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High-Protein Diet Improves Postoperative Weight Gain After Massive Small-Bowel Resection

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Abstract

Introduction—Short bowel syndrome (SBS) is a morbid clinical condition that results from massive small-bowel resection (SBR). After SBR, there is a dramatic weight loss in the acute postoperative period. Our aim was to determine the impact of a high-protein diet (HPD) on weight gain and body composition in mice after SBR.

Methods—C57BL/6 mice underwent 50 % proximal SBR. Postoperatively, mice were randomly selected to receive standard rodent liquid diet (LD) ($n=6$) or an isocaloric HPD ($n=9$) for 28 days. Mice weights were recorded daily. Body composition analyses were obtained weekly. Student's t test was used for statistical comparisons with $p<0.05$ considered significant.

Results—Mice that were fed HPD after SBR returned to baseline weight on average at postoperative day (POD) 8 versus mice that were fed LD that returned to baseline weight on average at POD 22. Total fat mass and lean mass were significantly greater by POD 14 within the HPD group. Both groups of mice demonstrated normal structural adaptation.

Conclusion—HPD results in greater weight gain and improved body composition in mice after SBR. This finding may be clinically important for patients with SBS since improved weight gain may reduce the time needed for parenteral nutrition.

Keywords

Short gut syndrome; Intestinal adaptation; High-protein diet; PEPT1

Introduction

Short bowel syndrome (SBS) is a clinical condition that occurs following a massive small-bowel resection (SBR). In patients with SBS, the gastrointestinal system is compromised since decreased mucosal surface area is associated with impaired absorption and digestion.

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Many SBS patients require total parenteral nutrition (TPN) which carries a significant morbidity and potential mortality¹.

Intestinal adaptation is a normal physiological compensatory response to massive SBR. Structurally, adaptation is highlighted by increases in crypt depth and villus height, thereby maximizing the mucosal surface area for absorption of nutrients.² During the acute postresection period, there is a dramatic weight loss.³ Ultimately, intestinal adaptation allows many patients to regain weight and wean from TPN completely.

Previously, our lab demonstrated that a high-fat diet is associated with amplified villus growth and accelerated weight gain after SBR.⁴ Similarly, others have confirmed this effect of fatty diets on the degree of structural adaptation after SBR.⁵⁻⁷ On the other hand, there have been limited studies investigating the effects of high-protein diet (HPD) after SBR. Theoretically, protein may be associated with greater lean mass, along the same lines that high-fat diets result in greater body fat accumulation. Feeding patients a high-fat diet after SBR therefore might potentially be detrimental to other organ systems. The purpose of the present study was to determine whether an HPD would be beneficial for weight gain and body composition after SBR.

Materials and Methods

Experimental Design

All experimental protocols were approved by the Washington University Animals Studies Committee (Protocol #20130038) and followed the standard National Institutes of Health animal care guidelines. C57BL/6 mice underwent 50 % proximal SBR. On postoperative day (POD) 1, mice were randomized to receive either standard rodent liquid diet (LD) ($n=6$) or an isocaloric high-protein (HPD) LD ($n=9$) (Dyets Inc., Bethlehem, Pennsylvania). LD consisted of 17 % protein compared to HPD which consisted of 45 % protein. The percentage and composition of fat remained the same in both diets (Table 1). Both diets are based on the Lieber-Decarli formula producing an isocaloric proportion of 1 kcal per gram of food⁸.

All mice were kept on their respective diet until POD 28, and daily weights were measured. Daily food intake and fecal output were recorded by caging mice individually on wire platforms from POD 21 to POD 28. Weekly MRI body composition analysis MRI (EchoMRI 3-1, Echo Medical Systems) was performed prior to bowel resection and on POD 7, 14, 21, and 28.

To evaluate for structural adaptation, a separate group of C57BL/6 mice underwent 50 % proximal SBR and was given either LD ($n=5$) or HPD ($n=5$). A 2-cm distal segment of the resected intestine was collected at the time of operation for baseline histology. Mice were harvested on POD 7, and postoperative histology was compared with baseline histology. Enterocytes were isolated as described below and used for protein and RNA analysis to evaluate for peptide transporters.

Animals and Small-Bowel Resection

Mice were kept in a standard animal-holding area with a 12-h light-dark schedule. SBR was performed on mice between 8 to 9 weeks of age. A 50 % proximal SBR was performed as previously described.² Briefly, our murine resection model consists of resecting the small intestine starting from 1–2 cm distal to the ligament of Treitz to 12 cm proximal from the ileocecal junction. A primary end-to-end anastomosis using 9-0 monofilament suture is performed to restore intestinal continuity. Animals were given free access to water for the first 24 postoperative hours. On POD 1, mice were randomized to LD or HPD and fed ad libitum.

Tissue Isolation

All mice were harvested as previously described.⁹ The entire distal small bowel was flushed with ice-cold phosphate-buffered saline (PBS) containing protease inhibitors (0.2 nM phenylmethanesulfonyl fluoride, 5 µg/mL aprotinin, 1 µM benzamidine, 1 mM sodium orthovanadate, and 2 µM cantharidin; EMD, Gibbstown, NJ) and excised from the mouse. A 2-cm segment of bowel distal to the anastomosis was cut and fixed in formalin to compare with histology collected intraoperatively. The remaining section of bowel distal to the anastomosis (approximately 10 cm in length) was used to isolate crypt and villus enterocytes using our previously published protocol.⁹ RNA and protein were further isolated for analysis of peptide transporters.

Histology

After formalin fixation and H&E staining, the villus height and crypt depth were measured using the MetaMorph computer program (Molecular Devices, Downingtown, PA). At least 20 well-orientated crypts and villi were measured per section. Postoperative and intraoperative crypt depth and villus height were compared to calculate a percentage change in evaluating the degree of adaptation after resection.

RT-PCR

RNA was isolated as previously described.^{9,10} Total RNA was extracted from the intestinal tissue by following the manufacturer's protocol for the RNeasy kit (Life Technology, Grand Island, NY). Total RNA concentration was determined spectrophotometrically. A TaqMan RNA-to Ct 1-Step kit (Applied Biosystems, Foster City, CA) was used per the manufacturer's protocol to determine relative gene expression directly from the isolated RNA. Equal amounts of RNA were used for real-time PCR with beta-actin as an endogenous control and a standard whole bowel sample used as the calibrator. PEPT1 gene expression was examined using a 750 Fast Real-Time PCR instrument (Applied Biosystems; Foster City, CA).

Statistical Analysis

Repeated measures ANOVA with a first-order autoregressive covariance structure was used (SAS 9.3, Cary, NC) to compare weight gain over time for the two groups. Other group comparisons were performed using Student's *t* test. All values are reported as means ± standard error of mean. A *p* value of less than 0.05 was considered significant.

Results

Postoperative Weight Changes and Body Composition

Mice were weighed daily after SBR. Mice in both groups experienced significant weight loss, but the weight loss in mice provided HPD was significantly less. Mice that were fed HPD after SBR returned to baseline weight on average at POD 8 versus mice that were fed LD which returned to baseline weight on average at POD 22. Weight change was reported as a percentage using POD 1 as the baseline reference weight (Fig. 1). Repeated measures ANOVA revealed a significant difference between the two groups for weight gain over time starting at POD 8.

Weekly body composition analysis was performed using MRI to determine total fat and lean mass. Both total fat mass and lean mass were significantly higher by POD 14 in the HPD group (Fig. 2a, b). By POD 14, the average fat mass of mice in the standard LD group was 2.8 ± 0.3 g whereas the average fat mass of mice in the HPD group was 3.7 ± 0.2 g (p value=0.036). Similarly by POD 14, the average lean mass for mice in the standard LD group was 15.5 ± 0.9 g compared to mice from the HPD group that was 17.6 ± 0.4 g (p value=0.024). This trend in differences between the two groups continued to the end of the study at POD 28 for both fat and lean mass.

Daily Food Intake and Fecal Output

To ensure that mice were consuming equal amounts of calories and to confirm that neither diet affected satiety, we individually caged mice on wire platforms for 1 week. This method allowed us to measure accurate daily food consumption and fecal excretion. The average daily food intake was identical between groups (13 ± 0.9 g for LD versus 13 ± 0.3 g in the HPD group). Since LD and HPD are both isocaloric, equivalent caloric intake was assured. The average fecal output was 0.3 ± 0.04 g per day for the LD group and 0.4 ± 0.01 g per day for the HPD group. These differences were statistically insignificant.

Structural Adaptation

To determine the effect of different diets on structural adaptation, we did a preliminary experiment to compare preoperative to postoperative histology at POD 7. Mice fed LD had an average of 51 ± 13 % increase in crypt depth and an average of 53 ± 3 % increase in villus height after SBR. When compared with mice fed HPD, there was an average of 29 ± 8 % increase in crypt depth and 56 ± 6 % in villus height (Fig. 3). Although the mean percent crypt changes differed by 22 % between groups, this was not statistically different.

PEPT1 Peptide Transporter Expression

PEPT1 is a major peptide transporter found predominantly in the proximal bowel. After a proximal bowel resection, PEPT1 expression would therefore be expected to diminish. We performed RT-PCR for PEPT1 mRNA expression to evaluate whether there was a compensatory response in the distal remnant bowel after SBR. The average relative expression in unoperated proximal bowel was 1.1 ± 0.2 . This was used to measure as a baseline comparison. The relative expression for the distal remnant bowel of mice fed LD was 0.63 ± 0.12 and for mice fed HPD was 0.73 ± 0.03 (Fig. 4) and showed no significant

difference (p value=0.39). These findings were very similar to previously published results¹¹.

Discussion

In the present study, we demonstrated a normal weight loss pattern in resected mice during the acute postoperative period.³ This was significantly improved following the provision of HPD, despite equal amounts of food intake and fecal output. Simultaneously, we observed an increase in total lean and fat mass for mice fed HPD compared to mice fed LD. These findings were identified in face of no obvious structural effect of diet on crypt depth and villus height. Finally, the expression of PEPT1, a major peptide transporter responsible for protein absorption, had no compensatory change, despite massive SBR or composition of diet.

Managing SBS patients continues to be a challenge. Many proposed modified diets with different proportions of fat,^{12,13} carbohydrates, and protein^{14,15} have been used in clinical trials in an attempt to wean patients off of TPN. Most recently, a randomized control trial tested the efficacy of administering enteral high-fat diet to premature infants with an enterostomy.¹³ The premature infants given supplemental enteral fat feedings required less intravenous lipid supplementation, had reduced conjugated bilirubin levels, and increased caloric intake with overall greater weight gain and intestinal length.

Other groups have supplemented enteral diets with free fatty acids,¹⁶ short-chain triglycerides,¹⁷ glutamine,^{18–20} trophic factors,^{21–25} growth hormones,^{26,27} or fiber²⁸ with no strong conclusive evidence leading to a standard of care in SBS management.^{29,30} Therefore, the search for a proper diet continues with the goal of having adequate nutrition and absorptive capacity of the remnant bowel leading to patients with SBS enterally feeding independently without the need for parenteral nutrition.

Previously, our lab has characterized body composition and metabolic changes associated with SBR in mice.³ In parallel with our past observations of mice on a standard LD, the present study showed a preferential percentage increase of fat mass over lean mass in both LD and HPD mice. However, when fat mass and lean mass were analyzed separately in terms of total grams, both fat mass and lean mass had a greater rate of increase in the group of mice that were fed an HPD. During the first postoperative week of recovery, the changes in metabolism and body composition are similar to partial starvation-refeeding animal models.³¹ The provision of an HPD may prevent the deterioration of lean mass and loss of protein stores. Intuitively, this would explain why mice fed HPD had greater increases in lean and fat mass during the initial phase of recovery and throughout the subsequent weeks.

We measured daily food intake and fecal output to evaluate a possible difference in caloric intake/output between the two groups of mice and found no statistical difference. Since both LD and HPD were isocaloric, the phenotypic difference we observed between the two groups is not likely attributed to early satiety or intestinal malabsorption.

Protein is absorbed via two major mechanisms. Digested proteins are hydrolyzed to amino acids and absorbed through several amino acid transporters and eventually into the portal

circulation.²⁹ A second mechanism of protein absorption is via PEPT1, which is a major peptide intestinal transporter located predominantly in the proximal bowel.^{32,33} These peptides are hydrolyzed within the enterocytes to become free amino acids which ultimately are deposited into the portal circulation. It has been shown that the state of hydrolysis of the enteral protein source makes no difference in weight gain, intestinal permeability, and energy balance in SBS patients.¹⁵ This would lead us to believe that the unhydrolyzed casein we used in this study is a sufficient source of enteral protein.

Next, we confirmed the lack of PEPT1 in the distal remnant bowel by performing RT-PCR. Our findings are consistent with other published studies showing that there was no increase in PEPT1 expression in the distal remnant bowel after bowel resection.^{11,34} Madhavan et al. concluded that the ability to absorb peptides was not due to an increased expression of PEPT1 but instead through mucosal hyperplasia.¹¹ Similarly, our study demonstrates that both groups of mice were able to normally adapt with increases of villus height and crypt depth compared to their baseline histology. Furthermore, we did not observe an added degree of adaptation with those mice that gained more weight. This observation confirms previous suggestions that structural and functional adaptation may be separate, uncoupled responses.³⁵ This is supported by the fact that other animal experiments have shown dietary protein improves growth independent of increased intestinal peptide or amino acid transporter expression¹⁴.

Our study has its limitations as we did not identify a direct mechanism that would explain the greater weight gain in resected mice which were fed an HPD. However, it has been long known that the amino acid, glutamine is the primary nutrient of the small bowel and is in sufficient quantities in the higher-protein diets.²⁰ For decades, glutamine has been documented to improve bowel function and increase mucosal growth.^{18,27} Additionally, Thomson showed that an HPD facilitated glucose uptake in animals with partial intestinal resection which may influence weight gain as well³⁶.

Another possible mechanism to explain our results is a recent study suggesting that HPDs are associated with increased serum levels of insulin-like growth factor 1 (IGF1).³⁷ IGF1 is a known intestinotrophic factor that binds and signals through insulin-like growth factor 1 (IGF1R).³⁸ We recently published results concluding that intestinal specific IGF1R is not necessary for normal structural adaptation and enterocyte proliferation after SBR.³⁹ It would be logical to test whether high-protein-induced serum levels of IGF1 would still manifest the same phenotype with IGF1R intestinal specific knockout mice. If enterocyte-specific IGF1R knockout mice are able to sustain greater weight gain after SBR, this would indicate serum levels of IGF1 act outside the intestinal epithelium and in other organ systems to affect body weight gain.

Lastly, another process that would explain our findings is the emerging research on the gut microbiome and the diversity that is altered by specific dietary changes.^{40–43} Previously, we have demonstrated increased rates of bacterial translocation to the mesenteric lymph nodes, spleen,⁴⁴ and liver after SBR.⁴⁵ This bacterial overgrowth in the small bowel may induce sepsis and inflammatory changes that take place in the SBR model. These inflammatory changes may be additionally modified by the type of diet and composition of fat and

proteins fed post resection. The exact mechanism is still unknown, but it is widely accepted that different types of food intake can alter the gut microbiota which may affect the intestinal barrier, nutrient transporters, and ultimately the metabolic phenotype.⁴² It has been described that a high-fat diet can alter the bacteria profile to decrease carbohydrate and protein metabolism as certain amino acid-metabolizing enzymes are known to alter metabolic function.⁴¹ It would be rational to consider the metabolic adaptation in the gut microbiome ecosystem after feeding mice with HPD would also occur and that intestinal mucosal immune function and host response after the small-bowel microbiome profile changes may be a conceivable mechanism in contributing to fat and lean mass deposition.

In summary, we determined that an HPD was beneficial to weight gain after SBR in mice. It appears that HPD allows for greater increases in lean and fat mass despite having both groups of mice receiving an isocaloric diet with equal amounts of food intake. PEPT1 expression in the distal remnant bowel did not appear to compensate for the loss of this transporter in the proximal bowel after resection. Further experiments will need to be performed to investigate whether HPD-induced IGF1 levels in the serum affects adaptation through intestinal specific IGF1R. Potentially, we can also characterize the intestinal microbiota ecosystem when mice receive HPD after SBR. This study not only demonstrates the complexity of functional intestinal adaptation but also gives us some understanding of the nutritional value of HPD during the initial recovery phase after massive SBR.

Conclusion

Enteral HPD feeding after SBR results in greater weight gain in mice affecting both lean and fat mass. During the initial postoperative period, mice are prone to stress and sustain a dramatic decline in both fat and lean mass stores. Therefore, providing an HPD may promote early functional adaptation during the anabolic recovery phase. Our findings may be clinically relevant for patients with SBS as gaining weight and maintaining lean mass are clinical measures used to wean patients off of TPN.

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Fig. 1.

Postoperative weight change. Postoperative weight from POD 1 to POD 28 presented as a percent weight change using POD 1 as the baseline reference weight. A repeated measures ANOVA with a first-order autoregressive covariance structure was used to determine significance of weight change over time. Significance between the LD and HPD diet started at POD 8 and continued through the end of the study

Fig. 2.

Body composition was performed weekly starting from POD 0 to POD 28 using magnetic resonance imaging. Similar to total body mass, both fat mass (**a**) and lean mass (**b**) decreased during the first 1–2 weeks. Between POD7 and 14, both fat mass and lean mass increased at a greater rate in the group of mice fed HPD compared to mice fed LD

Fig. 3. Structural adaptation. Baseline histology was collected by taking a 2-cm segment from the most distal section of the proximal resected segment at the time of SBR. On POD 7, crypt depth and villus height were compared between intraoperative and postoperative histology to measure the degree of adaptation between LD ($n=5$) and HPD ($n=5$) mice. Both groups displayed an increase in crypt depth and villus height compared to baseline histology, with no statistical differences detected between the mice fed LD versus mice fed HPD

Fig. 4. Relative mRNA expression of PEPT1 in the distal remnant bowel. RT-PCR for PEPT1 mRNA expression was used to evaluate whether there was a compensatory response in the distal remnant intestine after SBR. There was no difference between the mRNA relative expression of the distal remnant bowel from mice fed LD ($n=4$) and HPD ($n=5$; p value=0.39)

Table 1
Different compositions of protein, fat, and carbohydrates between the standard liquid diet and high-protein diet

	Standard liquid diet (% kcal/g)	High-protein diet (% kcal/g)
Protein	17.3 %	45 %
Fat	35 %	35 %
Composition of the 35 % fat		
Corn oil	22 %	22 %
Olive oil	71 %	71 %
Safflower oil	7 %	7 %
Carbohydrates	47.7 %	20 %