohydrates.[17] Therefore, several investigators have recommended adding long-chain FAs to the diets of patients with SBS.[1]

Although the positive effects of FAs in stimulating small intestinal adaptation have been reported over the years in many preclinical and clinical investigations,[5-9]

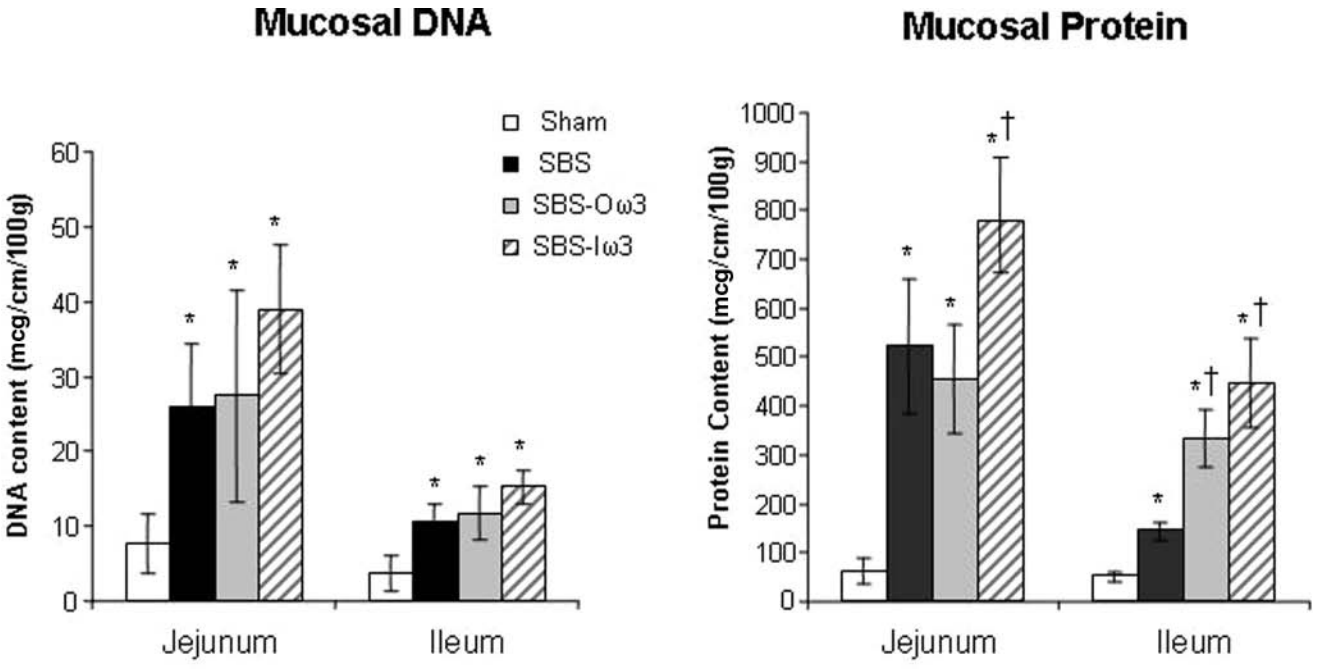

Figure 3. Effect of bowel resection and treatment with ω-3 fatty acid on mucosal DNA and protein content. SBS, short bowel syndrome; Oω3, oral ω-3; Iω3, intraperitoneal ω-3. SBS rats demonstrated a significant increase in mucosal DNA and protein in jejunum and ileum compared with sham animals. Whereas treatment with oral Omegaven significantly increased ileal protein content, parenteral Omegaven significantly increased ileal mucosal DNA content and jejunal and ileal mucosal protein content compared with SBS animals. Values are mean ± standard error of the mean. *$P < .05$ SBS vs sham rats; †$P < .05$ SBS-Oω3 and SBS-Iω3 vs SBS rats.

other studies have yielded conflicting conclusions regarding the benefits of ω-3 FAs in SBS.[10,23] Intravenous lipids are a critical component of PN solutions because they provide essential FAs and dense calories. However, fat emulsions have also been implicated as a significant cause of PNALD.[10,11] The main long-chain polyunsaturated FAs in PN solutions are ω-6 FAs and ω-3 FAs. ω-6 FAs and ω-3 FAs share metabolic pathways and thus interact with each other through a complex system involving dietary substrate availability, competition for the same metabolic enzymes for synthesis and membrane incorporation, and powerful negative feedback of the end products.[24] There is emerging evidence that ω-6 FAs may play a major role in the development of PNALD and that ω-3 FAs may ameliorate this effect by improving bile flow, inhibiting steatosis, and conferring immunomodulatory effects.[10] Gura and co-workers[23] recently reported the reversal of cholestasis in 2 infants with intestinal failure and PNALD after treatment with conventional intravenous fat emulsion, with 1 containing primarily ω-3 FAs.

Because liver disease may hinder intestinal adaptation,[11] we hypothesized that ω-3 FAs may ultimately stimulate intestinal adaptation in patients with SBS by treating their liver disease. In addition, ω-3 FAs may exert a direct stimulating effect on intestinal regrowth by increasing proliferation and decreasing apoptosis of crypt cells. The purpose of the present study was to evaluate the effects of enteral and parenteral ω-3 on structural intestinal adaptation following massive small bowel resection in rats. In a previous study, transperitoneal absorption of a commercial fat emulsion (Liposyn 10%; Abbott Laboratories, Chicago, IL) was studied in 12 beagle puppies.[25] In this experiment, serum triglycerides had increased 3-fold within 15 minutes after intraperitoneal fat injection and then decreased slowly, although remaining significantly elevated at 4 hours. Animals receiving no intraperitoneal fat showed no change in serum triglycerides. The authors concluded that a fat emulsion can be quantitatively absorbed from the peritoneal cavity over 4 hours and that intraperitoneal nutrition may be feasible.

In the current study, changes in bowel and mucosal weights, mucosal DNA and protein content, and histological appearance were measured to characterize intestinal adaptation. In addition, cell proliferation and apoptosis were measured to investigate enterocyte turnover. Two differentiation modulators of apoptosis (Bax and Bcl-2) were determined in tissue samples and quantified at mRNA levels relative to control levels. In the normal

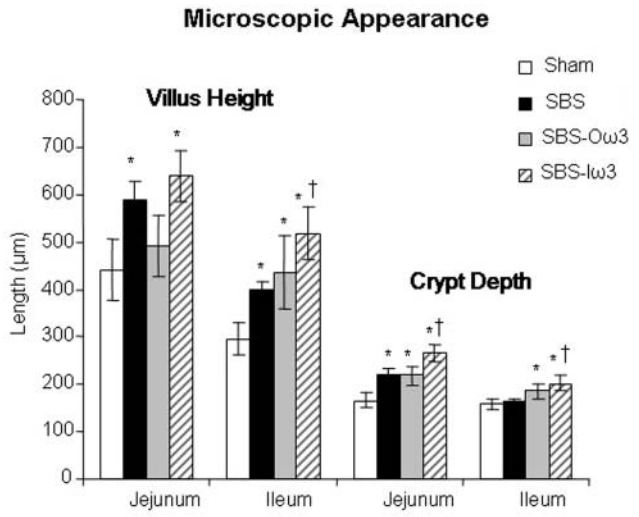

Figure 4. Effect of bowel resection and treatment with Omegaven on the microscopic appearance of the remaining small intestine. SBS, short bowel syndrome; Oω3, oral ω-3; Iω3, intraperitoneal ω-3. Small bowel resection markedly increased villus height in jejunum and ileum and crypt depth in jejunum compared with sham animals. Treatment with enteral ω-3 did not significantly change villus height and crypt depth compared with SBS untreated animals. Treatment with parenteral Omegaven significantly increased villus height in ileum as well as crypt depth in jejunum and ileum compared with SBS animals. Values are mean ± standard error of the mean. $^{*}P < .05$ SBS vs sham rats; $^{\dagger}P < .05$ SBS-Oω3 and SBS-Iω3 vs SBS rats.

physiologic state, the production of crypt cells and loss of cells at the villus tip are balanced to maintain the normal mass of gut mucosa. Cell loss in the small intestine after bowel resection is mainly regulated by programmed cell death. The Bcl-2 family has been implicated in both positive and negative regulation of intestinal cell apoptosis. Augmented expression of Bcl-2 acts in situ on mitochondria to prevent the release of cytochrome *c* and thus caspase activation, whereas Bax localizes to mitochondria and induces the release of cytochrome *c*, activation of caspase 3, membrane blebbing, nuclear fragmentation, and cell death.[26] It is generally accepted that the Bax to Bcl-2 ratio determines the cell's fate, whereby excess Bcl-2 confers survival and excess Bax promotes cell death. In the gut, Bcl-2 is mostly expressed at the base of the colonic crypt but less in the small intestinal mucosa, whereas Bax is predominant in the crypts of the small intestine. The higher ratio of proapoptotic to antiapoptotic genes in the small intestine (compared with in the large intestine) is most likely related to the large volume of potential toxins received by the small intestine, most of which have been neutralized before arrival in the colon.

Consistent with our previous experiments,[14,15] our current findings showed that massive intestinal resection results in significant structural adaptation. This conclusion is supported by the observed increase in bowel and mucosal weight of the remnant bowel, increase in mucosal DNA and protein, and increase in villus height and crypt depth in this model. Decrease in body weight in rats that underwent resection may suggest malnutrition despite the adaptation process. Parallel increases in mucosal DNA and protein indicate that the greater intestinal mass of resected animals can be attributed to cellular hyperplasia. Because the DNA and protein content are directly proportional to mucosal cell number, these measurements exclude such factors as edema and vascular engorgement as responsible for differences in mucosal mass. Most significant differences were observed in the terminal ileum, whereas hyperplasia in the proximal jejunum was less prominent. We used 5-BrdU to determine an index of crypt cell proliferation. This analogue of thymidine is incorporated into the DNA of proliferating cells during the S phase of the cell cycle. Our findings suggest that proliferation of crypt cells increased significantly following bowel resection and was closely correlated with increased crypt depth. The enhanced cell production was accompanied by an increased cell apoptosis, suggesting accelerated cell turnover. Increased cell apoptosis may be considered a mechanism that counterbalances the increased enterocyte proliferation in order to reach a new homeostatic set during intestinal adaptation. In addition, increased apoptosis promotes disposal of genetically aberrant stem cells and prevents tumorogenesis.[27,28] The mucosal response to massive resection in our experiment is comparable to the changes previously observed in our laboratory.[14,15] Bax mRNA expression was upregulated, whereas Bcl-2 mRNA did not change significantly during intestinal adaptation for a specific period. As a result, the Bax to Bcl-2 ratio increased in SBS rats, which correlates with the enhanced enterocyte apoptosis in this group.

The present study demonstrates that enteral ω-3 did not significantly change structural intestinal adaptation. This conclusion is supported by unchanged bowel and mucosal weights of the remnant bowel. In contrast, in several mucosal parameters, a trend toward an inhibitory effect was observed. A decrease in villus height in the jejunum suggests a decreased absorptive surface area in this group of animals. This experiment does not address whether impaired structure is associated with impaired function. However, decreased absorptive surface area is assumed to be accompanied by decreased nutrient absorption. Decrease in body weight in rats receiving oral Omegaven may suggest malnutrition. In parallel with these morphological changes, the epithelial cell turnover of the small intestine of PO-treated rats was significantly affected. We should emphasize that intestinal mucosal hypoplasia is a result of hypoproliferation in intestinal

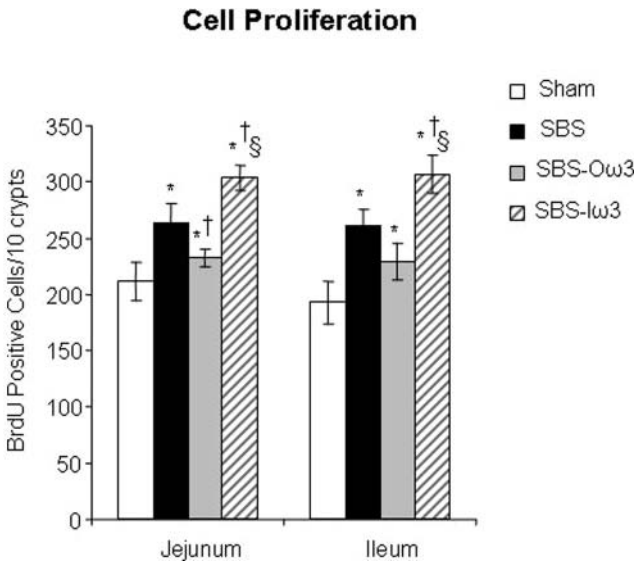

Figure 5. Effect of bowel resection and Omegaven on crypt cell proliferation. SBS, short bowel syndrome; Oω3, oral ω-3; Iω3, intraperitoneal ω-3; BrdU, bromodeoxyuridine. Incorporation of 5-BrdU into proliferating jejunal and ileal crypt cells was detected with a goat anti-BrdU antibody. A significant increase in cell proliferation was seen in SBS rats in jejunum and ileum compared with sham animals. Following oral Omegaven supplementation, SBS rats demonstrated a mild but significant decrease in jejunal proliferation rates compared with SBS untreated animals. Treatment with parenteral ω-3 significantly increased jejunal and ileal proliferation rates compared with the other 3 groups. Values are mean ± standard error of the mean. *$P < .05$ SBS vs sham rats; †$P < .05$ SBS-Oω3 and SBS-Iω3 vs SBS rats; §$P < .05$ SBS-Oω3 vs SBS-Iω3 rats.

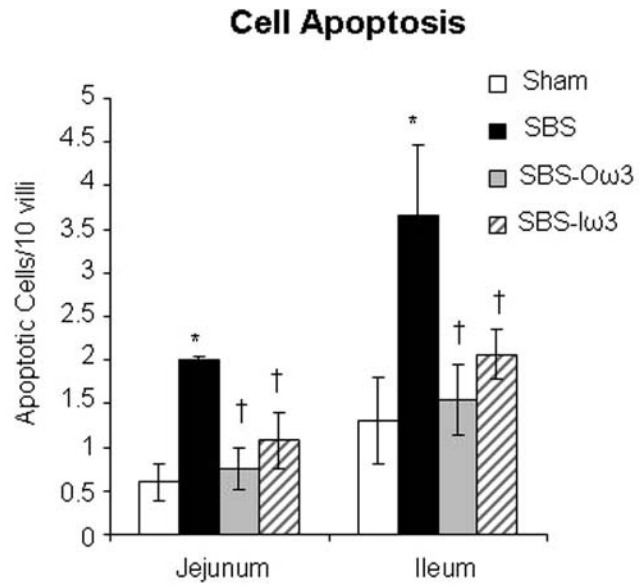

Figure 6. Effect of bowel resection and treatment with ω-3 fatty acids on enterocyte apoptosis in jejunum and ileum. SBS, short bowel syndrome; Oω-3, oral ω-3; Iω-3, intraperitoneal ω-3. Immunochemistry for caspase-3 was used to determine enterocyte apoptosis. SBS rats demonstrated a significant increase in cell apoptosis in jejunum and ileum compared with sham animals. Following enteral and parenteral ω-3 supplementation, SBS rats showed a significant decrease in cell apoptosis in jejunum and ileum compared with SBS untreated animals. Values are mean ± standard error of the mean. *$P < .05$ SBS vs sham rats; †$P < .05$ SBS-Oω-3 and SBS-Iω-3 vs SBS rats; §$P < .05$ SBS-Oω-3 vs SBS-Iω-3 rats.

crypts secondary to dietary treatment, which leads to villous atrophy and absorptive dysfunction. The mechanism of the decrease in cell apoptosis is poorly understood. The rate of cell death must be roughly equal to the rate of cell proliferation to maintain gut homeostasis. Thus, either cell death or cell proliferation was inhibited by oral Omegaven administration. The opposite factor then decreased in compensation. The alternative hypothesis is that both apoptosis and proliferation were inhibited by oral Omegaven. In this experiment, we could not assess which of these possibilities led to our findings. We found a mild decrease in Bax mRNA and increase in Bcl-2 mRNA levels of SBS rats treated with enteral Omegaven that correlated with decreased rates of cell apoptosis.

In the present study, administration of parenteral Omegaven in SBS rats significantly enhanced structural intestinal adaptation. Parenteral Omegaven significantly increased overall bowel and mucosal weights, which occurred with a synergistic increase in bowel circumference. Omegaven significantly increased ileal mucosal DNA and protein as well. Parallel increases in mucosal DNA and protein indicate that the greater ileal mass of animals treated with parenteral Omegaven can be attributed to cellular hyperplasia. Omegaven caused also a mild stimulation of cell proliferation and concomitant decrease in cell apoptosis compared with SBS nontreated animals, suggesting an activated enterocyte turnover, which may be considered a main mechanism of mucosal hyperplasia in recovering bowel. Bax mRNA levels were downregulated, whereas Bcl-2 mRNA was upregulated in Omegaven-treated rats compared with SBS nontreated animals. In accord, the Bax to Bcl-2 ratio decreased in SBS-Iω3 rats compared with SBS animals, suggesting increased enterocyte survival.

The reason Omegaven has opposite effects depending on the route of administration is poorly understood. Omegaven is not intended for enteral administration, and we are aware of no previous investigations that tested enteral administration of Omegaven in both animal and

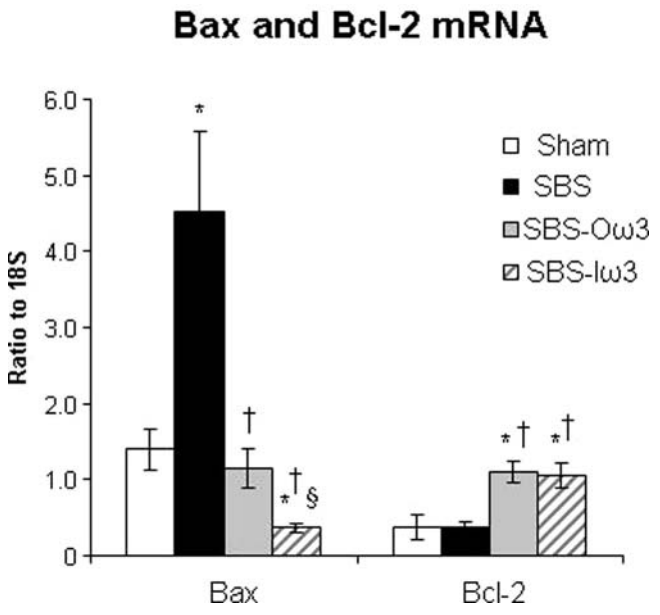

Figure 7. Effect of bowel resection and treatment with Omegaven on expression of Bax and Bcl-2 in ileal mucosal samples. SBS, short bowel syndrome; Oω3, oral ω-3; Iω3, intraperitoneal ω-3. Real-time polymerase chain reaction was used to determine Bax and Bcl-2 mRNA levels. In SBS rats, elevated rates of apoptosis were accompanied by a 3-fold increase in Bax mRNA expression and no change in Bcl-2 mRNA expression compared with control animals. SBS-Oω3 rats showed a significant decrease in Bax expression and a 3-fold increase in Bcl-2 mRNA expression compared with SBS animals. Parenteral administration of ω-3 resulted in a more significant decrease in Bax mRNA expression and, similar to oral ω-3, a 3-fold increase in Bcl-2 mRNA levels compared with SBS animals. Values are mean ± standard error of the mean. $^{*}P < .05$ SBS vs sham rats; $^{\dagger}P < .05$ SBS-Oω3 and SBS-Iω3 vs SBS rats; $^{\S}P < .05$ SBS-Oω3 vs SBS-Iω3 rats.

human models. The local exposure to Omegaven in the short bowel state with decreased absorption and faster transit time may negatively affect the bowel. This is further supported by the fact that intraperitoneal but not enteral Omegaven administration in control (nonoperated) rats resulted in a significant increase in serum triglyceride levels. In the present study, ω-3 FAs exerted the major stimulating effects on intestinal regrowth by bypassing the gut.

We conclude that parenteral Omegaven enhances cell proliferation and inhibits programmed cell death through upregulation of Bcl-2 and downregulation of Bax expression in this rat model. Enteral Omegaven does not change intestinal regrowth. Parenteral Omegaven stimulates enterocyte turnover and may be clinically beneficial as an agent to stimulate intestinal adaptation in patients with SBS.

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