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Effect of bowel rehabilitative therapy on structural adaptation of remnant small intestine: animal experiment

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Abstract

AIM To investigate the individual and the combined effects of glutamine, dietary fiber, and growth hormone on the structural adaptation of the remnant small bowel.

METHODS Forty-two adult male Sprague-Dawley rats underwent 85% mid-small bowel resection and received total parenteral nutrition (TPN) support during the first three postoperational days. From the 4 th postoperational day, animals were randomly assigned to receive 7 different treatments for 8 days: TPNcon group, receiving TPN and enteral 20 g·L^{-1} glycine perfusion; TPN+Gln group, receiving TPN and enteral 20 g·L^{-1} glutamine perfusion; ENcon group, receiving enteral nutrition (EN) fortified with 20 g·L^{-1} glycine; EN+Gln group, enteral nutrition fortified with 20 g·L^{-1} glutamine; EN+Fib group, enteral nutrition and 2 g·L^{-1} oral soybean fiber; EN+GH group, enteral nutrition and subcutaneous growth hormone (GH) (0.3IU) injection twice daily; and ENint group, glutamine-enriched EN, oral soybean fiber, and subcutaneous GH injection.

RESULTS Enteral glutamine perfusion during TPN increased the small intestinal villus height (jejunal villus height 250 μm ± 29 μm in TPNcon *vs* 330 μm ± 54 μm in TPN+Gln, ileal villus height 260 μm ± 28 μm in TPNcon *vs* 330 μm ± 22 μm in TPN+Gln, $P<0.05$) and mucosa thickness (jejunal mucosa thickness 360 μm ± 32 μm in TPNcon *vs* 460 μm ± 65 μm in TPN+Gln, ileal mucosa thickness 400μm ± 25 μm in TPNcon *vs* 490 μm ± 11 μm in TPN+Gln, $P<0.05$) in comparison with the TPNcon group. Either fiber supplementation or GH administration improved body mass gain (end body weight 270 g ± 3.6 g in EN+Fib, 265.7 g ± 3.3 g in EN+GH, *vs* 257 g ± 3.3 g in ENcon, $P<0.05$), elevated plasma insulin-like growth factor (IGF-I) level (880 μg·L^{-1} ± 52 μg·L^{-1} in EN+Fib, 1 200 μg·L^{-1} ± 96 μg·L^{-1} in EN ± GH, *vs* 620 μg·L^{-1} ± 43 μg·L^{-1} in ENcon, $P<0.05$), and increased the villus height (jejunum 560 μm ± 44 μm in EN ± Fib, 530 μm ± 30 μm in EN ± GH, *vs* 450 μm ± 44 μm in ENcon, ileum 400 μm ± 30 μm in EN+Fib, 380 μm ± 49 μm in EN ± GH, *vs* 320 μm ± 16 μm in ENcon, $P<0.05$) and the mucosa thickness (jejunum 740 μm ± 66 μm in EN ± Fib, 705 μm ± 27 μm in ENGH, *vs* 608 μm ± 58 μm in ENcon, ileum 570 μm ± 27 μm in EN ± Fib, 560 μm ± 56 μm in EN ± GH, *vs* 480 μm ± 40 μm in ENcon, $P<0.05$) in remnant jejunum and ileum. Glutamine-enriched EN produced little effect in body mass, plasma IGF-I level, and remnant small bowel mucosal structure. The ENint group had greater body mass (280 g ± 2. 2 g), plasma IGF-I level (1450 μg·L^{-1} ± 137 μg·L^{-1}), and villus height (jejunum 620 μm ± 56 μm, ileum 450 μm ± 31 μm) and mucosal thickness (jejunum 800 μm ± 52 μm, ileum 633 μm ± 33 μm) than those in ENcon,EN+Gln (jejunum villus height and mucosa thickness 450 μm ± 47 μm and 610 μm ± 63 μm, ileum villus height and mucosa thickness 330 μm ± 39 μm and 500 μm ± 52 μm), EN+GH groups ($P<0.05$), and than those in EN+Fib group although no statistical significance was attained.

CONCLUSION Both dietary fiber and GH when used separately can enhance the postresectional small bowel structural adaptation. Simultaneous use of these two gut-trophic factors can produce synergistic effects on small bowel structural adaptation. Enteral glutamine perfusion is beneficial in preserving small bowel mucosal structure during TPN, but has little beneficial effect during EN.

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INTRODUCTION

Various clinical conditions necessitate therapeutic massive small bowel resection, which is often followed by serious malabsorption, characterized by intractable diarrhea, steatorrhea, and weight loss. This malabsorptive state is defined as short bowel syndrome. Traditionally, parental nutrition (PN) is prescribed for these patients to assure adequate nutritional status and obtain enough time to wait for the remnant small bowel to undergo adaptation, with the hope of eventual transition from parental nutrition support to volitional enteral nutrition. Under this supportive therapeutic strategy, however, some patients may never achieve complete adaptation, i.e., sustaining completely on enteral nutrition, and life-long total parental nutrition (TPN) become lifesaving unless successful small bowel transplantation is conducted[1-3]. Unfortunately, long-term TPN is associated with great expense, vitamin or trace element malnutrition, recurrent catheter sepsis, and progressive cholestatic liver disease[4], whereas small bowel transplantation is still used currently as a salvage therapy for patients with severe metabolic complications, hepatic failure, or lack of venous access due to imperfect management of post-transplantational rejection[5]. Therefore, any therapy aimed at actively accelerating or enhancing the adaptive process in the residual bowel is likely to impact greatly on the patients' life style and the costs of medical care. Since 1995 Byrne *et al* have published a series of studies, which demonstrated that a combination of growth hormone, glutamine, and modified fiber-enriched diet can improve nutrient absorption, decrease stool output, and reducte TPN requirement[6-8]. These reports are the first to introduce the concept that further bowel adaptation can occur with the use of specialized nutrients and growth factors, and ushered in a new era in the treatment of short bowel syndrome. However, many questions about this therapy are to be answered, e.g., whether this therapy has enhanced the structural adaptation in the remnant small bowel and how each component of the remedy contributes to bowel adaptation.

Due to the heterogeneity in patient age, underlying diseases, and anatomy of the remnant bowel as well as ethic concerns, it is rather difficult to answer these questions directly from the patients. Consequently, we conducted this animal experiment in an attempt to assess the individual and combined effect of glutamine, dietary fiber and growth factor on the structural small bowel adaptation immediately after massive small bowel resection.

MATERIALS AND METHODS

Animals and surgical procedures

The animal protocols and procedures were approved by the Laboratory Animal Medicine Ethics Committee of Nanjing Military Area General Hospital of Chinese PLA. Male Sprague Dawley rats weighing between 220 g and 250 g (Shanghai Laboratory Animals Center, Chinese Academy of Sciences) were allowed 1 week to get acclimatized to our laboratory conditions before surgery. They were kept in individual stainless steel cages and fed a standard rat chow with free access to tap water in a room maintained at 22℃ on a 12 h day/night cycle (06:00/18:00).

The animals weighed about 260 g to 290 g at the end of acclimation period, and were fasted for 12 hours before surgery. Surgical procedures were performed using aseptic technique under anesthesia by intramuscular ketamine cocktail (ketamine, 100 $mg \cdot kg^{-1}$; and xylazine 8 $mg \cdot kg^{-1}$). Three surgeries were performed on each animal in the following sequence: placement of TPN catheter in the superior vena cava via the external jugular vein, installation of gastrotomy tube for liquid diet delivery, and 85% mid-small bowel resection. Both tubes were tunneled subcutaneously, and the dorsal cervical region exited through a spring-swivel apparatus. Normal saline was administered at 1 $mL \cdot h^{-1}$ through the TPN catheter with a minipump. Small bowel resection left the first 6 cm jejunum from the Treitz ligament and the terminal 6 cm ileum. The day when operations were performed was dated as day 0. The postoperative days were dated as day *n*.

Experimental design

After the operation, animals were placed back to stainless steel cages and supported with total parenteral nutrition (TPN), the composition of which is indicated in Table 1. TPN was administered at a half-rate of 1.25 $mL \cdot h^{-1}$ on day 1, and full-rate of 2.5 $mL \cdot h^{-1}$ on day 2 and 3. From day 4, animals were randomly allocated to seven experimental groups: ENcon group, receiving control liquid enteral nutrition (EN); EN+Gln group, receiving EN enriched with 20 $g \cdot L^{-1}$ glutamine; EN+Fib group, receiving control EN and fed with 2 g/d soybean fiber, containing 70% total dietary fiber (provided by Nanjing Military Area General Hospital of Chinese PLA, Nanjing, China), and was mixed with water to the consistency of porridge; EN+GH group, injected with control EN and 0.3IU recombinant human growth hormone (rhGH) (Saizen, provided by Laboratories Serona S.A., 1170 Aubonne, Switzerland) subcutaneously twice a day; ENint group, receiving subcutaneous injection of EN enriched with 20 $g \cdot L^{-1}$glutamine, 2 $g \cdot d^{-1}$ soy bean fiber, and 0.3IU rhGH twice a day; TPNcon group, receiving TPN, and 20 $g \cdot L^{-1}$glycine perfusion *via* gastrotomy tube; and TPN+Gln group, receiving TPN, and 20 $g \cdot L^{-1}$ glutamine perfusion *via* gastrotomy tube. Each experimental group contained 6 animals. The liquid EN was fiber-free with the compositions as indicated in Table 2, and was reconstituted with sterile water before

perfusion and used within 12 hours to avoid bacteria growth. Gastrotomy tube feeding of EN or amino acid solution was introduced gradually by the following schedule: half-strength solution at 1.25 mL·h^{-1} on day 4, full-strength solution at 1.25 mL·h^{-1} on day 5, and full-strength solution at 2.5 mL·h^{-1} from day 6-until day 12. PN was continued until full-strength EN was administered at full-rate, thus combined EN and PN to supply 251kJ calorie to the animals. Our formulas for TPN and EN supplied isocaloric and isonitrogenous nutrition to all animals, i.e., 251Kj nonprotein calories, 0. 414 g nitrogen from PN or Pepti-2000 variant, and 0. 272 g nitrogen from glutamine enrichment or glycine for control per day for each animal. Glutamine enrichment constituted 39.5% of the total nitrogen. Water was provided *ad lib* throughout the study.

Table 1 Total parenteral nutrition composition (400 mL)

Ingredients	mL
500 g·L^{-1} glucose	100.0
Fat (300 g·L^{-1} intralipid *)	67.5
Amino acid (114g·L^{-1} Novamin *)	150.0
100 g·L^{-1} NaCl	10.0
100 g·L^{-1} KCl	10.0
100 g·L^{-1} calcium gluconate	5.0
Multi-electrolytes (Addemel *)	2.5
Water-soluble vitamins (Soluvit *)	2.5
Lipid-soluble vitamins (Vitalipid *)	2.5
Nonprotein energy (kJ·mL^{-1})	4.2
Total nitrogen (g)	2.7
NPC / N (kJ·g^{-1})	620

*Provided by Sino-Swed Pharmaceutical Corp. Ltd., Wuxi, China. NPC / C: nonprotein energy per gram of nitrogen.

Table 2 Enteral nutrition composition (420 mL)

Ingredients	Control EN	Glutamine enriched EN
Pepti-2000 variant * (g)	126.0	126.0
Protein hydrolysate (g)	19.9	19.9
Nitrogen (g)	2.9	2.9
Fat (g)	4.9	4.9
Vegetable (g)	2.45	2.45
MCT (g)	2.45	2.45
Linoleic acid	1.12	1.12
Carbohydrates (g)	93.1	93.1
Malto^{a2}dextrin (g)	91.7	91.7
Lactose (g)	<1.12	<1.12
Organic acid (g)	0.01	0.01
Minerals (g)	2.6	2.6
Vitamins (g)	0.4	0.4
Glycine (g)	10.0	0
Glutamine (g)	0	10.0
Nonprotein energy (kJ·mL^{-1})	4.2	4.2
NPC / N	610	610

*A commercial, nutritional complete, short-chain peptide based elemental diet (Nutricia, the Netherland). NPC / N: nonprotein energy per gram of nitrogen from Pepti-2000 variant.

Plasma and tissue isolation

Body mass was monitored every three days after surgery as an index of nutritional status. At about 12:00 am on the 12 th postoperative day, laparotomy was performed on all animals under anesthesia with ketamine hydrochloride (100 mg·kg^{-1}). Blood was obtained from the inferior vena cava and placed on ice in a heparin-pretreated tube. Plasma was then isolated by centrifugation at 4℃ and stored at -20℃ for later analysis.

The residual small intestine was rapidly resected from peritoneal and vascular connections, and the luminal content was removed. After the intestine was flushed with ice-cold normal saline, 2 cm segments from both jejunum and ileum located between 2 and 4 cm from the anastomosis were removed and fixed in phosphate-buffered neutral formalin. The animals were then killed by exsanguination.

Plasma insulin-like growth factor (IGF-I) assay and histological image analysis

Total plasma IGF-I concentrations were measured after acid-ethanol extraction with the rat IGF-I RIA DSL-2900 kit (Diagnostic Systems Laboratories, Inc., Webster, USA) in duplication, following the instruction of the manufacturer. This measurement was taken both as indicator of nutritional status and evidence of rhGH treatment.

Villus height (from villus base to villus tip), crypt depth (from crypt base to villus base), and mucosal thickness (from crypt base to villus tip) were taken as indicators of structural adaptation. Routine hematoxylin and eosin stained sections were prepared, and HPIAS-1000 True Color Image Analysis System (Tongji Qianping Image Engineering Corp., Wuhan, China) was employed to analyze the image. Fifteen intact axially oriented villi and crypts selected from each specimen were measured.

Statistical analysis

Data were expressed as $\bar{x} \pm s$. Multiple comparison tests were performed after analysis of variance with the Student-Newman-Keuls test. Differences with P value less than 0.05 were considered to be statistically significant.

RESULTS

Body mass

As shown in Table 3 and Figure 1, the initial body mass on day 0 was similar among the 7 experimental groups. The body mass on day 3 was not significantly different among the 7 groups, which reflected similar operative stress and postoperative nutritional support remedy. The overall body mass loss during the first three postoperative days averaged 26.3 g. Marked body mass difference was evident from day 6. The two TPN-supported groups had gained remarkable body mass by day 6 which was kept steady during the remainder of the experimental period, suggesting the ebbing of stress and fixed nutrition supply. No significant body mass difference was observed between the two TPN groups throughout the experiment. In ENcon, EN+Gln, and EN+GH groups, the introduction of EN resulted in further body mass loss, which was accompanied by a moderate to a large amount of liquid stool production, indicating insufficient

nutrition assimilation from the remnant small bowel. From day 6, diarrhea in these groups became increasingly milder and almost disappeared after day 9, which was associated with gradual body mass gain. Each animal in two fiber-supplied groups ate up daily fiber supply, and fiber-containing solid stool instead of large liquid stool was observed after EN introduction. Continuous body mass gain began from day 3 in ENint group, and after day 6 in EN+Fib group. From day 3, the mean body mass of ENint group maintained higher than those of the other groups, which bore statistical significance on day 6 when compared with ENcon, EN+Fib, and EN+GH groups, and on day 9 and day 12 when compared with TPNcon, ENcon, EN+Fib, and EN+GH groups. Throughout the study, ENcon and EN+Gln groups had similar body mass, which remained significantly lower than that of TPNcon on day 6 and day 9 and rose to the similar level as TPNcon on day 12. From day 6, mean body mass of EN+Fib groups began to distinguish itself significantly from ENcon, and became markedly higher than both ENcon and TPNcon on day 12. On day 9, the mean body mass of EN+GH groups was significantly higher than that in ENcon group, and on day 12 significantly higher than both ENcon and TPNcon groups.

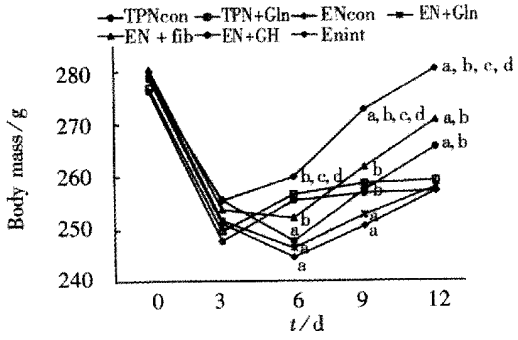

Figure 1 Body mass before and after surgery.
[a]*P*<0.05, *vs* TPNcon; [b]*P*<0.05, *vs* ENcon; [c]*P*<0.05, *vs* EN+GH; [d]*P*<0.05, *vs* EN+Fib. Day 0, refers to the day operation was performed, from which the time was dated.

Plasma IGF -I concentrations

Plasma IGF-I concentrations were similar among TPNcon, TPN+Gln, ENcon, and EN+Gln groups. Plasma IGF-I level in EN+fib group was significantly higher than that in ENcon group. GH treatment resulted in significant increase in plasma IGF-I concentration as compared with ENcon group. ENint group had the highest level of plasma IGF-I (Figure 2).

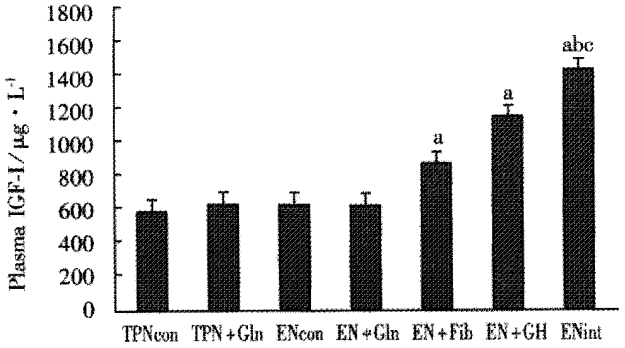

Figure 2 Plasma insulin-like growth factor level on day 12.
[a]*P*<0.05, *vs* Encon; [b]*P*<0.05, *vs* EN+Fib; [c]*P*<0.05, *vs* EN+GH.

Remnant intestinal mucosal structure

Compared with ENcon group, TPNcon group had significantly lower mean villus height and mucosal thickness in both remnant jejunum and ileum, and significantly shallower jejunal crypt (Figures 3 and 4). Luminal glutamine perfusion completely restored the villus height and mucosal thickness in ileum and partially restored the villus height, crypt depth, and mucosal thickness in jejunum. However, glutamine-enriched EN had a little impact on mucosal structural parameters whether in remnant jejunum or ileum when compared with ENcon group. The villus height and mucosal thickness in both remnant jejunum and ileum were more significantly increased by fiber supplementation than in ENcon group. GH treatment produced significant increase in villus height in both remnant jejunum and ileum, and mucosal thickness in remnant ileum when compared with ENcon group. Combined treatment with glutamine, fiber, and GH significantly deepened the crypt in remnant ileum as against TPNcon group, and further increased villus height and mucosal thickness in both remnant jejunum and ileum, which, when compared with EN+GH group, achieved statistical significance in villus height of both jejunum and ileum, and in mucosal thickness of ileum.

Table 3 Comparison of body mass before and after operation between each experiment groups (n = 6, $\bar{x} \pm s$, g)

Groups	Day 0	Day 3	Day 6	Day 9	Day 12
TPNcon	276.3 ± 9.0	247.8 ± 7.4	255.5 ± 3.6	256.8 ± 3.4	257.3 ± 2.9
TPN+Gln	277.0 ± 6.1	249.7 ± 6.5	256.7 ± 4.0	258.7 ± 3.7	259.3 ± 2.9
Encon	278.8 ± 7.8	251.2 ± 8.0	244.7 ± 3.2[a]	250.7 ± 3.5[a]	257.3 ± 3.3
EN+Gln	279.8 ± 8.9	251.8 ± 7.1	246.5 ± 3.4[a]	252.8 ± 2.7	258.0 ± 2.5
EN+fib	280.5 ± 8.0	253.8 ± 6.6	252.2 ± 2.0[b]	261.8 ± 3.4[b]	270.7 ± 3.6[a,b]
EN+GH	278.7 ± 12.9	255.7 ± 6.2	247.8 ± 2.3[a]	257.5 ± 3.3[b]	265.7 ± 3.3[a,b]
ENint	278.8 ± 8.1	255.5 ± 6.2	260.5 ± 2.4[b,c,d]	272.7 ± 2.3[a,b,c,d]	280.5 ± 2.2[a,b,c,d]

[a]*P*<0.05, *vs* TPNcon; [b]*P*<0.05, *vs* ENcon; [c]*P*<0.05, *vs* EN+Fib; [d]*P*<0.05, *vs* EN+GH.
Day 0, refers to the day operation was performed, from which the time was dated.

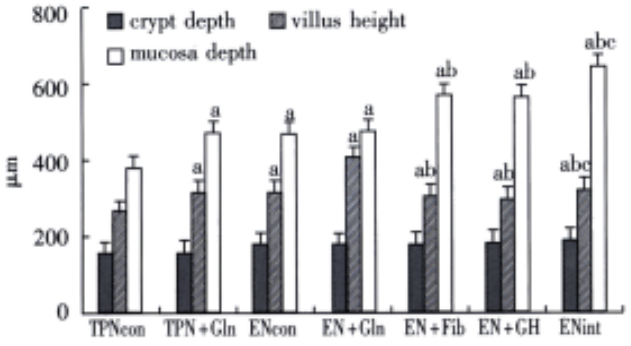

Figure 3 Crypt depth, villus height and mucosal thickness of the remnant ileum.
[a]*P*<0.05, *vs* TPNcon; [b]*P*<0.05, *vs* ENcon; [c]*P*<0.05, *vs* EN+GH.

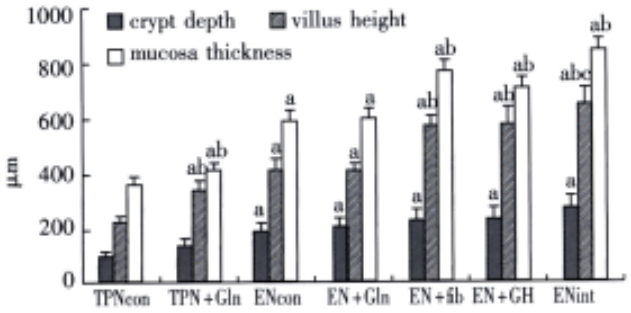

Figure 4 Crypt depth, villus height and mucosal thickness of the remnant jejunum.
[a]*P*<0.05, *vs* TPNcon; [b]*P*<0.05, *vs* ENcon; [c]*P*<0.05, *vs* EN+GH.

DISCUSSION

After massive small bowel resection, the structural adaptation of the remnant small bowel was characterized by mucosal hyperplasia. The villus height and crypt depth are both increased, while cell hypertrophy is considered unimportant[9]. This process is influenced by many factors, among which, both the quantity and route of nutrient intake are important regulators. Luminal nutrient supply, which is essential for structural adaptation, serves as not only energy source, but also signal for endogenous secretions and the release of various gut-trophic hormones and growth factors. On the other hand, normal nutritional status, which usually needs the facilitation of TPN to maintain, favors bowel adaptation[1]. In this study, great efforts were made to ensure strict control on the quantity and route of nutritent supply, and TPN was used immediately after bowel resection and during the period of reintroduction of enteral nutrition to improve the nutritional status. The compositions and delivery schedules for TPN and EN were set to provide isocaloric and isonitrogenus nutrition and to produce comparable overall nutritional status in animals of each experimental group. And the goal of producing comparable nutritional status was attained, which was attested by similar body mass and plasma total IGF-I concentration among TPNcon, TPN+Gln, ENcon, and EN+Gln groups. The utilization of plasma IGF-I as index of nutritional status is justified by sensitive IGF-I response to dietary intake, close correlation with body composition, short half-life, and its nycthermeral stability[10]. Thus the risk of confounding effects of variation in nutritional supplementation had been minimized.

Substantial researches have been done to modify the formulation of enteral nutrition to optimize the postresectional small bowel adaptation. Pepti-2000 variant has often been recommended as fiber-free EN to patients with short bowel syndrome in Nanjing Military Area General Hospital of Chinese PLA and has been chosen in this experiment as the EN formulation. This is justified by its compositional feature as peptide-based, polymeric nutrient-complete diet, which provide equal energy from medium-chain triglycerides and long-chain triglycerides. Polymeric diets are more trophic for intestinal adaptive hyperplasia compared with monomeric diets. Partial hydrolysation or protein facilitates amino acid absorption from peptide transporters while preserves gut trophic effect. Medium-chain triglycerides are water-soluble and are better absorbed in the presence of bile acid or pancreatic insufficiency, while long-chain triglycerides are more effective in inducing adaptation[11].

Glutamine is an essential nutrient for intestinal mucosa. Besides serving as the structural unit of protein synthesis, a precursor for synthesis of nucleotides and other micromolecules, it is the major respiratory fuel for intestinal mucosa[12]. *in vitro* studies have indicated that intracellular mitogenic signal transduction can be modified by glutamine supply and metabolism[13]. Animal studies have consistently shown that total parenteral nutrition (TPN)-induced intestinal hypoplasia can be attenuated by parenteral glutamine supplementation[14-16]. Glutamine-fortified-parent-eral or enteral nutrition has also been demonstrated to accelerate small intestinal healing and improves survival outcome after chemotherapy and radiation[17,18].

Following massive bowel resection, malabsorption occurs and patients may be intolerable to enteral nutrition and have to sustain on TPN for a certain period. Efforts made to preserve intestinal mucosal mass and absorption area during TPN will assist early reintroduction of enteral nutrition, which is essential for the initiation of intestinal adaptation. Parenteral glutamine supplementation has been shown to prevent TPN induced mucosal atrophy in the remnant small intestine after 85% resection[19]. Since enteral glutamine can be readily absorbed by small intestinal epithelium, and glutamine oxidation

stimulates enterocyte Na^+/H^+ exchange, leading to a high rate of electroneutral NaCl absorption in healthy and diseased jejunum[20], we studied the effect of enteral glutamine perfusion on the remnant intestinal mucosal structure during TPN. Because previous studies have not confirmed the beneficial effect of enteral glutmine supplementation during EN[21-25], we also studied the effect of adding extra glutamine to nutrition-complete enteral diet. Our results confirmed the existence of TPN induced intestinal hypoplasia, which was indicated by significantly lower villi in both jejunum and ileum and markedly shallower crypts in jejunum in TPNcon group compared with ENcon group. Luminal glutamine perfusion was effective to reverse TPN-induced hypoplasia in ileum and partially reverse TPN-induced hypoplasia in jejunum. In contrast, glutamine-supplemented enteral nutrition has little impact on small intestinal structural parameters. This finding is surprising, yet is similar to the results of several other studies[19-23]. The dosage ranged approximately from 2 $g \cdot kg^{-1} \cdot day^{-1}$ to 5.6 $g \cdot kg^{-1} \cdot day^{-1}$. And a dosage of more than 4 $g \cdot kg^{-1} \cdot day^{-1}$ was found effective in preventing TPN-induced intestinal hypoplasia in rats[12]. In all these studies, glutamine-fortified enteral nutrition was compared with standard rat chow or non-glutamine-containing elementary diet, or EN with insufficient glutamine due to partial hydrolysation of protein. Therefore, the lack of effect cannot be interpreted as insufficient glutamine delivered to intestine, or overload of glutamine. Different routes of administration were tried. Extra glutamine was mixed with enteral diet to form *ad lib* diet, or was administrated separately in bolus form, or administered as 24 h continuous enteral perfusion. The remnant intestines were analyzed 2, 7, 14 or 21 days after small bowel resection. The time points represent the beginning, active, and maximal remnant intestinal hyperplasia. In most of those studies, the animals gained body mass. In our study, the animals maintained the body mass lower than that of preoperation, reflecting our restrict nutrition supply. Nevertheless, despite different experimental protocols, similar results were obtained that during enteral nutrition, enteral glutamine supplementation produced little effect on remnant small intestinal morphological parameters. Therefore, the current available data raise the hypothesis that for otherwise healthy small intestine, with adequate enteral nutrition stimulation, glutamine delivered by arterial blood is sufficient for optimal intestinal growth. In consistent with this hypothesis, *in vitro* studies have shown that in a number of cell lines, maximal proliferation occurs when glutamine concentrations are maintained at 0.5 $mmol \cdot L^{-1}$ or above, a concentration approximates normal plasma concentration[26]. With the maximal mitogenic stimulation of epidermal growth factor, the optimal proliferation of IEC-6, a rat jejunum cell line, occurred at a glutamine concentration of 1.0 $mmol \cdot L^{-1}$ in the cultural medium, a concentration within the physiologic ranges of glutamine found in rat plasma[27]. Under normal nutritional status, plasma glutamine homeostasis can be maintained without nutritional glutamine supply[28]. However, in the absence of luminal nutrition stimulation during TPN, glutamine delivered by blood flow may be insufficient for optimal intestinal growth, as is attested by the fact that parenteral or enteral glutamine supplementation is effective in reducing TPN-induced intestinal hypoplasia. This insufficiency may be caused by decreased blood flow induced by TPN, or enteral nutrition can stimulate the uptake of glutamine through the basolateral membrane by modifying the activity of the transporters. However, our data do not exclude the possibility that when inadequate enteral nutrition is received, enteral glutamine may exert a trophic effect on small intestine, since minimum luminal nutrition has been found indifferent in stimulating small intestinal mucosal growth as compared with TPN[29].

Dietary fiber includes a wide variety of carbohydrates that, as a group, are resistant to enzymatic hydrolysis within the human gastrointestinal tract. The principal physiologic function of dietary fiber are regulating gastric emptying and intestinal transit time, based on the bulking action of the fiber. Insoluble fibers are minimally fermented and function almost solely as bulking agents that decrease colonic transit time and increase fecal mass. Soluble fibers are largely fermented by anaerobic gut flora, resulting in increase of the bacterial quantity, and fecal mass, and production of short-chain fatty acids (SCFAs), which are quickly absorbed by the colonic mucosa. The importance of dietary fiber in maintenance of normal colonic morphology and function has been widely acknowledged. However, its role in short-bowel syndrome has not been investigated thoroughly. Previous studies on the effect of dietary fiber on colon were mostly carried out in normal animals with intact small and large intestine. In contrast, after massive small bowel resection, the colon in continuity with the remnant small bowel will receive substantial unabsorbed carbohy drates and protein, which will undergo fermentation in colon by various anaerobic bacteria and produce SCFAs. This represents an important mechanism whereby the colon compensates some energy absorption function of the lost small intestine[30,31]. How colon with this altered environment responses to additional dietary fiber loading is not clear. Although there is the speculation that in carbohydrates malabsorption, dietary fiber supplementation seemed to be redundant in

enhancing the SCFAs production[11], available data from massive small bowel resection models showed the beneficial effect of dietary fiber supplementation on residual small bowel as well as colon adaptation[32-34]. In contrast, dietary fiber had little effect on intact intestinal function[35]. Therefore, it seems that the effect of dietary fiber is modified by the absorptive function of intestine.

We fed the animals with fiber-free enteral nutrition of 2 g soy bean fiber daily, containing about 70% total dietary fiber consisting predominantly of insoluble fiber, and found that soy bean fiber supply led to large solid fiber-containing stool, instead of large liquid stool as seen in non-fiber supplied EN groups. This indicates that fiber supply may alter the colon absorptive and moving function. Significantly improved body mass recovery was associated with this improved stool consistency. In addition, the plasma IGF-I concentration was simultaneously elevated, which implied that the improved body mass was not solely caused by colonic fiber retention. Both the residual jejunum and ileum displayed significantly greater mean villus height and mucosal thickness. The exact mechanism that soybean fiber enhanced the postresectional adaptation was not investigated in this study. Since the soy bean fiber consisted predominantly of insoluble fiber which mainly influences the intestinal transit, it is most likely that the presence of fiber in combination with unabsorbed carbohydrates and proteins in the colon had delayed the intestinal transit through direct bulk effect, hence promoting the colonic fermentation of unabsorbed carbohydrate and protein. Consequently, the secretion of colon-derived enteric hormones, such as enteroglucagon and peptide YY, might be augmented, which in turn exerted their small bowel trophic effect and stimulated further structural adaptation in small bowel.

Besides gut-special nutrients such as glutamine and dietary fiber, the possible role of many growth factors has met with intense research interest. Strong evidence has demonstrated that growth hormone (GH) is an important growth factor for intestine. Complete GH depletion due to hypophysectomy caused pronounced hypoplasia of small intestinal mucosa with decreased villus height and reduced crypt cell proliferation[36]. Simple replacement of GH can restore mucosal proliferative activity[37]. The study of the transgenic mice overexpressing the bovine growth hormone demonstrated that chronic excessive GH can produce hyperplasia in small intestinal mucosa whether food intake was *ad lib* or restricted[38]. Hypophysectomy could impair the adaptive hyperplasia in response to small bowel resection[39]. In an unpaired-fed study, exogenous GH was found to enhance mucosal hyperplasia after extensive small bowel resection[40]. Our pair-fed study revealed that exogenous GH administration significantly elevated the plasma IGF-I level, increased villus height in both remnant jejunum and ileum, and significantly increased ileum mucosal thickness. Postresectional body mass recovery was also accelerated by GH administration, suggesting the enhanced nutrient absorption. Our data further confirmed the gut-trophic property of GH. The more important findings of our study are that concomitant fiber supplementation further enlarged the villus height and mucosal thickness in both remnant ileum and jejunum, enhanced body mass gain, and increased the plasma IGF-I concentration in ENint group. These results suggest that gut-trophic growth factor, GH, can be used in combination with gut-special nutrient, dietary fiber, to bring about synergistic effect on postresectional remnant small bowel structural adaptation.

In summary, our study demonstrated that both dietary fiber and growth hormone can enhance the postresectional small bowel structural adaptation, promote bodymass-gain, and increase plasma IGF-I level when used separately. Simultaneous use of these two gut-trophic factors produced synergistic effect on structural adaptation parameters, body mass, and plasma IGF-I concentration. In contrast, the gut essential nutrient, glutamine when enterally administered with enteral nutrition, showed little beneficial effect on remnant small bowel structural adaptation, and the body mass did not alter in comparison with the control enteral nutrition. However, enteral glutamine is effective in preserving intestinal mucosal structure during TPN. These findings raise the doubt about the necessity of enteral glutamine supplementation during enteral nutrition. On the other hand, they provide the evidence favoring the combined utilization of GH and dietary fiber with enteral nutrition in patients with short bowel syndrome.

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