Effect of Enteral Nutrition Formula on Fat Absorption and Serum Free Fatty Acid Profiles in Rat with Short-Bowel Syndrome

ZHOU, Jian-Nong* 鞠建农（周建农） TAN, Li† 谭力 WANG, Xue-Hao‡ 王学浩 JU, Huang-Xian* 鞠熀先

The First Clinical Medical College, Nanjing Medical University, Nanjing, Jiangsu 210029, China
Key Laboratory of Analytical Chemistry for Life Science (Ministry of Education of China), Department of Chemistry, Nanjing University, Nanjing, Jiangsu 210093, China

Introduction
Short bowel syndrome (SBS) occurs upon massive resection of the small intestine and usually results in the malabsorption of nutrients and fluids.1 A combination of significant reduction in absorptive area with severely compromized enterohemal circulation increases the possibility of steatorrhea and fat malabsorption. Intestinal adaptation after massive small bowel resection is an important response of the remnant bowel to compensate for the acute loss of intestinal mucosal surface area. Patients with SBS often require parenteral and enteral nutrition for survival. Lipid emulsions are commonly used to supplement such enteral nutrition. Their major role as energy donor, as well as carriers of essential fatty acids are the precursors of prostaglandins and other eicosanoids and therefore have important metabolic functions.2 Despite minimal contents in vivo, fatty acids are the main sources of energy substrates for some vital organs such as muscle, heart, liver and renal cortex. Any alteration in their serum levels can affect the supply of energy substrates for these vital organs. Therefore, an evaluation of serum free fatty acid (FFA) profiles in some morbid status will helpful to assess the metabolic changes in the whole body. However, few studies have involved in the effect of MCT supplemented enteral nutrition on serum FFA profiles.

On the other hand, L-arginine (Arg) is a dibasic nonessential amino acid processed metabolically by the urea cycle. It plays an important role in many physiologic and biologic processes beyond its role as a protein-incorporated amino acid. Dietary supplementation with L-arginine has been shown to enhance wound healing, regulate endocrine activity, and potentiate immune activity.3 Thus L-arginine may be considered to have a beneficial effect in preventing septic complications to occur frequently on patients with SBS. However, very few studies have examined the role of L-arginine following massive small bowel resection.

This study examined the changes of fat absorption, absorbed from the small bowel under conditions in which the absorption of LCT is impaired. Some fatty acids are the precursors of prostaglandins and other eicosanoids and therefore have important metabolic functions.2 Despite minimal contents in vivo, fatty acids are the main sources of energy substrates for some vital organs such as muscle, heart, liver and renal cortex. Any alteration in their serum levels can affect the supply of energy substrates for these vital organs. Therefore, an evaluation of serum free fatty acid (FFA) profiles in some morbid status will helpful to assess the metabolic changes in the whole body. However, few studies have involved in the effect of MCT supplemented enteral nutrition on serum FFA profiles.

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Keywords serum fatty acid profile, fat absorptivity, gas chromatography, short bowel syndrome, enteral nutrition formula, rat
intestinal morphological parameters and serum fatty acid profiles in SBS rats treated with enteral nutrition containing LCT, MCT/LCT or Arg/LCT by using gas chromatography equipped with a flame-ionization detector (GC-FID). L-Arginine enhanced enteral nutrition showed the elevation of fat absorptivity. This work uses a traditional analytical method to obtain data for guiding enteral nutrition supplementation of patients with SBS, which changes the action of analytical techniques from obtaining data to dissolving some medical problems. It is significant and provides a way for studying the physiological function of supplementary nutrition and elucidating physiological and pharmacological process of drugs.

Methods and materials

Materials and reagents

Enteral nutrition formulas (Table 1) were obtained from Nutricia Pharmaceutical (Co. Ltd. Wuxi, China). Fatty acid standards were purchased from Sigma (St. Louis, MO, USA). n-Hexane and isopropanol (HPLC grade) were from Merck (Darmstadt, Germany).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>LCT</th>
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<th>Arg/LCT</th>
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<tr>
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</table>

Animals and surgical models

Male Sprague-Dawley rats weighing 220—250 g were individually housed in stainless-steel cages and provided with an unrefined diet and water ad libitum. Following overnight fasting, 28 rats were randomly assigned to 4 groups with 7 rats each: (1) sham operation fed with LCT (Con); (2) 85% small bowel resection fed with LCT (LCT); (3) 85% small bowel resection fed with MCT and LCT (MCT/LCT); and (4) 85% short bowel resection rats fed with LCT and L-arginine (Arg/LCT).

Surgical procedures were performed using aseptic technique under anesthesia by intraperitoneal ketamine (100 mg/kg). The abdomen was opened through a midline incision, and the total small bowel length was measured with a cotton thread along the anti-mesenteric margin. For SBS rats, 85% mid-gut resection was performed by removing the small bowel from a point 5 cm distal to ligament of Treitz to a point 10 cm proximal to the ileocecal valve. The mesenteric blood supply was tied off with 3-0 silk. An end-to-end single layer anastomosis was performed using 6-0 Vicryl interrupted sutures with a total of 8 to 10 stitches. For control, the small bowel was transected and re-anastomosed at a site 5 cm distal to the ligament of Treitz. A gastrostomy tube was installed for liquid diet delivery in all groups. The tube was tunneled subcutaneously and exited the dorsal cervical region through a spring-swivel apparatus.

Rats of each group were treated with both the same nitrogen (1.392 g/kg) and the same calorie (920 kJ•kg⁻¹•d⁻¹) through the gastrotomy using a continuous infusion pump. Enteral nutrition was initiated on postoperative day 2 and continued till postoperative day 14. Enteral nutrition was 1/4 on postoperative day 2, 1/2 on postoperative 3, and full amount from postoperative day 4 on. The rats were given the enteral nutrition containing 9% LCT in the Con and LCT groups, or 4.5% MCT and 4.5% LCT in the MCT/LCT group. For Arg/LCT group, rats were fed with an enteral nutrition containing 9% LCT supplemented with 200 mg/kg L-arginine, and protein content was cut down correspondently to ensure iso-nitrogen compared with other groups.

Sampling and measurements

The rats were killed by cervical dislocation and thorax cavities were opened at 8:00 AM on postoperative day 15. Blood sample was drawn from the right ventricle, and serum was separated for free fatty acid profile. Free fatty acid profiles were quantified by gas chromatography using an HP4890 GC equipped with a flame-ionization detector (GC-FID) and an HP3398A GC workstation (Hewlett Packard, Bracknell, UK).

The extraction of free fatty acid serum samples was performed with a modified method reported previously. Briefly, a 0.5 mL sample was added with 10 µg of internal standard (heptadecanoic acid, C 17:0) and 30 µL of 2 mol/L HCl solution. FFAs were then extracted with 3 mL of n-hexane-isopropanol mixture (3 : 1, V/V), and the organic layer was evaporated to dryness under a stream of nitrogen at 30 °C. The residue was derivatized with 0.5 mL of BF₃-Et₂O with methanol (1 : 3, V/V) at 70 °C for 10 min. After cooling down to room temperature, 0.5 mL of hexane and 0.5 mL of purified water were added to the vial that was vortex-mixed and centrifuged later. The hexane layer was transferred into another vial and was evaporated to about 50 µL under nitrogen. A 2 µL residue solution was injected into GC-FID system. An FFAP fused-silica capillary column with 0.25 µm film thickness (30 m×0.25 mm I. D., Agilent Co., USA) was used. The GC oven temperature was programmed from 100 to 180 °C at a rate of 8 °C/min and held at 180 °C for 10 min. Then it was programmed to 230 °C at a rate of 10 °C/min and held for 8 min, and increased to 250 °C at 10 °C/min and held.
for 36 min. The temperatures of detector and injector were 300 °C and 260 °C, respectively. High purity nitrogen was used as carrier gas (flow-rate 10 psi).

**Bowel weight, mucosal weight and morphological measurements**

The small bowel was removed rapidly, rinsed with cold isotonic saline, and divided into 2 segments: jejunum proximal to anastomosis and terminal ileum. Each segment was weighed, mucosa was scraped using a glass slide, and they were collected and weighed. Histological sections were prepared from bowel just 4 cm distal to the ligament of Treitz (jejunum) and 4 cm proximal to the ileocecal junction (distal ileum) in resected animals and from comparable sites in control rats. Segments of small bowel were fixed in 10% buffered formalin, embedded in paraffin, and cut longitudinally into 5-µm sections. The slides were stained with hematoxylin and eosin. Mucosal thickness, villus height, and crypt depths were quantified and villus surface areas were calculated.

**Fat absorptivity measurements**

All excrements of rats were collected respectively after postoperative day from 12th to 14th day. And then determine the content of fat in excrement (amount of fat eliminated) using Soxhlet extraction method. The amount of fat enteral nutrition supplied can be calculated through amount of enteral nutrition supplied from 12th to 14th day after operation.

\[
\text{Fat absorptivity} = \left(1 - \frac{\text{Amount of fat eliminated in excrement}}{\text{Amount of fat enteral nutrition supplied}}\right) \times 100\%
\]

**Statistical analysis**

All data are expressed as means ± standard deviation for animals in each group. Differences among groups were tested using one-way ANOVA test. \( P \) values less than 0.05 were considered to be statistically significant. All analyses were performed with SPSS 11.0 Software.

**Results and discussion**

**Changes of the small intestine weight and intestinal mucosa weight**

Short bowel syndrome is a clinical condition characterized by a loss of intestinal mass or competence, resulting in a diminished ability to digest and absorb a regular diet. Decreased absorption of nutrients frequently occurs following bowel resection; and lipid absorption is generally considered to be the most vulnerable function. It results in loss of body weight. All rats in four groups showed weight loss during the first 3 days after surgery. From postoperative day (POD) 3 to POD 15, the weight increases at similar rates. On POD 15, the weight of SBS rats in each group was significantly lower than that of control group, but did not vary significantly among the three SBS groups. No death was observed during experimental procedure.

The small intestine and intestinal mucosa weights of rats in SBS groups were significantly higher than those in control group \( (P<0.01) \) (shown in Figure 1). Additionally, the ileal mucousa weight in the group of Arg/LCT was 18.0±3.5 mg cm\(^{-1}\) 100 g\(^{-1}\), which was significantly higher than in LCT and MCT/LCT groups.

**Morphological parameters**

After surgery and then treatment with different enteral nutrition formula for 14 d, the significant increases in villus height, crypt depth, mucosal thickness and villus surface area of jejunum and ileal were observed in SBS groups in comparison with control group. Additionally, the increases in villus height, crypt depth, mucosal thickness of jejunum and ileal of rats treated with Arg/LCT showed significant difference from those treated with LCT and MCT/LCT (Table 2).

**Fat absorptivity in treated SBS rats**

In short bowel syndrome, the combination of the loss of absorptive area with a comprised enterohepatic circulation results in inefficient fat absorption and steatorrhea. Essential fatty acid deficiency may progress as a result, and kidney stone or osteoporosis may develop. Optimal enteral composition may play a major role in patients with SBS and might contribute to optimal conditions for fat absorption. Although the effect of high-fat diet versus low-fat diet on the overall condition of SBS patients has been examined, it is still unclear whether other nutrients, such as MCT or L-Arginine, may produce any beneficial effect on SBS patients.

Fat absorptivities in SBS rats were 77.7%±2.1% in LCT group, 81.3%±3.8% in MCT/LCT group and...
After extensive small bowel resection, the adaptation of the remnant small bowel has been characterized by an early increase in blood flow to the remnant segment. It is shown that basal NO production is important in minimizing the mucosal dysfunction associated with reperfusion of post-ischemic intestine. L-Arginine is a semi-essential amino acid especially in stress situations such as trauma or infection. It can be hydrolyzed into urea and ornithine in the presence of L-arginine decarboxylase located at the intestinal epithelial cells, and ultimately produce polyamine in the presence of ornithine decarboxylase. These metabolites intimately participate in permeability and adaptive responses of the gut. At the same time, by increasing somatostatin release, L-arginine plays a role in secretion of growth hormone, which has been shown to promote small bowel mucosal growth in previous studies. Another possible role of L-arginine in SBS is that it may exert a negative influence on gastric acid hypersecretion, which causes mucosal injury, decreases the adaptive process, and lowers the absorption of lipids and proteins due to inactivation of lipase and trypsin. In a rat model with 90% small bowel resection L-arginine can increase villous height and crypt cell mitosis in small bowel mucosa and improve weight gain. These changes suggest a function of L-arginine in the mucosal adaptive response after bowel resection. Thus after fed with LCT and L-arginine, the marked increases in intestinal morphological parameters and fat absorptivity was observed, which was possibly due to the enterotrophic effect of L-arginine and the increased fatty acid take-up of remnant small bowel cells. It should be pointed out that further experiments were needed to understand the effect of L-arginine on long chain fatty acid transport.

### Total FFA levels in treated SBS rats

Serum FFAs exert an important effect on the supply of energy substrates. Despite minimal in vivo, they are the main sources of energy substrates for some vital organs such as muscle, heart, liver and renal cortex. Any alteration in their serum levels can affect the supply of energy substrates for these vital organs. Therefore, an evaluation of serum FFA profiles in some morbid status will be helpful to assess the metabolic change in the whole body.

Figure 2 shows the levels of total serum FFA in four groups after treated with different kinds of enteral nutrition for 15 d. Total FFA levels in SBS rats were significantly lower than those in control group except in LCT/LCT group. The mean total FFA level in SBS rats fed with LCT only was (995.5 ± 188.4) µmol/L, which was the lowest among these groups. The total serum FFA levels were obviously improved in SBS rats supplemented with MCT. The mean value increased to (1576.2 ± 536.0) µmol/L. But this value was still lower than 89.2% ± 3.4% of Con group (P < 0.05). The decrease of fat absorptivity and the increases of physiologic intestinal morphological parameters were obvious in LCT group. Fat absorptivities in MCT/LCT and Arg/LCT groups were greater than that in LCT group (P < 0.05). Thus, the loss of absorptive area resulted in fat malabsorption, and then performed intestinal adaptation.
than (2299.2±518.1) µmol/L of control group. After the SBS rats were treated with Arg/LCT their total serum FFA levels were significantly raised to (2727.0±505.6) µmol/L.

Circulating FFAs are derived mainly from adipose tissue lipolysis without absorbed lipid. However, in this work, the observed serum fatty acid profiles showed some correlation to absorption of fat. Firstly, there was a coincidence between change of total FFA and fat absorptivity in the four groups. The decrease in serum levels of polyunsaturated fatty acids, especially EFA in LCT group, may be due to the malabsorption of long chain fatty acid. So EFAD may progress as a result. The increase of PUFA, especially EFA in serum, may be the result of the improvement of fatty acid absorption in MCT/LCT and LCT/Arg groups.

Essential fatty acid levels

Medium chain triglycerides are saturated fatty acids from 8C to 12C length that can be easily hydrolyzed into water-soluble glycerol and medium chain fatty acids at a rate 6 times that of LCT in stomach and duodenum. In the intestinal epithelium, MCT can be absorbed in the form of glycerol and medium chain fatty acids without bile emulsification. It is observed that HIV patients have fewer stools, decreased stool fat and weight, and show a significant increase in urine nitrogen after fed with MCT-based diet. It is observed that animals given MCT/LCT diet showed more profound epithelial proliferation with a higher mucosal mass and protein content and increased villous length and crypt depth in the proximal part of the small intestine compared with the LCT diet groups. But Vanderhoof found that MCT might not stimulate the same degree of mucosal adaptation as LCT in SBS rats. In this work we did not observe any significant change in rat small intestine weights, intestinal mucosal weights and morphology. The gas chromatogram for one blood sample of 85% SBS fed with MCT/LCT is shown in Figure 3. Partial substitution of LCT with MCT could significantly increase fat absorptivity in SBS rats, suggesting the favored absorption of MCT-based enteral nutrition in the remnant small bowel compared with LCT-based enteral nutrition. This result was possible due to the easier absorption of MCT/LCT compared with LCT. It was noticed that the content of medium chain fatty acid increased in MCT/LCT group in comparison with Arg/LCT group, which may be due to the adding of MCT in MCT/LCT group. It was possible that there was a dynamic equilibrium between fatty acid in adipose tissue and serum FFAs. After a period of nutrition supplement, the compositions of fatty acids in adipose tissue and serum FFAs were changed with different enteral nutrition formula.

The concentrations of essential fatty acid in sera, including linoleic acid (C18:2n-6), arachidonic acid (C20:4n-6), α-linolenic acid (C18:3n-3), eicosapentaenoic acid (C20:5n-3) and docosahexaenoic acid (C22:6n-3), were given in Figure 4. The concentrations of C18:2n-6, C20:4n-6, C18:3n-3 and C22:6n-3 in LCT group showed significantly lower than those in control group with P values less than 0.01 for C18:2n-6, C20:4n-6 and C18:3n-3 and 0.05 for C22:6n-3, while the concentration values of C20:5n-3 between LCT and control groups did not show statistical difference. The concentrations of C20:4n-6 and C20:5n-3 in MCT/LCT group had no statistical difference from control group. The concentrations of C20:4n-6 and C20:5n-3 in MCT/LCT group were higher than those in LCT group (P<0.05). Most of the essential fatty acids in those treated with Arg/LCT were close to the levels in control group, only C20:4n-6 concentrations in Arg/LCT group showed significantly higher value than those in control group (P<0.01).
FFA profiles in treated SBS rat sera

Table 3 presents the results of FFA profile levels in control rats and short bowel syndrome (SBS) treated with enteral nutrition based on LCT, supplementation MCT or L-arginine in LCT. Essential fatty acid (EFA), such as C18-2n6 and C18-3n3, in LCT group were significantly lower than those in Con group with $P$ values less than 0.001. No significant difference of these fatty acids in MCT/LCT and Arg/LCT groups except a higher C18-3n3 level in Arg/LCT group were observed when compared with Con group. Polyunsaturated fatty acids, such as C20:4n6, C20:5n3 and C22:6n-3, also showed similar phenomena. Levels of medium chain fatty acids, such as C8:0, C10:0 and C12:0, in MCT/LCT group did not show statistical difference from those in LCT group, but were higher than those of Arg/LCT group. Additionally, serum C20:4n6 level was much higher in Arg/LCT group than in LCT group, which is the precursor of prostaglandin that may regulate intestinal adaptation in SBS.$^{26}$

Table 3 Comparison of free fatty acid profile levels (unit: %) in control rats and SBS rats treated with enteral nutrition based on LCT, supplementation MCT or L-arginine in LCT

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Control group</th>
<th>LCT</th>
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<td>Caprylic acid</td>
<td>C8:0</td>
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<td>0.077±0.056$^{a}$</td>
<td>0.031±0.028</td>
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<td>0.078±0.020$^{c}$</td>
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<td>Lauric acid</td>
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<td>0.119±0.022$^{e}$</td>
<td>0.104±0.3$^{f}$</td>
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<td>Myristic acid</td>
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<td>1.44±0.07$^{f}$</td>
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<td>Palmitic acid</td>
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<td>40.11±2.61$^{r}$</td>
<td>25.59±1.08$^{f}$</td>
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<td>Stearic acid</td>
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<td>12.54±2.38</td>
<td>21.27±1.11$^{f}$</td>
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<td>Arachidic acid</td>
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<td>0.12±0.03$^{f}$</td>
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<td>4.84±0.47</td>
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<td>Palmitoleic acid</td>
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<td>cis-Vaccenic acid</td>
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<td>17.74±5.60</td>
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<td>Linoleic acid</td>
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<td>γ-Linoleic acid</td>
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<td>Dihomo-γ-linolenic acid</td>
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<td>Docosapentaenoic acid</td>
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<td>8.1±0.59</td>
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$^{a}P<0.05$ vs. control group. $^{b}P<0.01$ vs. control group. $^{c}P<0.001$ vs. control group. $^{d}P<0.05$ vs. LCT group. $^{e}P<0.01$ vs. LCT group. $^{f}P<0.001$ vs. LCT group. $^{g}P<0.05$ vs. MCT/LCT group. $^{h}P<0.01$ vs. MCT/LCT group. $^{i}P<0.001$ vs. MCT/LCT group.
Enteral nutrition formula

7 total FFA and the decreased essential fatty acid content, which was improved in other two SBS groups. It was possible due to the change of fat absorption. This work extends the application of gas chromatography from providing significant data to elucidating physiological and pharmacological process, which possesses guidable significance to applying analytical techniques in dissolving some medical problems.

References


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Effect of Enteral Nutrition Formula on Fat Absorption and Serum Free Fatty Acid Profiles in Rat with Short-Bowel Syndrome

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The effects of enteral nutrition formula containing triglycerides or L-arginine on fat absorbability, serum free fatty acid profiles and intestinal morphology in rats with short-bowel syndrome were studied by using gas chromatography. This work can provide information for guiding enteral nutrition supplementation of patients with SBS.