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Peptide Absorption after Massive Proximal Small Bowel Resection: Mechanisms of Ileal Adaptation

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Abstract

BACKGROUND—Protein absorption occurs as di- and tri-peptides via H⁺/peptide cotransporter-1 (PepT1).

AIM—To identify mechanisms of ileal adaptation after massive proximal enterectomy.

HYPOTHESIS—Ileal adaptation in uptake of peptides is mediated through upregulation of PepT1 gene expression.

STUDY DESIGN—Rats underwent 70% jejunoileal resection. Total mucosal cellular levels of mRNA and protein and transporter-mediated uptake per cm of the di-peptide Gly-Sar were compared in remnant ileum 1 and 4 wk postoperatively to control and 1-wk sham laparotomy rats. Histomorphology; food consumption, and weights of rats were monitored.

RESULTS—After 70% resection, although mRNA per cell for PepT1 decreased at 1-wk ($p=0.002$), expression of mRNA at 4 wk and protein at 1 and 4 wk in remnant ileum were unchanged ($p>0.1$). Ileal Gly-Sar uptake ($V_{\max}$ -nmol/cm/min, i.e. number of transporters per cm) increased at 1 and 4 wk compared to control and 1-wk sham ($P<0.05$ each); K_m (i.e. transporter function) was unchanged. Villous heights (mm) in remnant ileum increased at 1-wk and 4-wk time-points over controls (0.45 and 0.57 vs 0.21, resp; $p<0.001$).

CONCLUSIONS—Ileal adaptation to proximal resection for peptide absorption occurs through cellular proliferation (hyperplasia) and not through cellular upregulation of PepT1 mRNA or protein per enterocyte.

Keywords

Peptide absorption; PepT1; short bowel syndrome; small bowel resection; adaptation

INTRODUCTION

Extensive operative resection of the small intestine can be imperative in the management of several pathologic conditions but may result in the devastating malabsorptive state of “short bowel syndrome (SBS)” [1–2]. A similar state can follow traumatic loss of intestinal surface area, also leading to SBS. Despite recent advances in treatment options for patients with short bowel, current therapies still carry substantial risk of morbidity and mortality [3–6].

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Several studies conducted in laboratory animals as well as human subjects have shown that after massive, proximal-based, small bowel resection, the remnant ileum undergoes morphologic and functional adaptive changes in an attempt to maintain nutritional health by increasing net ileal absorption of enteral nutrients [7–12]. This inherent capability of the ileum to “adapt” has created interest in studying the cellular mechanisms responsible for ileal adaptation to formulate novel therapies for treatment of these patients. In our laboratory, we investigated previously the mechanisms of ileal adaptation for glucose absorption after extensive resection of the proximal intestine in a rat model [13]. Recently, we examined the response of the proximal jejunum to a massive, mid-small bowel resection by evaluating gene expression of the peptide transporter PepT1 and peptide absorption [14], but to the best of our knowledge, this current study is the first comprehensive investigation of the effect of massive, proximal, jejunoileal resection on ileal gene expression of peptide transporter-1 (PepT1), the exclusive peptide transporter in the brush border of enterocytes and peptide uptake. Colonic gene expression of PepT1 was also investigated in this study before and after massive proximal small bowel resection.

The physiologic and clinical importance of intestinal PepT1 has been well recognized only recently; PepT1 mediates the uptake of essentially all di- and tri-peptides (the major protein digestion products) in addition to several peptide-like drugs from the intestinal lumen [15–19]. Indeed, PepT1 appears to account for the majority of protein absorbed. Absorption of short peptides and peptide-like substances under normal conditions occurs predominantly in the proximal intestine (primarily the jejunum), with the ileum absorbing a substantially lesser amount of peptides. Colonic absorption of peptides in the normal physiologic state is insignificant [17–21]. Our aim in this study was to identify the mechanisms of ileal adaptation (and colonic adaptation, if any) for peptide absorption after massive, proximal small bowel resection. We hypothesized that intestinal adaptation occurs via increased enterocyte expression of mRNA and protein for PepT1 in the enterocytes, resulting in increased absorption of small peptides.

METHODS

After approval from our Institutional Animal Care and Use Committee and in accordance with the NIH guidelines for the humane use and care of laboratory animals, 30 male, Lewis rats weighing 200–250 g (Harlan, Indianapolis, IN) were maintained in a 12-h light/dark room (lights on from 6AM to 6PM). Twelve rats underwent a 70%, proximal-based, small bowel resection (see below, Small Intestinal Resection); these rats were then studied at 1 or 4 wk postoperatively (n=6, each group). An additional group of 6 rats were studied 1 wk after sham celiotomy to control for anesthesia and other postoperative changes; a group of 6 control rats (NC) housed in similar conditions were studied after 1 wk. In addition, 6 unoperated rats were observed for 4 wk to monitor weight gain and food consumption throughout the entire period of study (no tissue was harvested from this monitor group). All rats were housed in the 12-h photoperiod facility and allowed free access to water and standard rat chow (5001 Rodent Diet, PMI Nutrition International LLC, Brentwood, MO). Daily weights of the rat and the chow consumed (per light/dark cycle) were tabulated until the time of harvest. Because gene expression and transport function of PepT1 are known to vary diurnally [18–21], a set time-point of the light/dark cycle (3PM) was chosen to harvest the intestinal tissue; we selected this time-point, because we have shown previously that gene expression and transport function of PepT1 peak 3 h before the dark cycle [20–21].

Small Intestinal Resection

Rats were anesthetized initially using 2% inhaled isoflurane followed by intraperitoneal injection of 50 mg/kg sodium pentobarbital. A short-celiotomy (1 cm) was performed, and the small bowel was exteriorized. The proximal 70% of the small intestine starting from the

ligament of Treitz was resected after ligating the mesenteric blood supply which left about 15 cm of distal ileum. An end-to-end, single layer anastomosis was then performed using running 7-0 polypropylene sutures. The intestine was then returned into the peritoneal cavity, and the abdominal wall was closed in two layers with running 5-0 polyglactin suture. Sham celiotomy was performed under similar anesthesia using a short celiotomy with exteriorization of the entire small bowel. The intestine was manipulated manually for 5 min to simulate the resection procedure prior to reduction back into the abdomen. Abdominal closure was performed as above. Rats were deprived of food but not water for 24 h before any procedure. Postoperatively, all animals were maintained on water containing acetaminophen for 24 h after which they were allowed free access to chow.

Tissue Harvest

All tissue was harvested at a fixed time of the day due to known diurnal patterns in expression and function of PepT1 [20–21]. At the time of tissue harvest, rats were anesthetized with inhaled isoflurane and intra-peritoneal pentobarbital. After celiotomy, the duodenum was cannulated just distal to pylorus, and the bowel was flushed with cold (4°C) Ringer's solution (in mM: 128 NaCl, 4.7 KCl, 2.5 CaCl₂, 1.2 KH₂PO₄, 1.2 MgSO₄, 20 NaHCO₃; pH 7.3–7.4; 290 mOsm). The remnant ileum and the proximal 5 cm of colon were harvested. In NC and sham rats, the distal 15 cm of ileum was harvested which corresponded to the same segment of ileum harvested from the resection group. The harvested tissue was placed immediately in cold (4°C), oxygenated (95% O₂/5% CO₂) Ringer's solution. The distal portion of remnant ileum was used for measuring peptide uptake using the everted sleeve technique (see below, Uptake Function), and the proximal segment of remnant ileum was used for mRNA and protein measurements. There was not enough ileal length to evaluate differences in the proximal and distal aspects of the remnant ileum. Only PepT1 expression was studied in the colonic mucosal samples; colonic uptake was not evaluated, because our prior study [14] showed virtually no colonic absorption of the model dipeptide. The mucosa was scraped bluntly using a glass slide into cold, phosphate-buffered saline (PBS); although the mucosal scrapings include some non-enterocytes in the sample, PepT1 is expressed almost exclusively in the enterocyte only. Samples for mRNA analysis were placed in RNA stabilization buffer (RNALater, Qiagen, Valencia, CA) and stored immediately at –80°C. The samples for protein analysis were collected separately, placed in cold RIPA buffer containing protease inhibitors (Halt protease inhibitor cocktail, Pierce, Rockford, IL) and PMSF (phenylmethane sulfonyl fluoride solution; Sigma Aldrich, St. Louis, MO), and stored at –80°C. For histomorphometry, 0.5-cm portions were excised from the middle of remnant ileum, pinned out carefully on a support, and fixed in 10% buffered formalin.

mRNA Measurement

Real-time polymerase chain reaction (RT-PCR) was used to quantitate mRNA levels of PepT1 as described previously [20–21]. Mucosal samples stored in RNA stabilization buffer were thawed on ice, and homogenized RNA was isolated using the RNeasy Midi kit (Qiagen). RNA was then reverse transcribed into cDNA using the Super Script III kit (Invitrogen, Carlsbad, CA) and random hexamer primers. The cDNA levels of PepT1 and the stably expressed housekeeping gene glyceraldehyde-6-phosphate dehydrogenase (GAPDH) were then determined quantitatively using RT-PCR in a 7500 Thermocycler using TaqmanR chemistries with primers and fluorescently-labeled probes in assay mixes (Applied Biosystems, San Francisco, CA). Standard curves from serial dilutions of known copy numbers were used to calculate copy numbers of cDNA for each sample. All samples were run as duplicates with 2 µl of cDNA added to 23 µl of master mix. PCR was carried out at 50 °C for 2 min, then 95°C for 10 min, followed by 40 cycles of 15 s at 95°C, and 1 min at 60°C during which fluorescence measurements were made. Transporter copy

numbers were normalized to copy numbers of GAPDH from each sample in attempt to determine relative expression per enterocyte.

Protein Measurement

Levels of total cellular protein for PepT1 were measured semi-quantitatively using our well-characterized technique with Western blots [14,20–21]. Tissue samples stored in RIPA buffer containing Halt protease inhibitors and PMSF were thawed on ice; the presence of protease inhibitors was used to minimize protein degradation. Samples were homogenized using a Kontes Pellet Pestle (Fisher Scientific, Pittsburg, PA), and the protein-containing supernatant was separated by centrifugation at 5000 x g for 15 min. Protein concentrations were measured by the bicinchoninic acid method (Pierce); 200 µg of protein was resolved on a 10% SDS-PAGE gel (Bio-Rad, Hercules, CA) and transferred electrically to a PVDF membrane (Millipore, Bedford, MA). Membranes were blocked using 5% milk in Tris-buffered saline with Tween (TBS-T). GAPDH was used as a stably expressed “housekeeping” protein. Membranes were incubated overnight at 4°C with primary antibody for PepT1 (Santa Cruz Biotechnology, Santa Cruz, CA), and GAPDH antibody (US Biological, Swampscott, MA). After incubation with primary antibody, membranes were rinsed 3 times with TBS-T and incubated with secondary antibody in TBS-T containing 5% milk using horseradish peroxidase-conjugated, goat anti-rabbit IgG for PepT1 and anti-mouse IgG for GAPDH (Sigma). Protein bands were visualized by a colorimetric reaction using Opti-4CN Substrate kits (Bio-Rad) for GAPDH and amplified with Opti-4CN for PepT1. Scion Image (Scion Corp, Frederick, MA) was used for semiquantitative measurements based on band densitometry. Protein measurements were normalized to GAPDH as a technique designed to estimate relative amount of PepT1 per enterocyte.

Uptake Function

We measured transporter-mediated uptake of the di-peptide Glycyl-Sarcosine (Gly-Sar), a non-hydrolysable substrate for PepT1 [19], using a modified everted sleeve technique as we described previously [14,20–21]. Ileal segments (1 cm) were everted over a pre-grooved steel rod and secured with silk ties, thereby exposing the mucosal surface externally. Colonic uptake studies were not performed, because our prior work showed very little if any colonic uptake of dipeptides [14]. The intestinal “sleeves” were kept in cold (4°C) Ringer’s solution bubbled with 95% O₂/5% CO₂. The sleeves were transferred into 8 ml of warmed (38°C), Gly-Sar-free incubation medium (in mM: 129 NaCl, 5.1 KCL, 1.4 CaCl₂, 1.3 NaH₂PO₄, and 1.3 Na₂HPO₄; pH 6.0) [14, 19–20] bubbled with 95% O₂/5% CO₂ and stirred at 1,200 rpm for 5 min. Then, the sleeves were placed in 8 ml of 38°C incubation medium containing 0.2, 1, 3, 20, or 40 mM Gly-Sar maintaining iso-osmotic conditions by replacement with appropriate amounts of NaCl in the test solutions. One µCi of ¹⁴C-Gly-Sar was included in the test solution to measure total uptake of Gly-Sar from which the transporter-mediated uptake by PepT1 was calculated (see below). After 1-min incubation, sleeves were removed, rinsed in 30 ml of ice-cold (Gly-Sar-free) incubation medium stirred at 1,200 rpm for 20 s, placed in glass scintillation vials, and solubilized in 1 ml of tissue solubilizer (Perkin-Elmer, Boston, MA) at 50°C in a water bath for 3 h. After complete solubilization, 15 ml of scintillation counting cocktail (Opti-Flour, Perkin-Elmer, Waltham, MA) was added, and disintegrations per minute (DPM) of ¹⁴C were determined using liquid βscintillation counting.

Transporter vs. non-transporter mediated uptake—The method of estimating transporter vs. non-transporter mediated uptake of Gly-Sar was described previously [20–21]. To calculate transporter-mediated uptake, total uptake has to be corrected for passive diffusion and mucosal adherence (non-transporter mediated “uptake”). Non-transporter-mediated uptake at lesser concentrations can be estimated from observed uptake at markedly

greater concentrations [20–22]. As the substrate concentration increases, non-transporter mediated passive uptake increases linearly before and after the transporter is saturated; thus, the linear increase in total uptake after the transporter is saturated is attributed only to non-transporter-mediated “uptake”, i.e. passive diffusion and mucosal adherence. We used 20- and 40-mM concentrations of Gly-Sar (at which a linear increase in total uptake was observed) to estimate non-transporter-mediated “uptake” at the lesser concentrations (0.2, 1, 3 mM). Subtraction of the estimated, non-transporter-mediated uptake from observed total uptake allowed estimation of the transporter-mediated uptake.

Villous Height

The formalin-fixed ileal tissues from all groups were embedded in paraffin, sectioned parallel to the villous axis, and stained with hematoxylin and eosin. Maximum villous height was measured from above the crypt to the tip of the villous at 10x magnification using an optical reticule with a micrometer. A minimum of 6 sections were studied from each specimen with at least three measurements made per section.

Statistical Analysis

PepT1 mRNA and protein levels were expressed as the ratio of PepT1 to the housekeeping gene (GAPDH) in an attempt to estimate gene expression per enterocyte. Transporter-mediated uptake of Gly-Sar was measured in nmol/cm/min with Lineweaver-Burke plots used to calculate $V_{\max}$ and K_m . Data are reported as median $\pm$ interquartile range (IQR) unless otherwise specified. Kruskal-Wallis analysis was used to compare non-parametric data across multiple groups; Wilcoxon rank sums were used for direct comparisons between individual groups. P-values were corrected according to the Bonferroni method, only corrected p values of < 0.05 were considered significant, and n values are number of rats.

RESULTS

Food Intake/Weight Gain

Rats in both NC and sham celiotomy groups followed a nocturnal-based feeding pattern; greater than 70% of ingestion of chow occurred between 6PM and 6AM ($p < 0.001$). The non-resected rats consumed similar amounts of food and had similar weight gain by the end of 1-wk study period (Figure 1A). In contrast, rats in both resection groups (1 and 4 wk) ingested significantly less chow compared to NC and sham rats; in addition, diurnal rhythmicity in the feeding pattern was disrupted during the first week postoperatively ($p < 0.05$; Figure 1A). Starting from the second week postoperatively, however, rats in the 4-wk resection group followed a nocturnal-based feeding pattern and consumed daily-amounts of food similar to the non-operated control rats monitored for 4 wk (data not shown).

Resected rats had a net weight loss at 1 wk postoperatively compared to NC and sham rats (Figure 1B; $p < 0.001$); nevertheless, rats in the 4-wk resection group started to gain weight by the second week postoperatively until the time of tissue harvest in a rate similar to the non-operated, monitored control group (Figure 1B). By the end of 4-wk period, rats that had undergone resection had gained less weight (median gain 44g; IQR: 36–65g) compared to monitor group (median gain 91g; IQR: 84–98g); this difference in total weight gain occurred due to the initial weight loss in resected rats during the first week postoperatively.

None of the rats (in all groups) developed diarrhea or other post-operative complications that required exclusion from the study. Although total water intake and lean body mass were not measured in this study, the matched daily food intake and weight gain between non-resected and resected rats after the first week postoperatively suggest that there were no differences in total water intake or total body water.

mRNA Expression

Ileum—There was no change in relative expression of PepT1 mRNA after sham celiotomy. At 1 wk after 70% proximal small bowel resection, cellular mRNA levels of PepT1 in the remnant ileum decreased (about 3-fold decrease in median relative expression compared to NC; $p \leq 0.002$; Figure 2A). In the 4-wk post-resection group, however, ileal mRNA expression increased back toward “pre-resection” levels with no difference compared to NC and sham groups ($p > 0.05$).

Colon—Cellular mRNA expression of colonic PepT1 remained unchanged both at 1 wk and 4 wk after massive small bowel resection compared to NC and sham rats ($p > 0.5$; Figure 2B). Furthermore, in each individual group, we compared relative cellular expression of PepT1 mRNA in colon versus remnant ileum. As expected, mRNA expression in colonic enterocytes was much less compared to remnant ileum in all four groups (about 1000 fold difference; $p < 0.0001$).

Protein Expression

Ileum—Despite the decreases in mRNA expression for ileal PepT1 at 1 wk post-resection, relative protein expression by Western blotting for total cellular PepT1 did not differ among all four groups ($p > 0.6$; Figure 3A).

Colon—Similarly, there was no change in colonic protein expression per enterocyte after sham celiotomy or massive small bowel resection (both at 1 and 4 wk) compared to control levels ($p > 0.4$; Figure 3B). Moreover, we compared total cellular expression for PepT1 protein in colon versus remnant ileum. Unexpectedly, although mRNA expression in colon was 1000-fold less than in ileum, there was no difference between colonic and ileal enterocytes in total protein expression of PepT1 across all four groups ($p > 0.05$).

Transporter-Mediated Uptake of Gly-Sar

Uptake values—Ileal uptake of Gly-Sar in all four groups demonstrated saturation kinetics consistent with transporter-mediated uptake. Transporter-mediated uptake of Gly-Sar at all three concentrations (0.2, 1, 3 mM) was not different between NC and sham groups; however, uptake increased markedly in the remnant ileum at 1 and 4 wk after 70% proximal small bowel resection ($p < 0.05$; Figure 4). There was no further increase in transporter-mediated uptake of Gly-Sar from 1 wk to 4 wk after resection ($p > 0.2$).

Uptake kinetics—Similar to uptake values, the calculated $V_{\max}$ (nmol/cm/min, a function of the number of apical transporters participating actively in uptake) remained unchanged after sham celiotomy compared to NC; however, in the 1- and 4-wk post-resection groups, the median $V_{\max}$ increased markedly; there was no difference between the 1- and 4-wk groups (1 and 4 wk vs NC and Sham: 44 and 52 vs 22 and 23; $p < 0.05$, Figure 5A). K_m (mM), a function of transporter affinity to its substrate did not differ amongst all the groups ($p > 0.05$; Figure 5B).

Villous Height and Intestinal Length

In the ileal segment, median villous height (mm) was markedly greater at 1 and 4 wk after resection compared to NC and sham rats (0.45 and 0.57 vs 0.21 and 0.24; $p \leq 0.01$; Figure 6). There was no difference between NC and sham ($p > 0.8$). Additionally, there was no difference in villous height in rats at 1 and 4 wk after resection ($p > 0.1$). Changes in villous height were compared to changes in $V_{\max}$ among all groups. At 1 wk after resection, median villous height in remnant ileum increased by 114% compared to NC (0.45 vs 0.21 mm), while median $V_{\max}$ increased by 100% (44 vs 22 nmol/cm/min). At 4 wk time point, median

villous height increased by 171% compared to NC (0.57 vs 0.21 mm), while median $V_{\max}$ increased by 136% (52 vs 22 nmol/cm/min).

All resected animals were left with a remnant ileum of 15 cm in length at the time of resection. One week after resection, this length appeared to have shortened to a median 13.5 cm (range, 12.5–14.5 cm; $p < 0.01$); after 4 wk, the intestinal length appeared to have increased to a median of 17 cm (range, 14.5–19.5 cm; $p < 0.01$). In addition to intestinal length, gross luminal diameter also increased notably over time, and while actual measurements could not be measured reliably, the everted sleeve technique necessitated larger caliber steel rods for eversion (4 mm in NC and shams, 5 mm 1 wk after resection, and 6 mm 4 wk after resection).

DISCUSSION

Ileal adaptation after massive proximal small bowel resection has been the focus of increasing interest in recent years, especially as it pertains to exploring potential therapies for short bowel patients. Despite several treatment options, such as long-term parenteral nutrition, intestinal rehabilitation, various intestinal lengthening procedures, and, more recently, small bowel transplantation, the outcomes with current therapies are still not optimal for patients with SBS [1–4]. Preserving the distal ileum with the ileocecal junction in patients undergoing massive small bowel resection is associated with better surgical outcomes; however, the adaptive mechanisms occurring in the distal ileum for improving nutrient absorption, and especially for proteins as peptides, has not been characterized. Our laboratory has focused recently on the early adaptive changes after massive intestinal resections. Our studies have been designed to improve our understanding of the potential cellular mechanisms underlying the ileal adaptive response by studying gene expression and transport function of major nutrient transporters, including the recently recognized and important transporter PepT1. This topic has direct implications in our understanding of the pathophysiology of SBS and may lead potentially to improvements in our management of this condition.

Our data confirm that the ileum, which in the normal, intact gut does not account for much of the peptide absorption from the lumen, is highly adaptable and can increase its peptide transport after a proximal-based, 70% small bowel resection. This ileal adaptation was associated with a rapid increase in the villous height (i.e. hyperplasia) with increased numbers of enterocytes/cm in the remnant ileum in the resected groups. This adaptive response occurred as early as 1 wk postoperatively in this rat model and also was sustained 4 wk after resection. Interestingly, however, was that there was no cellular upregulation of mRNA and total protein expression for PepT1 in the ileal or colonic enterocytes after massive proximal small bowel resection at either time point (1 and 4 wk post-resection) despite the marked increase in peptide uptake per cm of bowel. These data are consistent with our previous findings of marked, mid-ileal adaptation for glucose absorption and gene expression of hexose transporters as well as the response of the proximal jejunum to a 70% mid small bowel resection for gene expression and transport function of PepT1 [14]; in contrast, they differ from the response of the distal-most ileum which underwent hyperplasia but did not increase its absorption of dipeptides per enterocyte [14].

PepT1 has emerged as a very important transporter in the gut mucosa. Not only is PepT1 the primary transport pathway for absorption of the majority of ingested proteins (via transport of short peptides after luminal protein hydrolysis), but PepT1 also is involved in the absorption of multiple therapeutic pharmacologic agents, including certain antibiotics, antiviral agents, antihypertensives, and other drugs as well. Indeed, much of the prior

research on function and control of expression of PepT1 has been in the field of pharmacology.

Our interest focused on the control of PepT1 gene expression as an adaptive response to an acute massive loss of intestinal absorptive surface area. Intestinal adaptation to states of massive loss of absorptive area by disease or intestinal loss is well known to occur by a marked increase in absorptive capacity per unit length of bowel. This adaptation occurs in part secondary to a marked cellular hyperplasia (increased height of villi) and intestinal dilation, but whether the enterocyte itself “upregulates” gene expression (cellular levels of mRNA and/or protein), modifies posttranscriptional or posttranslational processing, or alters the membrane levels of the transporter(s) (and thus $V_{\max}$) remains poorly understood.

Our hypothesis was that ileal adaptation to a proximal, 70% jejunoileal resection would occur both by marked hyperplasia and intestinal dilation as well as by cellular upregulation of both mRNA and protein expression of PepT1. Our findings did not completely confirm our pre-experiment hypothesis. While hyperplasia (increased villous height) and intestinal dilation did occur and uptake of the dipeptide was markedly increased, cellular levels of mRNA measured by quantitative real time RT-PCR and protein by semi-quantitative Western blot (corrected by relative expression against the constant expression of the housekeeping gene GAPDH) remained unchanged at 4 wk post-resection. Moreover, although hyperplasia and increased uptake were evident even at 1 wk post-resection, cellular mRNA levels in the ileal enterocyte decreased 3-fold at 1 wk post-resection, while cellular protein levels remained unchanged. This decrease in gene expression of PepT1 despite the strong catabolic stress with a 70% proximal small bowel resection might be related to some counterregulatory hormones (such as epidermal growth factor and triiodothyronine) or other negative regulators of PepT1 gene expression as has been suggested by other *in vitro* studies (in cell cultures) [23–24]; however, we have no objective data to support this statement. Similar findings of a decrease in jejunal mRNA for PepT1 were reported after enterectomy in rabbits, but no protein or uptake studies were carried out [25].

Our data showed that the increase in uptake of dipeptides and $V_{\max}$ were proportionately less when compared with the changes in total absorptive surface area (villous heights and luminal caliber). This observation might suggest differential distribution of the population of PepT1 transporters along the crypt-villous axis in the remnant ileum. In fact, differences in distribution of PepT1 along the villi were reported previously in rat jejunum via measurements of immunohistochemistry showing a relative increase in expression toward the tip of villous [26].

These combined observations suggest that either the increased uptake of dipeptide was related solely to the ileal mucosal hyperplasia and an increase in absorptive area or that the enterocyte regulated the apical levels of this transporter via still poorly understood intracellular mechanisms of PepT1 translocation without changing total cellular levels of PepT1. Indeed, there are good *in vivo* data [27–29] as well as our preliminary data in cell culture [30–32] supporting the concept of cellular regulation of translocation of membrane transporters such as glucose transporter 2 (GLUT2). The experimental data for PepT1 is suggestive of a similar cellular translocation of PepT1 from intracellular stores of pre-formed PepT1, but the lack of stereospecificity of the transporter and reliable techniques of quantitating selectively the apical levels of PepT1 protein has hindered progress in this field. Because we measured total cellular levels of PepT1 protein, our experimental design and techniques were unable to differentiate cellular from apical levels of PepT1 protein. We are actively investigating this form of cellular regulation of PepT1 currently.

Although these findings did not support our hypothesis, there are similar precedent data. We have studied previously the regulation of gene expression of the hexose transporters sodium-glucose co-transporter 1 (SGLT1) and GLUT2 after this same model of proximal 70% jejunoileal resection with similar findings of increased uptake of glucose associated with increased villous height and a lack of alteration in gene expression of the hexose transporters [13]. In contrast, we studied recently the regulation of gene expression of PepT1 after a much different form of resection (70% mid small bowel resection) with some different findings in the distal remnant ileum [14]. That recent work demonstrated a decrease in gene expression (mRNA and protein) of PepT1 after massive mid small bowel resection but did not show any increase in dipeptide uptake in the very short remnant ileum despite modest increase, although less dramatic when compared with our current model, in villous height. In contrast, that same study showed a significant increase in the uptake of dipeptides in the remnant proximal jejunum (associated with increased villous height) without any changes in gene expression of mRNA or protein at either 1 or 4 wk post-resection. Those differences in the response of remnant jejunum and ileum to proximal versus mid jejunoileal resection might be related to the presence of the proximal jejunum which has more capacity for peptide absorption and could decrease potentially the amount of dietary peptides reaching the terminal ileum and thereby alter ileal adaptation.. Another possibility is that the distal ileum responds differently than does the mid ileum; unfortunately, there was not enough ileum to compare the proximal and distal segments of the remnant ileum in this current study of massive proximal-based jejunoileal resection. This constellation of findings suggests that the small bowel adapts to loss of surface area primarily by hyperplasia and dilation to increase absorption per unit length of bowel by increasing absorptive area and not by increasing net uptake per enterocyte via regulating levels of functional PepT1 in the apical membrane. These findings may have important implications in the attempt to augment the absorptive adaptation of the small intestine during the various conditions of the short gut syndrome, at least in the early postoperative period after massive loss of small bowel.

In addition, our two studies of massive intestinal resection suggest potentially important regional differences in the adaptive response of the rat enterocyte in the gene expression of PepT1. The jejunum appears to respond by hyperplasia without any change in cellular (enterocyte) levels of PepT1 mRNA or protein to increase peptide uptake per unit length of jejunum. In contrast, the remnant ileum, after massive proximal jejunoileal resection, responds by a similar hyperplasia and increase in peptide uptake per unit length of bowel despite an overall paradoxical decrease in cellular levels of mRNA with no subsequent change in cellular levels of PepT1 protein. Our data are inadequate to determine whether there is a decrease in PepT1 transcription, a change in posttranscriptional processing, a more efficient synthesis of PepT1, or a change in PepT1 translocation to the apical membrane of the ileal enterocyte. Moreover, although mRNA expression in ileum was about 1000-fold more than in colon, there was, unexpectedly, no difference between ileal and colonic enterocytes in total protein expression of PepT1 among resected and non-resected rats. These findings might be explained by differences in post-transcriptional processing or stability of mRNA for PepT1 in ileal versus colonic enterocytes. Although total cellular protein levels were similar between these two segments, our experimental design and techniques were unable to differentiate intra-cellular from apical levels of PepT1 protein. Subsequent studies, both *in vivo* and *in vitro* (such as cell cultures), are needed in the future to further characterize the cellular mechanisms of intestinal adaptation for peptide absorption after massive proximal jejunoileal resection.

CONCLUSION

The ileum appears to be highly adaptable in its ability to increase dipeptide absorption via PepT1 after massive proximal-based, small bowel resection, but this increase in absorptive

capacity is due to cellular proliferation by villous hyperplasia and intestinal dilation to increase absorptive surface area as opposed to gene upregulation of PepT1 within the enterocyte. These findings might have important implications in the management of SBS as well as in the field of pharmacology.

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ABBREVIATIONS USED

cDNA	complementary DNA
DPM	Disintegrations per minute
GAPDH	Glyceraldehyde-6-phosphate dehydrogenase
GLUT2	Glucose transporter 2
Gly-Sar	Glycyl-Sarcosine
IQR	Interquartile range
mRNA	messenger RNA
NC	Naïve control rats
.PBS	Phosphate-buffered saline
PepT1	Peptide transporter 1
RT-PCR	Real time, polymerase chain reaction
RXN	Resection (used in illustrations only)
SBS	Short bowel syndrome
SGLT1	Sodium-glucose co-transporter 1
TBS-T	Tris-buffered saline with Tween

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FIGURE 1.

A) Patterns of food consumption over 1 wk in non-resected, normal control (NC) rats, rats that underwent sham celiotomy, and rats that underwent 70% proximal jejunoileal resection (1 wk RXN). NC rats and sham rats followed a predominately nocturnal feeding pattern; however, resected rats lost diurnal rhythmicity in feeding pattern and consumed less chow during the 1st wk postoperatively. Nevertheless, both resected and non-resected rats had a similar feeding pattern and weight gain, starting from the 2nd wk postoperatively. B) Weight changes over 4 wk in monitored control rats and rats that underwent resection (4 wk RXN). Resected rats had a net weight loss at 1 wk postoperatively compared to NC and sham rats.

FIGURE 2.

Relative expression levels of PepT1 mRNA in A) Ileum and B) Colon for four study groups (NC, sham, 1 wk RXN, and 4 wk RXN). Although mRNA levels per enterocyte decreased in the remnant ileum at 1wk post-resection, expression of ileal mRNA at 4 wk and colonic mRNA at 1 and 4 wk after resection were unchanged compared to NC and sham rats.

FIGURE 3. Relative expression levels of PepT1 protein in A) Ileum and B) Colon for four study groups (NC, sham, 1 wk RXN, and 4 wk RXN). There were no differences amongst all four groups in their ileal or colonic protein expression for PepT1 per enterocyte.

FIGURE 4.

Transporter-mediated uptake of Gly-Sar at three concentrations of Gly-Sar (0.2, 1, 3 mM) in the ileum of four study groups (NC, sham, 1 wk RXN, and 4 wk RXN). Ileal uptake of Gly-Sar increased significantly at 1 and 4 wk after resection compared to NC and sham groups. There was no further difference in Gly-Sar uptake between 1 wk and 4 wk RXN groups.

FIGURE 5.

Values for A) V_{max} (in nmol/cm/min) and B) K_m (in mM) of Gly-Sar in the ileum of four study groups (NC, sham, 1 wk RXN, and 4 wk RXN). Similar to uptake values, the calculated V_{max} increased markedly at 1 and 4 wk post-resection compared to NC and sham groups; B) K_m values did not differ amongst all groups.

FIGURE 6.

Villous heights (mm) in the ileum of four study groups (NC, sham, 1 wk RXN, and 4 wk RXN). Median villous height was markedly greater at 1 and 4 wk after resection compared to NC and sham rats. There were no differences between NC and sham rats ($p>0.2$) or between 1 wk RXN and 4 wk RXN.