ABSTRACT

Background: Intestinal adaptation after massive bowel resection in animal models is characterized by increased gut-mucosal growth and expression of nutrient transporters. Few data about these indexes exist in humans with short-bowel syndrome (SBS).

Objective: The objective was to compare small-bowel and colonic mucosal growth and expression of the peptide transporter PepT1 in adults with or without SBS.

Design: Mucosal biopsy specimens were obtained from the small bowel and colon of 33 control subjects with intact intestine and from 13 SBS patients dependent on parenteral nutrition because of chronic malabsorption. Gut-mucosal crypt depth, villus height, and villus width were measured, and expression of PepT1 was determined by Northern blotting, in situ hybridization, and immunohistochemistry.

Results: The indexes of small-bowel and colonic mucosal growth were not significantly different between the 2 groups. PepT1 expression was high in the apical region of duodenal, jejunal, and ileal villus epithelial cells; low in absorptive colonic crypts; and not significantly different in the distal small intestine of the 2 groups. However, the abundance of PepT1 mRNA in the colon of SBS patients was more than 5-fold that in control subjects (P < 0.01).

Conclusions: Gut adaptation in SBS patients does not appear to involve an increase in gut-mucosal crypt depth or villus size. PepT1 is abundant along the small-bowel brush border in humans; expression in the colon indicates that the large intestine has an adaptive mechanism for luminal di- and tripeptide transport. Up-regulation of colonic PepT1 in SBS may adaptively improve accrual of malabsorbed di- and tripeptides, independent of changes in the mucosal surface area.

KEY WORDS PepT1, intestinal mucosa, intestinal adaptation, peptides, transport, short-bowel syndrome

INTRODUCTION

Intestinal transport of luminal di- and tripeptides across the brush border of absorptive enterocytes is a major mechanism by which the digestion products of proteins are absorbed (1–8). The oligopeptide transporter PepT1 is responsible for the transport of di- and tripeptides in mammalian intestine (9). In vitro studies have shown that highly homologous rabbit, rat, and human PepT1 complementary DNAs (cDNAs) encode PepT1 proteins capable of transporting a broad array of neutral, acidic, and basic di- and tripeptides and peptidomimetic drugs by an H⁺-dependent, electrochemical active transport mechanism (9–11). After transport into cells, the small peptides primarily undergo intracellular hydrolysis to free amino acids for delivery into portal blood (8).

PepT1 expression has been little characterized in human tissues, but its messenger RNA (mRNA) has been shown in human ileum, liver, kidney, and pancreas and in the colon adenocarcinoma-derived cell line Caco-2 (8, 11). PepT1 protein and mRNA are abundant in the small-bowel villus epithelial cells of rats and rabbits (12, 13), but only small amounts of PepT1 mRNA are present in the proximal colon of rabbits (13).

Short-bowel syndrome (SBS)—after extensive small-bowel resection or a combination of small-bowel plus colonic resection—is a devastating clinical problem characterized by diarrhea, dehydration, micronutrient depletion, and generalized malnutrition (14, 15). In animal models of SBS, the residual small-bowel mucosa, and to a lesser extent the colonic mucosa, undergoes hyperplasia and concomitantly increased nutrient absorptive function (intestinal adaptation) (14–19). Clinical experience suggests that intestinal adaptation occurs in humans, as evidenced by a decrease in diarrheal episodes and an apparent increase in nutrient absorption over time (14). It is unknown whether compensatory intestinal adaptation in humans involves changes in...
the mucosal mass, in nutrient-transporter expression and function, or in both processes. Quantitative data on intestinal adaptation in humans are limited to case reports and a few small series in which nutrient absorption or growth indexes of small-bowel mucosa were measured (20–24).

Several early reports indicate that the colon of intact rabbits, pigs, and dogs absorbs small amounts of L-amino acids or protein hydrolysates placed within the lumen (25–29). However, whether the intact or adapting human colon has these classic small-bowel-associated mechanisms for amino acid or peptide transport is unknown. The aims of this study were 1) to determine the cellular localization of PepT1 in the small-bowel and colonic mucosa of humans and 2) to investigate whether growth indexes of gut mucosa and PepT1 expression are up-regulated in patients with SBS after massive bowel resection but not in unselected control subjects.

SUBJECTS AND METHODS

Subjects

We studied 13 adult patients with SBS who were able to consume an oral diet but required long-term home parenteral nutrition to maintain adequate nutritional and hydration status. The patients were referred by their primary physicians from different hospitals throughout the southeastern United States. All patients had intact stomach and duodenum and various lengths of residual small bowel and colon. The length of residual intestine was estimated by reviewing all prior surgical records and results of barium-contrast studies. Each patient’s oral dietary intake, weekly parenteral nutrition volume, and energy and protein prescriptions were stable for ≥2 mo before the study. Those patients with a history of Crohn disease were in clinical remission for ≥6 mo before entry.

Seven SBS patients underwent all of the study procedures outlined below (eg, assessments of nutrient absorption, gut-mucosal growth, and intestinal PepT1 expression). In addition, histologic sections of small bowel, colon, or both were available from 6 additional parenteral nutrition–dependent SBS patients, whose etiology of SBS, sex, age (x ± SD: 54 ± 7 y), and duration of parenteral nutrition (18 ± 4 mo) were not significantly different from that of the original 7 SBS patients.

A total of 33 control subjects (20 women and 13 men) aged 54 ± 2 y with no history of SBS were recruited from among outpatients undergoing clinically indicated upper endoscopy or colonoscopy for colon cancer screening or evaluation of abdominal pain. All control subjects were clinically stable, had no previous history of bowel resection, and had no inflammatory disease, malabsorptive disorders, active infection, or intestinal carcinoma. Studies were performed in 15 of the control subjects to determine the proximal to distal gut-mucosal gradient of PepT1 mRNA expression (n = 5 each for studies of the duodenum, ileum, and colon). Biopsy specimens from the remaining control subjects were used to compare PepT1 mRNA expression in the small-bowel mucosa or colonic mucosa with that in specimens from age- and sex-matched SBS patients. All SBS patients and control subjects gave informed consent, and the study was approved by the Human Investigations Committee of Emory University, Atlanta.

Intestinal absorptive function in SBS patients

Seven patients with parenteral nutrition–dependent SBS were admitted to the General Clinical Research Center (GCRC) of Emory University Hospital for nutrient absorption studies (Table 1). These patients received their usual home parenteral nutrition regimen and oral diet for 7 consecutive days in the GCRC. The composition of the oral diets prepared and administered was based on a 7-d prediagnosis food recall performed by a registered dietitian (KU). After an initial 3-d period of equilibration to the GCRC, daily stool and ostomy outputs were collected for 4 consecutive days and pooled. Identical portions of all food consumed were prepared for analysis of actual oral dietary intake during the nutrient absorption study. The nitrogen, energy, and fat contents of the intestinal output and of the oral diet were analyzed to calculate net intestinal nutrient absorption. The nitrogen assays were performed at the GCRC Metabolic Core Laboratory by micro-Kjeldahl digestion. The fat and energy assays were performed at Covance Laboratories (Madison, WI) by acid hydrolysis and bomb calorimetry, respectively. The percentage absorption of nitrogen, energy, and fat was calculated as [(oral intake − intestinal output)/oral intake] × 100.

Endoscopic gut-mucosal biopsy procedures

After completion of the nutrient absorption studies, gut-mucosal biopsy specimens were obtained from the SBS patients on the morning of day 8. The specimens were obtained from the distal 10 cm of residual small intestine and from the midportion of residual colon, if the colon was present. Biopsy specimens were also obtained from a colonic mucous fistula in patient 3.

### Table 1

<table>
<thead>
<tr>
<th>Patient and sex</th>
<th>Percentage of ideal body weight</th>
<th>Cause of short-bowel syndrome</th>
<th>Estimated length of residual intestine</th>
<th>Duration of PN before study</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, M</td>
<td>32</td>
<td>108</td>
<td>Blunt trauma/bowel infarction</td>
<td>70 cm jejunum + left colon</td>
</tr>
<tr>
<td>2, M</td>
<td>36</td>
<td>115</td>
<td>Gunshot wound/bowel infarction</td>
<td>50 cm jejunum + left colon</td>
</tr>
<tr>
<td>3, F</td>
<td>55</td>
<td>115</td>
<td>Multiple resections for postoperative complications (fistulas)</td>
<td>105 cm jejunum</td>
</tr>
<tr>
<td>4, F</td>
<td>51</td>
<td>105</td>
<td>Multiple resections for Crohn disease</td>
<td>350 cm jejunum-ileum</td>
</tr>
<tr>
<td>5, F</td>
<td>35</td>
<td>142</td>
<td>Multiple resections for Crohn disease</td>
<td>180 cm jejunum-ileum + left colon</td>
</tr>
<tr>
<td>6, F</td>
<td>57</td>
<td>110</td>
<td>Multiple resections for postoperative complications (fistulas)</td>
<td>75 cm jejunum-ileum + intact colon</td>
</tr>
<tr>
<td>7, F</td>
<td>37</td>
<td>116</td>
<td>Multiple resections for postoperative complications (fistulas)</td>
<td>35 cm jejunum + left colon</td>
</tr>
</tbody>
</table>

1PN, parenteral nutrition.
(Table 1). In the control subjects, gut-mucosal biopsy specimens were obtained from either the second portion of the duodenum (in those undergoing upper endoscopy) or from the distal 10 cm of ileum or midtransverse colon (in those undergoing colonoscopy). For ethical reasons, the Human Investigations Committee of Emory University allowed no more than 4 total gut-mucosal biopsy specimens to be obtained from the control subjects. In some cases, only PepT1 analysis or histologic assessment was performed in the control subjects, depending on tissue availability. However, mucosal endpoints were compared between age- and sex-matched control and SBS groups. A single gastroenterologist (LML) obtained the intestinal biopsy specimens from all subjects.

Gut-mucosal morphology

Small-bowel crypt depth, villus height, villus width at the base, and colonic crypt depth were determined as mucosal growth indexes. The morphology of the small-bowel mucosa was assessed in 13 SBS patients and 7 control subjects and that of the colonic mucosa in 9 SBS patients and 13 control subjects.

The biopsy specimens were fixed in 4% paraformaldehyde and 0.1 mol Na2PO4 and embedded in paraffin. Sections (5 μm) were mounted on SuperFrost Plus slides (Fisher Scientific, Pittsburgh) and stained with hematoxylin and eosin. A total of 10–20 sagittally well-oriented small-bowel crypt villus units or colonic crypts in each section were quantitated by gastrointestinal pathologists, who were blinded to patient diagnosis, using an Olympus BH-2 light microscope (Global Medical Instrumentation Inc, Clearwater, MN) equipped with a calibrated ocular micrometer. All morphology data were analyzed by a senior gastrointestinal pathologist (RRP) with the assistance of gastrointestinal pathology fellows (TMW, EED, and KER). Both intra- and interobserver variability in the measurement of the morphologic indexes were <10%.

Northern blot analysis to determine PepT1 mRNA expression

Gut-mucosal biopsy specimens for analysis of PepT1 mRNA expression were rapidly frozen in liquid nitrogen and stored at −70°C. Total RNA was isolated with use of the method of Chomczynski and Sacchi (30). The final RNA concentration was measured spectrophotometrically at 260 nm, and the integrity was confirmed by analysis of ethidium bromide–stained 28S and 18S ribosomal RNA bands in 1% agarose gels.

For the Northern blot analysis, denatured RNA (25 μg) from individual tissues was resolved by electrophoresis in agarose-formaldehyde gels, transferred to nylon filters by overnight capillary blotting, and fixed by ultraviolet light. Filters were prehybridized for 4–5 h at 42°C in a solution containing 5X sodium chloride: sodium citrate, 50% (wt:vol) deionized formamide, 5X Denhardt’s solution, 1% sodium dodecyl sulfate, and 10% dextran sulfate. Hybridization was performed with the use of specific human cDNA probes. The human PepT1 cDNA was a 1200–base pair (bp) EcoRI fragment of the full-length 2.2-kilobase (kb) human PepT1 cDNA subcloned into the plasmid vector pBluescript SK(−) II (11). Human glyceraldehyde-3-phosphate dehydrogenase (GapDH) and human β-actin cDNAs were 1.2-kb and 1.9-kb fragments, respectively, and were purified from commercially purchased plasmids (American Type Culture Collection, Rockville, MD).

cDNAs were labeled with [32P]CTP by random priming (Prime-It II Random Primer Labeling Kit; Stratagen, La Jolla, CA) and purified on a resin column (MicroSpinG-25 Column; Pharmacia, Kalamazoo, MI). The filters were hybridized for 16–18 h at 42°C in hybridization solution (prehybridization solution containing salmon sperm DNA and labeled probe). After hybridization, the filters were washed and autoradiographed with XOMAT film (Kodak Inc, Rochester, NY) with intensifying screens. To verify equal RNA loading and to determine the specificity of PepT1 mRNA expression, all filters were stripped and sequentially rehybridized with human β-actin and GapDH cDNA probes. PepT1, β-actin, and GapDH mRNA transcripts were quantitated by laser densitometry.

In situ hybridization

Riboprobes were prepared by the method of Melton et al (31) with use of [35S]UTP (44.4 TBq/mmol; Amersham, Arlington Heights, IL) (31). In situ hybridization was performed essentially as described by Wilcox (32). The full-length 2.2-kb human PepT1 cDNA was linearized by SauI for transcription of antisense and NorI for transcription of sense (negative control) riboprobes, respectively. [35S]UTP was dried under vacuum, and transcription was begun by sequential addition of transcription buffer (Promega, Madison, WI), dithiothreitol, RNAse inhibitor, linearized plasmid DNA, and a nucleotide stock solution containing ATP, CTP, GTP, and sterile water. T3 and T7 DNA-directed RNA polymerases were added to direct the synthesis of antisense (T3) and sense (T7) PepT1 riboprobes, respectively (31). After transcription, DNase was added to degrade the DNA template and the labeled riboprobes were purified on a G-50 Sephadex column (Quick Spin Column; Boehringer Mannheim, Indianapolis). Because shorter riboprobes (<300 bp) give superior results in our experience, the antisense and sense riboprobes were digested by alkaline hydrolysis to ~200 bp and stored at −70°C as previously described (32).

Tissue sections were deparaffinized, washed, and permeabilized with protease K (Sigma, St Louis). Tissues were covered with 100 μL prehybridization solution containing dithiothreitol, sodium chloride, tris buffer, EDTA, Denhardt’s solution, dextran sulfate, and formamide, and then were incubated for 2 h at 42°C.

The hybridization solution was prepared by heating 6 × 105 cpmp [35S]-labeled riboprobe with transfer RNA at 95°C and then adding 17 μL prehybridization solution to it. The hybridization solution (20 μL) was mixed in the prehybridization solution bubble covering the tissue and the slides were hybridized overnight at 55°C.

After hybridization, the sections were washed and immersed in a solution containing RNase A to degrade single-stranded RNA (32). The sections were then washed, dehydrated in graded alcohols, dried under vacuum, coated with Kodak NTB2 nuclear track emulsion (International Biotechnologies Inc, New Haven, CT), and exposed in the dark at 4°C for 6–8 wk (small-bowel sections) or for 10–12 wk (colonic sections). The sections were developed with the use of Kodak D19 and Kodak Fixer, counterstained with hematoxylin and eosin, and photographed with a polarized light epiluminescence system (32).

Generation of polyclonal antibody to human PepT1

A polyclonal antibody was generated against the synthetic peptide NRLEKSNYFMSGANSEQQN, corresponding to residues 689–708 of the cytoplasmic carboxyl terminus of the predicted human PepT1 sequence (11). The anti-PepT1 antibody was raised in rabbits immunized with the peptide coupled to keyhole
PepT1 mRNA expression to compare expression in different
for
used for the analyses. The final mRNA results were controlled
(STATVIEW 512+; Abacus Concepts, Inc, Berkeley, CA) was
patients with an unpaired
differences. Fisher’s exact test was used to compare data
between the 6 SBS patients with residual left colon and the 3 SBS patients with intact colon (103 ± 13 compared with 86 ± 16 μm, respectively).

**PepT1 mRNA expression in human intestinal mucosa**

The 3 groups of 5 control subjects in whom the gradient of PepT1 mRNA expression in the intestine was determined were
trol and SBS patients. Data are presented as means ± SEMs, and
P values < 0.05 were considered statistically significant.

**RESULTS**

**Nutrient absorption in SBS patients**

Parenteral nutrition was administered 5–7 d/wk for an average of 2.5 ± 1.1 y in the 7 SBS patients undergoing the nutrient
absorption studies (Table 1). The daily oral energy intake of the
SBS patients averaged 9831 ± 1367 kJ (2352 ± 327 kcal), and the
daily oral protein intake averaged 99 ± 19 g. The weekly parenteral fluid intake of the SBS patients was 12 ± 2 L, and the intravenous feedings provided an average of 4548 ± 986 kJ/d
(1088 ± 236 kcal/d) and 73 ± 15 g protein/d.

The SBS patients had moderately severe diarrhea (stool weight: 2664 ± 547 g/d), which was associated with the malabsorption of dietary nitrogen, energy, and fat (Table 2). Marked
malabsorption of dietary nitrogen was confirmed (53 ± 7% of
oral intake absorbed; range: 29–76% absorption); normal
absorption in adults is known to exceed 90–95% of dietary prote
intake (1).

**Small-bowel and colonic mucosal histology and growth indexes**

The intestinal mucosa used for mRNA analysis was histologi
cally normal in all control subjects and SBS patients, except for
SBS patients 4 and 5, who had minor nonspecific histologic
changes in residual ileal mucosa compatible with inactive,
chronic Crohn disease. Two of the control subjects from whom ileal mucosal biopsy specimens were obtained had localized ade
nocarcinoma in a distal colonic polyp and in the rectal mucosa,
respectively, and 2 other control subjects had benign distal
colonic polyps. Two other control subjects from whom colonic mucosal biopsy specimens were obtained had benign rectal
polyps and one had a benign distal colonic tubular adenoma.

Small-bowel crypt depth, villus height, and villus width and
colonic crypt depth were not significantly different between the
control subjects and the SBS patients (Table 3). Colonic crypt
depth was not significantly different between the 6 SBS patients
with residual left colon and the 3 SBS patients with intact colon
(103 ± 13 compared with 86 ± 16 μm, respectively).

**TABLE 2**

Nitrogen absorption in the intestine of patients with short-bowel syndrome

<table>
<thead>
<tr>
<th>Patient</th>
<th>Nitrogen</th>
<th>Energy</th>
<th>Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>51</td>
<td>75</td>
<td>61</td>
</tr>
<tr>
<td>2</td>
<td>38</td>
<td>47</td>
<td>13</td>
</tr>
<tr>
<td>3</td>
<td>44</td>
<td>43</td>
<td>31</td>
</tr>
<tr>
<td>4</td>
<td>58</td>
<td>58</td>
<td>40</td>
</tr>
<tr>
<td>5</td>
<td>76</td>
<td>92</td>
<td>88</td>
</tr>
<tr>
<td>6</td>
<td>72</td>
<td>75</td>
<td>65</td>
</tr>
<tr>
<td>7</td>
<td>29</td>
<td>61</td>
<td>52</td>
</tr>
</tbody>
</table>

Mean ± SEM; n = 5 in brackets. There were no significant differences between groups.

**TABLE 3**

Growth indexes of the gut mucosa in control subjects with no history of bowel resection and in patients with short-bowel syndrome

<table>
<thead>
<tr>
<th>Growth index</th>
<th>Control subjects</th>
<th>Patients with short-bowel syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Villus width</td>
<td>133 ± 8 [7]</td>
<td>152 ± 10 [13]</td>
</tr>
</tbody>
</table>

Mean ± SEM; n in brackets. There were no significant differences between groups.

Immunohistochemistry for cellular localization of the PepT1 protein in the gut-mucosal sections was performed with the anti
human PepT1 antibody. This confirmed that the antibody detected a protein with an apparent molecular mass of
80–85 kDa, similar to the predicted molecular mass of 78 kDa of
the human PepT1 cDNA (data not shown) (11).

Immunohistochemistry

Immunohistochemistry for cellular localization of the PepT1 protein in the gut-mucosal sections was performed with the anti
human PepT1 antibody. An avidin-biotinylated enzyme complex kit (DAKO LSAB2) and an automated autostainer system, both
from DAKO Corp (Carpintera, CA), were used as described by
the manufacturer. The gut-mucosal sections were deparaffinized and rehydrated, and antigen retrieval was accomplished in citrate buffer in a pressure cooker at 120°C. Cooled sections were
exposed to 0.3% hydrogen peroxide, primary antibody (1:400, by vol), biotinylated secondary linking antibody (rabbit anti
immunoglobulin G), and strepavidin-conjugated horseradish
peroxidase. 3,3-Diaminobenzidine tetrahydrochloride was used
as the chromogen, and sections were counterstained with hema
toxulin. All studies incorporated negative controls for each tissue
section in which the primary antibody was replaced by buffer.
Additional negative control studies were performed in which
secondary antibodies were replaced by buffer and showed no
positive staining (data not shown).

**Statistics**

PepT1 mRNA expression in the duodenum, ileum, and colon of control subjects was compared with use of analysis of
variance. The post hoc Fisher’s protected least-significant-difference test with Bonferroni correction was used to analyze intergroup differences. Fisher’s exact test was used to compare data
between the sexes. Gut-mucosal morphology and PepT1 mRNA
expression were compared between control subjects and SBS
patients with an unpaired t test. A standard statistical package
(STATVIEW 512+; Abacus Concepts, Inc, Berkeley, CA) was
used for the analyses. The final mRNA results were controlled
for β-actin mRNA expression and expressed as the ratio of
PepT1 to β-actin in arbitrary densitometry units. Data from the
Northern blotting experiments were normalized to duodenal
PepT1 mRNA expression to compare expression in different
intestinal segments or to compare mean values between the con

Additional negative control studies were performed in which
secondary antibodies were replaced by buffer and showed no
positive staining (data not shown).
not significantly different with regard to age or sex (data not shown). The total RNA yield was 2–3 μg total RNA/mg tissue (=10–15 μg RNA per 5-mg biopsy specimen). The 3.3-kb PepT1 mRNA transcript was abundant in normal duodenal and ileal mucosa and, unexpectedly, was also faintly detectable in normal colonic mucosa (Figure 1). PepT1 mRNA expression in the ileal and duodenal mucosa was not significantly different. Mucosal PepT1 mRNA expression in the small bowel compared with the colon was significantly different.

**Cellular localization of PepT1 mRNA in intestinal mucosa**

In situ hybridization showed that PepT1 mRNA was localized to human villus epithelial cells in the duodenum (Figure 2, A and B) and the ileum (Figure 2, C–E). In the duodenum, increased labeling was observed toward the villus tip relative to the villus base (arrow in panel A). In the ileum, an abundant and relatively even distribution of PepT1 mRNA was observed in epithelial cells extending from the crypt-villus junction (arrow in panel E) to the villus tip. Small-intestinal goblet cells were not labeled.

In the colonic mucosa, low-level labeling of PepT1 mRNA above background labeling was observed in the absorptive colonocytes, but not in the goblet cells (Figure 3). In situ hybridization performed in 4–5 sets of age- and sex-matched control subjects

---

**FIGURE 1.** Steady state expression of PepT1 messenger RNA (mRNA) in the duodenal (Duo; n = 5), ileal (Ile; n = 5), and colonic (Col; n = 5) mucosa of adult control subjects with normal bowel mucosa and no history of intestinal resection. Expression is shown by Northern blotting (left panel) and as a percentage of the expression in the duodenum corrected for β-actin mRNA expression (right panel). *Significantly different from Col: *P = 0.008, **P = 0.001.

**FIGURE 2.** PepT1 messenger RNA (mRNA) expression in the duodenal and ileal villus epithelial cells by in situ hybridization in adult control subjects with normal bowel mucosa and no history of intestinal resection. A: Specific localization of PepT1 to duodenal villus epithelial cells; PepT1 expression was higher toward the duodenal villus tip than at the villus base (arrow). B: Nonspecific background labeling in duodenum with the PepT1 sense riboprobe. C: Antisense in situ hybridization of PepT1 mRNA expression in the ileum, specifically localized to villus epithelial cells in the mid and upper villus; PepT1 mRNA expression was abundant and relatively evenly distributed along the entire villus epithelia and was not present in goblet cells. D: Minimal nonspecific labeling in the ileum with the sense control probe. E: PepT1 mRNA expression along the epithelial cells of the lower villus to the ileal villus-crypt junction (arrow). In situ hybridization performed in 4–5 sets of age- and sex-matched control subjects and SBS patients and indicated no significant between-group difference in the cellular localization of PepT1 mRNA.
and SBS patients indicated no significant difference in cellular localization of PepT1 mRNA in the small bowel or colon.

**Intestinal PepT1 mRNA expression in SBS patients and control subjects**

PepT1 mRNA expression in the distal residual small bowel of the SBS patients (72 ± 14% of the control value) was not significantly different from that in the distal ileum of 9 control subjects (100 ± 15%) (Figure 4). No significant difference in the steady state expression of \( \beta \)-actin and GapDH mRNA was observed between the 2 groups: \( \beta \)-actin, 100 ± 22% in the control subjects and 95 ± 10% in the SBS patients; GapDH, 100 ± 13% in the control subjects and 159 ± 45% in the SBS patients).

In contrast with observations in the small bowel, PepT1 mRNA expression in the colonic mucosa of the SBS patients (512 ± 127%) was more than 5-fold that in the control subjects (100 ± 23%) (Figure 5). The colonic expression of \( \beta \)-actin and GapDH mRNA was not significantly different between the control subjects and the SBS patients: \( \beta \)-actin, 100 ± 7% in the control subjects and 148 ± 46% in the SBS patients; GapDH, 100 ± 13% in the control subjects and 159 ± 45% in the SBS patients). These findings indicate that the up-regulation of colonic PepT1 in the SBS patients was mRNA specific. PepT1 mRNA expression was low in the colonic mucosa of the mucous fistula of patient 3.

**PepT1 protein expression in intestinal mucosa**

We detected PepT1 protein in both the small-bowel and colonic mucosa by immunostaining with a specific rabbit anti-human PepT1 antibody. In ileal sections of control and SBS patients, abundant PepT1 protein expression was detected along the apical brush border of villus epithelial cells, from the crypt-villus junction to the villus tip (Figure 6). The cellular localization of PepT1 protein in the duodenal mucosa of the control subjects and in the jejunal mucosa of the SBS patients was not significantly different (data not shown). Consistent with the in situ hybridization data, PepT1 protein was not present in small-intestinal goblet cells. Qualitatively, there was no apparent difference in the abundance or cellular localization of PepT1 protein in the distal small-bowel mucosa between the SBS patient (Figure 6, A) and the unresected control subjects (Figure 6, B). Studies performed in the 4–5 sets of age- and sex-matched control subjects and SBS patients indicated no significant difference in cellular localization of PepT1 protein in the small bowel.

In the colon, PepT1 protein was localized to absorptive colonocytes along the entire colonic crypt extending onto the luminal surface in the control subjects and in the SBS patients (Figure 7). PepT1 was not detected in colonic goblet cells. The qualitative abundance of PepT1 protein expression was higher in

---

**Figure 3.** PepT1 messenger RNA (mRNA) expression in colonocytes by in situ hybridization in the colonic mucosa from an adult with short-bowel syndrome (SBS; patient 1) with the use of antisense (A) and sense (B) probes. The sense probe showed only nonspecific labeling. In situ hybridization was also performed in 4–5 sets of age- and sex-matched control subjects and SBS patients and indicated no significant between-group difference in the cellular localization of PepT1 mRNA. Magnification is 100 X.

**Figure 4.** PepT1 messenger RNA (mRNA) expression in the distal residual jejunal and ileal mucosa of adults with short-bowel syndrome (SBS; patients 3 and 4) and in the distal ileum of 2 adult control subjects (C) with normal bowel mucosa and no history of intestinal resection (C) with normal bowel mucosa and no history of intestinal resection. Expression is shown by Northern blotting (left panel) and as a percentage of control values in 7 SBS patients and 9 age- and sex-matched control subjects, corrected for \( \beta \)-actin mRNA expression (right panel). PepT1 mRNA was abundant in the small-bowel mucosa but was not significantly different between the control subjects and SBS patients.

**Figure 5.** PepT1 messenger RNA (mRNA) expression in the mid-residual colon of adults with short-bowel syndrome (SBS; patients 1, 2 and 6) and in the mid-transverse colon of 2 control subjects (C) with normal bowel mucosa and no history of intestinal resection by Northern blotting (left panel). Quantitative expression in the colonic mucosa of 5 SBS patients and 12 age- and sex-matched unresected control patients as a percentage of the control value corrected for \( \beta \)-actin mRNA expression is shown (right panel). *Significantly different from C, \( P < 0.01 \).
SBS patients (Figure 7, A) than in the control subjects (Figure 7, B). This finding is consistent with the results of the quantitative Northern blotting experiments performed in the age- and sex-matched control subjects and SBS patients (Figure 5). The small-bowel and colonic tissues incubated without primary or secondary antibodies were negative for PepT1, indicating the specificity of the immunostaining (Figure 6, C; Figure 7, C).

DISCUSSION

After massive small-intestinal resection in humans, an adaptation process manifested clinically by decreased diarrhea and apparently improved nutrient absorption occurs over time. The underlying mechanisms involved in this process have not been elucidated. In the current study, we found no evidence that adaptation to extensive loss of intestinal surface area in humans with SBS involves hyperplasia of the small-bowel or colonic mucosa. We showed that expression of PepT1, the H⁺-dependent mechanism for transport of di- and tripeptides in the gut, is up-regulated in the colonic mucosa of SBS patients. To our knowledge, this is the first report on nutrient-transporter expression in humans with SBS and the first to indicate that up-regulation of gut epithelial nutrient transporters is a potential mechanism of human gut adaptation. The current study was limited, however, by its small sample size and heterogeneity of the SBS patients.

Our data that showed no significant differences in the growth indexes of the gut mucosa between the SBS patients and the control subjects were surprising in light of the often marked increase in gut-mucosal growth observed in animal models of SBS (18, 19). The current study is the first to our knowledge to compare colonic crypt depth between SBS patients and unresected control subjects. However, our small-bowel data are consistent with data from the few studies of changes in human gut mucosa after massive bowel resection. In 1965 Porus (23) reported that histologic growth indexes of the mucosa of the proximal small bowel are not significantly different between control subjects and age- and sex-matched SBS patients with <75% of the small bowel resected. In that study, the number of villus epithelial cells increased modestly and only in

FIGURE 6. PepT1 protein expression in the ileal mucosa of an adult with short-bowel syndrome (patient 5; A) and in a control subject with normal bowel mucosa and no history of intestinal resection after staining with (A and B) or without (C) PepT1 antibody. Note the heavy expression of PepT1 protein along the brush border of villus epithelial cells (thick arrows) in A and B and no staining in the goblet cells (thin arrow; B).

FIGURE 7. PepT1 protein expression in the colonic mucosa of an adult with short-bowel syndrome (patient 1; A) and in a control subject with normal bowel mucosa and no history of intestinal resection after staining with (A and B) or without (C) PepT1 antibody. Note that PepT1 is localized in the absorptive colonocytes located between the goblet cells along the colonic crypt (white arrow, B). In the SBS patient, PepT1 expression appears to be more abundant than in the control subjects along the absorptive cells of the villus crypt and on the luminal surface (arrow; A).
patients who had >75% of their small bowel resected. O’Keefe et al (24) showed that crypt depth, villus height, and villus width in the duodenum of SBS patients with jejuno-stomies and no residual colon were similar to those in healthy control subjects. Larger sample sizes at different time points after bowel resection and assessment of microvillus length are indicated to further evaluate possible changes in gut-mucosal growth in humans with SBS.

The in situ hybridization and immunohistochemistry studies showed that PepT1 mRNA and protein expression in small bowel was abundant and localized exclusively to the apical border of human duodenal, jejunal, and ileal villus epithelium. This pattern of expression is similar to that observed by Walker et al (33) in the duodenum and ileum of healthy humans and in the intact small bowel of rabbits and rats (12, 13, 34). These data are consistent with the high capacity for di- and tripeptide absorption in human small bowel shown by luminal peptide perfusion studies (1–7).

Diets high in protein or dipeptides up-regulate the expression of small-bowel PepT1 and PepT1-mediated brush border dipeptide transport in intact rodents (10, 35, 36). Previous studies in animal models of SBS indicate that certain nutrient-digestive and nutrient-transport functions in the small bowel may be up-regulated, independent of cellular hyperplasia (16, 17). Therefore, we hypothesized that the residual small-bowel expression of PepT1 in our SBS patients would increase as an adaptive response to small-bowel resection or to chronic malabsorption of high-protein diets. In contrast, we found that PepT1 mRNA expression in the distal small-bowel mucosa of the SBS patients was quantitatively not significantly different from that in the distal ileum of the unresected control subjects. We recently found that proximal jejunal PepT1 mRNA expression was significantly lower in rabbits 2 wk after 70% resection of the mid-small bowel than in controls, whereas values in the distal ileum were not significantly different (37). Studies to determine the time course of PepT1 expression and the functional activity of PepT1 in human intestine after intestinal resection are needed.

In 1939 Rhoads et al (29) showed that casein or peptone hydrolysates, which probably contain di- and tripeptides, could be absorbed from isolated colonic loops in dogs to a greater extent than the absorption of free amino acids. In the current study we showed that PepT1 mRNA and protein are expressed in human colonic epithelia, indicating for the first time that humans have a mechanism for di- and tripeptide transport across the large intestine (38). PepT1 mRNA was also detected in the proximal colon of rabbits (13). In addition, expression of PepT1 protein was recently reported in the colonic epithelial cells of patients with inflammatory bowel disease (39).

It is unknown whether human colonocytes adaptively develop new absorptive functions not characteristic of that site. In our study, the SBS patients showed up-regulated colonic expression of PepT1, a nutrient-transporter system classically associated with the function of the small intestine. Given our patients’ dietary protein intakes and degree of nitrogen malabsorption, increased colonic exposure to diet-derived small peptides may have contributed to the increase in PepT1. We found no significant differences in the steady state expression of β-actin or GapGH mRNA between unresected control subjects and SBS patients. This finding suggests that changes in colonic PepT1 mRNA were not due to a generalized postresection increase in mRNA species. Thus, evaluation of additional gut-mucosal nutrient-transporter and nutrient-exchanger mRNAs and of the genes involved in cell proliferation and apoptosis in human SBS would be of interest. We also detected a low expression of PepT1 mRNA in the colonic mucous fistula of patient 3. This finding suggests that the basal expression of PepT1 mRNA in the colon of SBS patients is not completely dependent on exposure to luminal diet-derived peptides. Additional studies are needed to define specific transcriptional regulation of the human PepT1 gene by nutrients and systemic factors.

The low abundance of colonic PepT1 mRNA observed in the current study suggests that, under normal circumstances, only minor transport of PepT1 solutes occurs in humans. However, our observation that the abundance of PepT1 mRNA in the colon of SBS patients was more than 5-fold that in control subjects could be of nutritional importance over time in SBS patients. It will be important to confirm the functional activity of colonic PepT1 transporters and whether gut-mucosal PepT1 is up-regulated in other types of malabsorptive disorders or is specific to SBS.

PepT1 may play a role in inflammatory processes. Merlin et al (40) showed that PepT1 transports the bacterial-derived tripeptide n-formyl-Met-Leu-Phe (fMLP) into human colon-derived Caco-2 cells and that PepT1-mediated fMLP transport stimulated neutrophil transmigration across Caco-2 monolayers. In addition, PepT1 protein is up-regulated in the colonic epithelial cells of patients with chronic ulcerative colitis and Crohn disease (39). It is possible that exposure to luminal peptides derived from nondietary sources, such as bacterial-derived fMLP, may regulate PepT1 expression. This does not explain the up-regulated expression of colonic PepT1 in our SBS patients because none had histologic evidence of colonic inflammation. However, given the possible role of PepT1 in mediating gut-mucosal inflammatory events, one could hypothesize that up-regulated PepT1 expression in the colon may be deleterious in SBS patients who have bacterial overgrowth or inflammatory bowel disease.

In summary, our data suggest that adaptation to intestinal resection in humans does not necessarily involve mucosal proliferative responses, as has been observed in experimental animal models. We showed that PepT1 mRNA and proteins are abundant in human small-bowel mucosa but are expressed at low levels in colonic absorptive cells. Up-regulation of this oligopeptide transporter in the colon of SBS patients suggests that the human colon can increase the luminal transport of di- and tripeptides derived from the diet or other sources during intestinal failure. An elevated expression of colonic PepT1 is an example of gut adaptation in SBS patients, independent of changes in the mucosal surface area. Additional studies are needed to define the physiologic functions and regulation of PepT1 in humans with SBS and other gastrointestinal tract disorders.

We are grateful to FH Leibach, V Ganapathy, and Y-J Fei of the Medical College of Georgia, Augusta, for providing the human PepT1 cDNA and for helpful discussions; to the nurses of the Emory GCRC for the care of the SBS patients; and to Glynda Gerron and Joni Barlow (GCRC Core Laboratory) for nutrient analysis.

REFERENCES

