Role of luminal nutrients and endogenous GLP-2 in intestinal adaptation to mid-small bowel resection

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1Department of Nutritional Sciences, University of Wisconsin, Madison, 53706; 2Department of Pathology and Laboratory Medicine, University of Wisconsin Hospitals and Clinics, Madison, Wisconsin 53792; and 3Department of Medical Physiology, The Panum Institute, University of Copenhagen, DK-2200 Copenhagen N, Denmark

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Dahly, Elizabeth M., Melanie B. Gillingham, Ziwen Guo, Sangita G. Murali, David W. Nelson, Jens J. Holst, and Denise M. Ney. Role of luminal nutrients and endogenous GLP-2 in intestinal adaptation to mid-small bowel resection. Am J Physiol Gastrointest Liver Physiol 284: G670–G682, 2003.—To elucidate the role of luminal nutrients and glucagon-like peptide-2 (GLP-2) in intestinal adaptation, rats were subjected to 70% midjejunooileal resection or ileal transection and were maintained with total parenteral nutrition (TPN) or oral feeding. TPN rats showed small bowel mucosal hyperplasia at 8 h through 7 days after resection, demonstrating that exogenous luminal nutrients are not essential for resection-induced adaptation when residual ileum and colon are present. Increased enterocyte proliferation was a stronger determinant of resection-induced mucosal growth in orally fed animals, whereas decreased apoptosis showed a greater effect in TPN animals. Resection induced significant transient increases in plasma bioactive GLP-2 during TPN, whereas resection induced sustained increases in plasma GLP-2 during oral feeding. Resection-induced adaptive growth in TPN and orally fed rats was associated with a significant positive correlation between increases in plasma bioactive GLP-2 and proglucagon mRNA expression in the colon of TPN rats and ileum of orally fed rats. These data support a significant role for endogenous GLP-2 in the adaptive response to mid-small bowel resection in both TPN and orally fed rats.

Despite the multifactorial regulation of intestinal adaptation, the literature suggests that luminal nutrients are fundamental to the adaptive response such that no adaptive intestinal growth will occur in the absence of luminal nutrients (9, 10, 30). This conclusion is largely based on studies that show significant resection-induced adaptive growth in rats (10, 30) and dogs (9) fed orally compared with those fed parenterally. However, given the clear evidence that total parenteral nutrition (TPN) causes dramatic intestinal atrophy in rats (5, 7, 31, 32) and mucosal hypoplasia in humans (2), the interpretation of these reports (9, 10, 30) is limited by the absence of appropriate parenterally fed transection control groups. We have corrected for this in the present study.

The presence of ileum or colon is important in intestinal adaptation and appears to determine whether resection-induced adaptation occurs in humans (20, 28). In fact, the majority of individuals with resection of the ileum and colon resulting in an end-jejunooileostomy are dependent on permanent parenteral nutrition due to intestinal failure (28). The ileum and colon are the primary sites of synthesis and secretion of glucagon-like peptide-2 (GLP-2), a posttranslationally spliced product of the proglucagon gene that is produced in the L-cells of the intestine (8). The lack of a meal-stimulated increase in plasma bioactive GLP-2 levels in individuals without ileum and colon supports the notion that GLP-2 is produced from the distal bowel (20). Evidence that exogenous GLP-2 increases enterocyte proliferation and decreases intestinal apoptosis (4, 46) and stimulates adaptive growth after intestinal resection (39) establishes GLP-2 as one of the strongest candidate enteroendocrine hormones to date. The role of endogenous circulating bioactive GLP-2 in adaptation to bowel resection has not been extensively studied due to its rapid cleavage by dipeptidyl peptidase IV and the subsequent cross-reactivity of inactive GLP-2 species in radioimmunoassays that are not specific to the NH2 terminus of bioactive GLP-2 (8). There are, however,
two reports (25, 45) showing increases in endogenous plasma bioactive GLP-2 and concomitant resection-induced intestinal adaptation in orally fed rats, suggesting that GLP-2 is a plausible mediator of the adaptive response to resection in the presence of luminal nutrients.

There are gaps in our understanding of how luminal nutrients modulate intestinal adaptation to small bowel resection (SBR) and GLP-2 responses. Previous investigations (37, 42) of the role of luminal nutrients in stimulation of adaptation and proglucagon expression have utilized fasting or fasting-refeeding in rats subjected to SBR without determination of circulating levels of bioactive GLP-2. Rountree et al. (37) noted nutrient-independent increases in proglucagon mRNA at 12 h after SBR in rats fasted for the previous 36 h. We have chosen to use TPN, rather than fasting, because this allows investigation of the acute, as well as extended, effects of the absence of luminal nutrients on intestinal adaptation and GLP-2 responses without the negative consequences of starvation. Moreover, TPN provides a clinical context.

This study was conducted to further understanding of how luminal nutrients and the GLP-2 system regulate intestinal adaptation by using a rat model of massive mid-SBR, a 70% midjejunoileal resection. We hypothesized that intestinal adaptation would occur in the absence of luminal nutrients in mid-small bowel-resected rats due to the ability of the residual ileum or colon to produce GLP-2. Our objective was to determine the intestinal adaptive response to mid-SBR in the absence of luminal nutrients due to TPN and the association of endogenous GLP-2 responses with the hyperplastic intestinal growth observed in both parenterally and orally fed rats. We characterized structural adaptation in the entire residual small bowel, sucrase activity in the jejunum and ileum, enterocyte proliferation and apoptosis in the jejunum, proglucagon expression in the ileum and colon, as well as plasma bioactive GLP-2 in growing, parenterally or orally fed rats after mid-SBR or small bowel transection control surgery.

MATERIALS AND METHODS

Animals and Experimental Design

Two experiments, utilizing the same mid-SBR model, were conducted to assess the role of luminal nutrients and endogenous GLP-2 in intestinal adaptation to mid-SBR in rats. The University of Wisconsin–Madison Institutional Animal Care and Use Committee approved the animal facilities and protocols. Male Sprague-Dawley rats (Harlan, Madison, WI) initially weighing 200–225 g were housed in individual stainless steel, wire-bottom cages with free access to water in a room maintained at 22°C on a 12:12-h light-dark cycle. All animals were adapted to the facility for 3–4 days while being fed a semipurified, powdered diet ad libitum (7). Three days before surgery, animals were fed a semielemental, residue-free, liquid diet ad libitum to minimize intestinal contents at the time of operation (Vital; donated by Ross Labs, Columbus, OH).

Experiment 1: Comparison of Intestinal Adaptation and GLP-2 Response in Rats Nourished Exclusively with TPN or Oral Feeding for 7 Days After Mid-SBR

The ability of 70% midjejunooileal resection to induce intestinal adaptation in the absence or in the presence of luminal nutrients was assessed in two separate studies by using TPN or oral feeding. These two studies were conducted under similar conditions, as reflected by nonsignificant differences in overall body weight change between the transaction controls for each feeding method (12 ± 2 and 10 ± 3 g/7 d for parenteral and oral feeding, respectively). The TPN study included two parenterally fed treatment groups: gut resection (R) + TPN (n = 13) and gut transection (T) + TPN (n = 13). Gut transection is necessary in the control group because cutting and suturing of the bowel, as opposed to laparotomy alone, results in transient hyperplastic changes (49, 52). Fourteen animals were initially assigned to each TPN group; 7 days after surgery, 13 animals remained in each treatment group. Two animals were unable to complete the study due to loss of catheter patency. The oral feeding (ORAL) study served as a positive control for assessing bowel growth and included gut resection (R + ORAL; n = 10) and gut transection (T + ORAL; n = 6) groups. All orally fed animals completed the study.

Experiment 2: Early Time Course of Intestinal Adaptation and GLP-2 Response in Rats Nourished Exclusively with TPN After Mid-SBR

Experiment 2 was conducted to assess the early time course of 70% midjejunoileal resection to induce intestinal adaptation and GLP-2 responses in TPN animals. The experimental design was a time course study that included parenterally fed rats killed at 8, 12, 24, 48, or 72 h after gut resection or transection surgery. Four to five resection and four to five transection animals were included at each time point. The survival rate was 98%; one animal was unable to complete the study due to loss of catheter patency. A nonsurgical, age-matched group (0 h; n = 14) was killed after 3 days after liquid feeding (Vital) to provide a “baseline” for animals immediately before surgery. All baseline animals completed the study.

We conducted a preliminary study to assess plasma bioactive GLP-2 concentrations and intestinal adaptation in orally fed rats 72 h after resection or transection surgery. Results showed a significant increase in plasma bioactive GLP-2 (T + ORAL, 62 ± 4; R + ORAL, 112 ± 8 pmol/l, P = 0.0003) and confirmed the presence of small bowel mucosal growth (jejunal mucosal dry mass: T + ORAL, 6 ± 1; R + ORAL, 12 ± 1 mg/cm, P = 0.0001; ileal mucosal dry mass: T + ORAL, 7 ± 1; R + ORAL, 11 ± 1 mg/cm, P = 0.0010). Because proglucagon expression was noted to be elevated at 48 and 96 h after resection and oral feeding (37, 42), we did not pursue further time course studies in orally fed resected rats.

Surgical Procedures and Animal Care

On the day of surgery (day 0), animals were anesthetized by an intraperitoneal injection of 2.5 mg acepromazine (Fermenta Veterinary Products, Kansas City, MO), 75 mg ketamine (Phoenix Pharmacy, St. Joseph, MO), and 0.02 mg/kg body wt atropine (Phoenix Pharmacy) (TPN animals) or by inhalation of isoflurane (IsoFlo, Abbott Laboratories, North Chicago, IL) via an anesthesia machine (orally fed animals). All animals were given 200 mg/kg body wt ampicillin subcutaneously as a perioperative antibiotic. The abdominal, neck, and subscapular areas were clipped, cleansed with betadine...
surgical scrub and solution (Purdue Frederick, Norwalk, CT), and covered with a sterile drape. Resected animals underwent a 70% midjejunooileal resection similar to that used to study intestinal adaptation in orally fed rats (39). Resected animals had bowel removed (from 15 cm distal to the ligament of Treitz to 15 cm proximal to the cecum). The jejunum and ileum were measured by using a 15-cm length of sterile silk suture placed along the antimesenteric border of the bowel so that all resected animals had an equivalent amount of proximal jejunum (15 cm) and distal ileum (15 cm) remaining. Continuity between the residual 30 cm of jejunum and ileum was restored with an end-to-end jejunooileal anastomosis by using 6-0 silk suture. Five milliliters of sterile saline was placed into the peritoneal cavity for fluid resuscitation. Transected animals received a single cut in the ileum (15 cm proximal to the cecum) and suturing to reestablish continuity. The midline abdominal incision was closed by continuous suturing of the peritoneum and stapling of the skin. After closure of the abdomen in the parenterally fed animals, the TPN catheter was placed in the superior vena cava via the external jugular vein as previously described (24).

Immediately after surgery (day 0), infusion of TPN solution was initiated (TPN animals) or the semipurified diet was provided (orally fed animals). Water was provided ad libitum to all animals. For 24 h after surgery, all TPN animals received 0.03 mg oxymorphone HCl·kg body wt−1·h−1 iv for pain management concurrent with continuous infusion of TPN (12), and orally fed animals received 0.30 mg oxymorphone HCl/kg body wt sc every 4 h. For 48 h after surgery, prophylaxis antibiotic was continued with intravenously (TPN animals) or subcutaneously (orally fed animals) administration of 200 mg/kg ampicillin every 12 h. Body weights were recorded daily. In experiment 1, changes in body weight due to resection were calculated as the difference between body weights on the day animals were killed (day 7) and the preoperative body weights on the day of surgery (day 0). Resected rats lost ~5 g of intestinal tissue due to surgery.

Composition of TPN and Oral Diet

TPN animals were given a nutritionally complete TPN solution (7). The infusion rate of the TPN solution was gradually increased from 24 g on day 0 to 40 g on day 1 and 60 g on days 2–6, providing the sole source of nutrition until the end of the experiment. Infusion bags containing the TPN solution were weighed daily to calculate the amount of TPN solution infused. Average caloric intake over the 7 days was 205 kcal·kg−1·day−1. TPN solution contained (in g/l): 45 amino acids, 180 dextrose, and 28 lipid (142 ml Intralipid), providing 32% nonprotein energy from fat and 68% nonprotein energy from dextrose (7). Daily and cumulative energy intakes between resected and transected animals maintained with oral feedings were not significantly different.

Small Intestine Composition and Histology

After either exclusive TPN or oral feeding, rats were anesthetized by intravenous administration of 20 mg ketamine/kg body wt (TPN animals) or by inhalation of isoflurane (orally fed animals). The abdomen was opened, and the rats were killed by exsanguination from the portal vein (experiment 1) or heart (experiment 2). The remaining small bowel and colon were removed. The small intestine was sectioned into the duodenum (pylorus to ligament of Treitz), jejunum (the 15 cm distal to the ligament of Treitz, proximal 15 cm) and ileum (anastomosis to ileocec junction, terminal 15 cm). Tissue (2 cm) on either side of the anastomosis was discarded. Segments were flushed with ice-cold 0.9% saline and placed onto an ice-cold glass plate where tissue sections were cut into defined lengths. The first centimeter of each segment from the proximal end was fixed in HistoChoice (Amresco, Solon, OH) and transferred to 70% (vol/vol) ethanol before processing for histological analyses. A 2-cm section, derived from the third and fourth centimeter of each small bowel segment, was used to determine mucosal mass; there were no significant differences between groups in water content of the intestine. The following 3 cm in the small intestine segments were used for the analysis of mucosal protein (bicinchoninic acid protein assay; Pierce Chemical, Rockford, IL) and DNA (23) contents. Jejunal and ileal sucrase activity (6) was analyzed in the same segment as protein and DNA. Small intestine mucosa was used in the analyses for tissue weights, concentrations of protein and DNA, and sucrase activity. Mucosa was obtained by slitting the small bowel segments lengthwise and scraping the mucosa from the muscularis with a glass slide. We acknowledge the limitations of assessing some of our parameters of intestinal adaptation (e.g., mass, protein, and DNA contents, and sucrase activity) on the basis of 2 or 3 cm of bowel because it is clear that the adaptive response involves changes in all layers of the bowel wall (25).

Conventional light microscopy of hematoxylin and eosin-stained specimens was used to detect mitosis and apoptosis 7 days after resection or transection surgery in the TPN and oral groups (Experiment 1) as previously reported (7). This method of quantitating apoptosis, presently considered the “reference standard” by Potten (34), is extremely precise if representative morphological changes are observed (19) and was used by others (40) to draw conclusions about the effects of resection on the degree of apoptosis. This method was chosen to avoid the nonspecific staining associated with terminal deoxyuridine nick-end labeling (TUNEL) (22), which shows considerable variability in intestinal sections (54). Briefly, jejunal sections as used in the histomorphometric assessment were examined in a blinded fashion by an experienced human pathologist (by Z. Guo) on the basis of the characteristic findings of mitotic and apoptotic cells (7).

Data are presented two ways to account for the effects of resection to increase the total number of cells in the crypt and villus columns. First, data are presented as the mean number of apoptotic cells per crypt or villus column; this is calculated by dividing the total number of apoptotic cells in 50 well-oriented crypt or villus cell columns by 50 for each animal. The mean number of mitotic cells per crypt column
was calculated similarly. Second, data are presented as an apoptotic index. The apoptotic index in the crypt or villus compartments was quantified by counting the total number of apoptotic cells in the 50 well-oriented crypt or villus columns and expressing this as a percentage of the total number of cells in the 50 crypt or villus columns for each animal. To identify locations of apoptosis along the crypt-villus axis, we determined the apoptotic index in the lower [cell positions (cp), 1–10], middle (cp, 11–20), and upper (cp, 21–30) crypt and in the bottom (cp, 1–40) and top (cp, 41–80) of the villus. Cell position 1 is defined as the cell at the base of the crypt column and the cell at the crypt-villus junction for the crypt and villus data, respectively.

### RNA Extraction

Total RNA was isolated from intact jejunum, ileum, and colon using TRIzol reagent according to the manufacturer’s instructions (GIBCO-BRL, Gaithersburg, MD). RNA was quantified spectrophotometrically by measuring absorbance at 260 nm. RNA integrity was confirmed by ethidium bromide staining of 28S and 18S ribosomal RNA using agarose/formaldehyde electrophoresis. RNA was stored at −70°C until further analysis.

### Proglucagon mRNA

NorthernMax Kit (Ambion, Austin, TX) was used according to the manufacturer’s instructions for quantification of mRNA. Ten-micrometer aliquots of total RNA were fractionated on formaldehyde agarose gel containing ethidium bromide. The amount of 18S ribosomal RNA was measured by densitometry of Polaroid negatives of these gels before Northern blot analysis.

A cDNA for rat proglucagon (16) was kindly provided by Dr. P. K. Lund (University of North Carolina, Chapel Hill, NC). This 503-bp cDNA was subcloned into the SacI/BamHI site of pGEM4z (Promega, Madison, WI). The plasmid was linearized with NdeI and used to generate an antisense RNA probe using n-[32P]UTP and T7 RNA polymerase (Ambion MaxiScript).

Proglucagon mRNA bands were visualized by exposing phosphor screens (Packard Instruments, Meriden, CT) to the blots. Quantitation was performed using the OptiQuant software (Packard Instruments, Meriden, CT) to the phosphor screens (Packard Instruments, Meriden, CT) to identify locations of apoptosis along the crypt-villus axis, we divided the total number of apoptotic cells by the total number of cells at each position, and expressed this as a percentage of the total number of cells in the crypt column. The apoptotic index in the crypt or villus data, respectively.

### Plasma GLP-2-(1–33)

Blood was collected into chilled tubes containing a final concentration of 1 mg/ml EDTA (Fisher, Pittsburgh, PA) and 0.1 mM diprotin A (Calbiochem, San Diego, CA) and stored on ice. Blood was spun at 1,000 g × 15 min at 4°C. Plasma was collected and frozen at −70°C until the radioimmunoassay of GLP-2-(1–33) as previously reported (53). Briefly, an antibody specific to the NH₂ terminus of GLP-2 was used to measure intact bioactive GLP-2 produced exclusively by the intestine. Each study was assayed separately.

### Statistical Analysis

Treatment groups in experiment 1 were compared using two-way ANOVA that determined the significance of main effects and interaction between resection and feeding method (SAS Institute, Cary, NC). Individual differences between groups were determined by one-way ANOVA (P < 0.05) on the four treatment combinations followed by the protected least significant differences technique. Some caution needs to be used in interpreting conclusions between TPN and oral feeding, because the TPN and oral feeding studies were done as separate experiments and some of the effects apparently due to feeding method might actually be due to changes in experimental conditions over time, although there are no specific data to support this concern. Group means (transsection vs. resection) at each time point in experiment 2 were considered to be significantly different at P < 0.05, as determined by a two-tailed t-test in SAS. For the proglucagon data, the Wilcoxon signed-rank analysis in SAS was used to test for a significant difference from 1 (P < 0.05) (37), followed by assessing the correlation of proglucagon message and plasma GLP-2 using SAS. Statistics were performed on log-transformed data for mitotic index and apoptotic index in the villus, because residual plots indicated unequal variance between groups. Data are presented as means with standard errors.

### RESULTS

#### Experiment 1: Comparison of Intestinal Adaptation and GLP-2 Response in Rats Nourished Exclusively with TPN or Oral Feeding for 7 Days After Mid-SBR

#### Body weight

There were no significant differences in body weights between the TPN or orally fed resected groups compared with their respective transection controls from before surgery through the day after surgery. Despite the loss of ~5 g of intestinal tissue due to resection surgery, cumulative body weight gains in resected and transected animals were not significantly different after 7 days of exclusive TPN (Fig. 1). In contrast, the orally fed resected animals had diarrhea that was not clearly apparent in the TPN groups, and their cumulative body weight change after 7 days of...
Oral feeding was significantly reduced with resection compared with orally fed transection animals (Fig. 1). Net body weight gains among all groups suggest that the nutrition was sufficient to promote whole body anabolism.

Small intestine composition and histology. The absence of luminal nutrients due to TPN induced significant jejunal mucosal hypoplasia on the basis of significantly lower concentrations of jejunal mucosal protein and DNA in transected animals receiving TPN compared with orally fed transected rats (Fig. 2) similar to our previous observations (31, 32). The TPN mucosal hypoplasia is markedly apparent in the jejunal histology, because transected animals maintained with TPN had villi 25% shorter than orally fed transected rats (Fig. 3; Table 1). Thus the transected animals receiving TPN established a baseline for comparison of the potential hyperplastic effects of resection in the absence of luminal nutrients due to TPN.

Resection, in the absence of exogenous luminal nutrients, prevented or reversed the TPN mucosal hypoplasia such that jejunal mucosal protein content and villus height were not significantly different between resection animals given TPN and orally fed transected animals (Figs. 2 and 3; Table 1). In fact, resection significantly increased mucosal protein content and villus height in the duodenum, jejunum, and ileum; mucosal DNA content in the duodenum and jejunum; and mucosal dry mass in the jejunum during TPN compared with TPN transection controls (Figs. 2 and 3; Table 1). As expected with oral feeding, resection significantly increased mucosal dry mass, mucosal protein content, and villus height in the duodenum, jejunum, and ileum; mucosal DNA content in the duodenum and jejunum; and mucosal dry mass in the ileum during TPN compared with orally fed transected rats. On the basis of increases in mucosal protein and/or DNA contents, resection induced mucosal hyperplasia in the duodenum and jejunum and hypertrophy in the ileum of both TPN and orally fed rats.

Overall, the resection-induced adaptive increases in mucosal mass and concentrations of protein and DNA in the duodenum, jejunum, and ileum reached significantly greater absolute levels with oral feeding compared with TPN (Fig. 2). Thus resection induced significant small bowel adaptive hyperplasia in the absence of exogenous luminal nutrients, and the presence of luminal nutrients enhanced the adaptive response.

Enterocyte proliferation in the jejunum. Resection significantly increased the number of mitotic cells per crypt column by 35% in TPN rats and by 73% in orally fed rats (Table 2). The mitotic index, or the percentage

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**Fig. 2.** Mucosal dry mass (top), protein content (middle), and DNA content (bottom) in the duodenum (A), jejunum (B), and ileum (C) in rats maintained exclusively with TPN or ORAL for 7 days after T or R. Values are means ± SE; n = 6–13 animals per group. Means with different letters (a–c) are significantly different on the basis of one-way ANOVA and PLSD. NS, non-significant.

**Fig. 3.** Light micrographs of jejunum stained with hematoxylin and eosin in rats maintained exclusively with TPN or ORAL for 7 days after T or R.
of mitotic cells in the crypt, was significantly greater in resected compared with transected animals during oral feeding but not TPN. This is not surprising given that constant proliferative indices, in the presence of an increase in both total number of cells and number of labeled cells in the crypt column, are sometimes seen after resection in oral feeding (15, 27, 49). The resection-induced increase in proliferation in TPN rats was confirmed with bromodeoxyuridine (BrdU) incorporation into proliferating cells (13), because resection significantly increased the number of BrdU-labeled cells by 29% compared with transected controls (T + TPN, 7 ± 1; R + TPN, 9 ± 1 BrdU-labeled cells per crypt column, P = 0.007). Thus resection increased enterocyte proliferation in both TPN and oral feeding, but the response was significantly greater with oral feeding.

**Enterocyte apoptosis in the jejunum.** **AMOUNT.** As we previously reported, TPN significantly increases the apoptotic index in the crypt 3.5-fold and in the villus 13-fold compared with oral feeding (7). Here, we show similar significant ~8- and 12-fold increases in the apoptotic index in the crypt and villus, respectively, in parenterally compared with orally fed transected rats. Resection, however, significantly decreased the apoptotic index in the crypt by 67% in parenterally fed animals, despite the significantly elevated background of apoptosis due to TPN itself (Table 3). Resection also decreased the number of apoptotic cells in the villus column and the apoptotic index in the villus by ~50% in parenterally fed animals; however, these decreases were not significant (Table 4). In contrast to TPN, resection did not significantly affect the relatively low number of apoptotic cells per crypt or villus column in orally fed animals nor did it affect the apoptotic index in the crypt or villus (7). Thus resection decreased apoptosis during TPN but not oral feeding. The resection-induced reductions in apoptosis in the crypt and villus during TPN resulted in levels of apoptosis not significantly different from orally fed rats.

**LOCATION.** To ascertain where within the crypt the reductions in apoptosis due to resection during TPN occurred, we determined the apoptotic index in the lower (cp, 1–10), middle (cp, 11–20), and upper (cp, 21–30) thirds of the crypt (Table 3) and in the bottom (cp, 1–40) and top (cp, 41–80) halves of the villus (Table 4). Peak incidences of apoptosis typically occur in the base of the crypt near the stem cell zone, cp ~4 from the crypt base (33), consistent with the location of peak apoptosis in parenterally fed, transected animals (Table 3). Resection significantly decreased apoptosis almost 80% in the base of the crypt during TPN compared with transected animals maintained with TPN. Additionally, the resection-induced reduction in apoptosis in the villi occurred in the bottom half of the villus in TPN animals (Table 4).

**Sucrase activity.** Resection significantly increased sucrase segmental activity in the jejunum during TPN (Fig. 4), suggesting that the additional cellularity in the resected animals is functionally mature after 7 days of TPN and may thus facilitate weaning from parenteral to enteral nutrition. Resection also resulted in almost two and five times the sucrase segmental activity in the jejunum and ileum, respectively, during oral feeding (Fig. 4) in agreement with a previous report (39). The greater magnitude of increase in the ileum compared with the jejunum is consistent with the resection-induced loss of the aboral gradient as the ileum takes on functional characteristics of the jejunum in response to enteral nutrients. Resection did not significantly affect jejunal sucrase specific activity during TPN or oral feeding, but it did significantly increase ileal sucrase specific activity during oral feeding but not TPN (data not shown). Overall, resection resulted in significantly greater jejunal and ileal sucrase segmental activities during oral compared with parenteral feeding, consistent with induction of disaccharidase activities by the presence of luminal substrate.

### Table 1. Small bowel histology at 7 days following resection

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Duodenum</th>
<th>Jejunum</th>
<th>Ileum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Villus Height</td>
<td>Crypt Depth</td>
<td>Villus Height</td>
</tr>
<tr>
<td>T + TPN</td>
<td>390 ± 11&lt;sup&gt;c&lt;/sup&gt;</td>
<td>183 ± 9</td>
<td>275 ± 14&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>R + TPN</td>
<td>477 ± 16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>196 ± 9</td>
<td>384 ± 10&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>T + ORAL</td>
<td>460 ± 12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>186 ± 8</td>
<td>365 ± 9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>R + ORAL</td>
<td>521 ± 18&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>221 ± 15</td>
<td>428 ± 10&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are micrometers expressed as means ± SE; n = 6–13 animals per group. Means with different superscripts are significantly different based on one-way ANOVA and PLSD. Results of the two-way ANOVA indicate the following: resection showed significant main effects (P < 0.003) to increase duodenal, jejunal, and ileal villus height and jejunal crypt depth. Oral feeding compared with TPN showed significant main effects (P < 0.006) to increase duodenal, jejunal, and ileal villus height but not crypt depth. TPN, total parenteral nutrition; R, resection; T, transection; ORAL, oral feeding.

### Table 2. Enterocyte proliferation in the jejunal crypt at 7 days following resection

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cells Per Crypt Column, n</th>
<th>Mitotic Cells Per Crypt Column, n</th>
<th>Mitotic Index, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>T + TPN</td>
<td>26 ± 1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.65 ± 0.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.5 ± 0.2&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>R + TPN</td>
<td>29 ± 1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.85 ± 0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.0 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>T + ORAL</td>
<td>30 ± 1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.74 ± 0.07&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>2.5 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>R + ORAL</td>
<td>32 ± 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.28 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.0 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE; n = 5–6 animals per group. Means with different superscripts are significantly different based on one-way ANOVA and PLSD. Resection and oral feeding both showed significant main effects to increase the above 3 parameters of jejunal enterocyte proliferation (2-way ANOVA, P < 0.04–0.0001).
Table 3. Enterocyte apoptosis in the jejunal crypt at 7 days following resection

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Amount</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Apoptotic Cells Per Crypt Column, n</td>
<td>Apoptotic Index, %</td>
</tr>
<tr>
<td>T + TPN</td>
<td>0.22 ± 0.06a</td>
<td>0.85 ± 0.24a</td>
</tr>
<tr>
<td>R + TPN</td>
<td>0.08 ± 0.02b</td>
<td>0.28 ± 0.08b</td>
</tr>
<tr>
<td>T + ORAL</td>
<td>0.03 ± 0.01b</td>
<td>0.09 ± 0.03b</td>
</tr>
<tr>
<td>R + ORAL</td>
<td>0.04 ± 0.01b</td>
<td>0.12 ± 0.04b</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE; n = 5–6 animals per group. Means with different superscripts are significantly different based on one-way ANOVA and PLSD. Results of the two-way ANOVA indicate significant interaction (P < 0.05) for resection and method of feeding such that the amount and location of apoptosis in the jejunal crypt were reduced by resection in TPN but not ORAL rats.

GLP-2 system. Resection significantly doubled ileal proglucagon mRNA in orally fed animals but did not significantly affect proglucagon mRNA in parenterally fed animals (Fig. 5A). Colon proglucagon mRNA levels were not significantly different between resected and transected animals after 7 days of exclusive TPN or oral feeding (Fig. 5B). Resection significantly doubled plasma bioactive GLP-2(1–33) levels in orally fed animals but did not significantly affect plasma GLP-2 levels in TPN animals (Fig. 5C). Increases in plasma bioactive GLP-2 in orally fed animals were significantly correlated with proglucagon mRNA levels in the ileum (Fig. 5D) but not the colon.

Experiment 2: Early Time Course of Intestinal Adaptation and GLP-2 Response in Rats Nourished Exclusively with TPN After Mid-SBR

Small intestine composition and histology. Resection significantly increased mucosal wet mass, protein content, and/or DNA content in the jejunum at 8, 12, 24, 48, and 72 h and in the ileum at 12 and 48 h compared with transection controls (Fig. 6). No significant histological differences in villus height or crypt depth between resection and transection groups were observed at any time point from 8–72 h in the jejunum or ileum (data not shown). The lack of a consistent resection-induced increase in mucosal mass, protein, or DNA in the ileum at 8–72 h (Fig. 6) but a significant resection-induced increase in protein content and villus height at 7 days (Fig. 2; Table 1) may reflect the local, transient hyperplastic effects of transection alone (49, 52). We believe the ileum transection, which we and others (25, 37) have used, is a more rigorous test of the adaptive response to resection, because the ileum has the greatest potential of adapting to take on the appearance of the jejunum, as reported in orally fed animals (44). However, we refrain from making comparisons between individual small bowel segments regarding the relative effects of resection on adaptive growth, because the location of the transection cut influences the baseline for interpretation of the magnitude of the results. Thus on the basis of significant resection-induced increases in jejunal mucosal protein and DNA contents, resection induced jejunal mucosal hyperplasia. The significant resection-induced adaptive hyperplasia in the absence of exogenous luminal nutrients occurred as early as 8 h after SBR. This rapid response is consistent with the significant increase in ileal wet mass as early as 28 h after bowel resection (and 4 h after refeeding) in orally fed rats (37).

GLP-2 system. Resection significantly doubled plasma bioactive GLP-2(1–33) at 12, 24, and 48 h after massive mid-SBR in the absence of luminal nutrients (Fig. 7C). Furthermore, resection significantly doubled colonic proglucagon mRNA at the combined 12-, 24-, and 48-h time points (Fig. 7B), the same time points at which plasma GLP-2 levels were elevated. Resection did not significantly affect proglucagon mRNA levels in the jejunum (data not shown) or in the ileum (Fig. 7A) at any time point. Resection-induced increases in plasma bioactive GLP-2 at 12, 24, and 48 h in TPN animals were significantly correlated with proglucagon mRNA levels in the colon (Fig. 7D) but not the ileum.

Table 4. Enterocyte apoptosis in the jejunal villus at 7 days following resection

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Amount</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Apoptotic Cells Per Villus Column, n</td>
<td>Apoptotic Index, %</td>
</tr>
<tr>
<td>T + TPN</td>
<td>0.29 ± 0.09a</td>
<td>0.40 ± 0.14a</td>
</tr>
<tr>
<td>R + TPN</td>
<td>0.15 ± 0.07b</td>
<td>0.20 ± 0.10b</td>
</tr>
<tr>
<td>T + ORAL</td>
<td>0.03 ± 0.01b</td>
<td>0.03 ± 0.02b</td>
</tr>
<tr>
<td>R + ORAL</td>
<td>0.03 ± 0.01b</td>
<td>0.03 ± 0.01b</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE; n = 5–6 animals per group. Means with different superscripts are significantly different based on one-way ANOVA and PLSD. Results of two-way ANOVA indicate that resection did not show a significant main effect on apoptosis in the jejunal villus, and TPN compared with ORAL showed significant main effects (P < 0.007) to increase apoptosis in the jejunal villus. Although resection decreased villus apoptosis by 50% in TPN animals, these decreases were not significant.
DISCUSSION

Multiple factors, including luminal nutrients, pancreaticobiliary secretions, and humoral agents, such as GLP-2, have been implicated in controlling the intestinal adaptive response after bowel resection. Here, we demonstrate that mid-SBR induces structural adaptation in the entire residual small bowel and functional adaptation in the jejunum independent of exogenous luminal nutrients. This resection-induced hyperplasia during TPN was associated with stimulation of enterocyte proliferation and inhibition of apoptosis, whereas the resection-induced adaptation during oral feeding was associated with an increase in proliferation and no significant change in apoptosis. Additionally, we show for the first time that resection-induced adaptive growth in parenterally and orally fed rats was associated with a significant positive correlation between increases in plasma bioactive GLP-2 and proglucagon mRNA expression in the colon of TPN rats and ileum of orally fed rats. These data support a significant role for endogenous GLP-2 in the adaptive response to resection both in the absence and in the presence of exogenous luminal nutrients.

Several experimental approaches support our finding that luminal nutrients are not essential for resection-induced adaptive growth. For example in orally fed rats, intestinal resection in one rat produced intestinal hyperplasia in the vascular parabiosed partner (52), and ileal hyperplasia was seen in isolated Thiry-Vella fistulas after jejunectomy (51). Thus these studies suggest a role for a humoral factor that may have been stimulated by luminal nutrients in intestinal adaptation. Yet, even in the absence of exogenous luminal nutrition, animals maintained with TPN and subjected to pancreaticobiliary diversion (29), 85% small bowel bypass (41), or SBR (14, 38) had an adaptive response not seen in parenterally fed controls. Taken together, these studies suggest that a humoral factor that can be stimulated independently of exogenous luminal nutrients may play a role in resection-induced adaptive growth.

The resection-induced hyperplasia we observed in TPN resected rats compared with TPN transected controls was significant because resection completely prevented or reversed the TPN mucosal atrophy, similar to the intestinotrophic abilities of IGF-I or GLP-2 to prevent TPN atrophy (5, 32). Not surprisingly, luminal...
Fig. 6. Mucosal wet mass (top), protein content (middle), and DNA content (bottom) in the jejunum (A) and ileum (B) in rats maintained exclusively with TPN for 8, 12, 24, 48, or 72 h after T (○) or R (●). The 0-h time point represents animals immediately before surgery. Values are means ± SE; n = 4–5 animals per group per time point. *Significant difference (P < 0.05) between resected and transected animals at the same time point. #P = 0.058.

Fig. 7. Ratio of the abundance of proglucagon mRNA to 18S in the ileum (A) and colon (B) of R compared with T rats maintained exclusively with TPN for 8, 12, 24, 48, or 72 h after T or R. Insets show representative bands from Northern blot analysis of proglucagon mRNA. Plasma GLP-2 (1–33) concentration is shown in C. Correlation between colon proglucagon mRNA and plasma GLP-2 in TPN animals killed at 12, 24, and 48 h is shown in D. Values are means ± SE; n = 3–5 animals per group per time point. #Significant increase in proglucagon mRNA levels in the colon of TPN R compared with TPN T controls at the combined 12-, 24-, and 48-h time points. *Significant increase in plasma GLP-2 concentrations in R compared with T animals at the 12-, 24-, and 48-h time points.
nutrients heightened the adaptive response to resection as the resection-induced adaptive parameters reached greater absolute levels in orally fed compared with parenterally fed animals. However, the magnitude of the resection-induced increases in the various intestinal parameters compared with appropriate controls was not always greater with oral feeding compared with TPN. Moreover, it is difficult to determine whether orally fed animals reached maximal resection-induced adaptation, because additional intestinal growth occurs after GLP-2 or IGF-I administration in orally fed resected rats (39, 48). The greater amount of food intake needed to support body weight gain in the orally fed compared with parenterally fed animals does not appear to be a primary stimulus for the greater adaptive growth response, because significant intestinal adaptation occurs in intragastrically fed animals with net body weight loss over 7 days after resection (56).

Increases in enterocyte proliferation accompanied the resection-induced adaptation in both TPN and orally fed rats. We conclude that resection increased enterocyte proliferation during TPN on the basis of both a greater absolute number of mitotic cells and an increased total cell number in the crypt in the presence of a constant mitotic index (15, 27, 49). In the orally fed rats, resection increased enterocyte proliferation, similar to reports in orally fed rabbits (43) and mice (17, 18, 40). The greater relative increase in mitotic cells per crypt column with oral compared with parenteral feeding (73 vs. 35%, respectively) suggests that luminal nutrients are a strong stimulus for proliferation. Thus this heightened proliferation in the presence of luminal nutrients may be responsible for the enhanced adaptation in orally compared with parenterally fed rats.

Whereas increased proliferation seems to play a greater role in the resection-induced hyperplasia observed with oral feeding, decreases in apoptosis appear to play a greater role in the resection-induced hyperplasia noted with TPN, because resection decreased enterocyte apoptosis in TPN rats but did not significantly affect apoptosis during oral feeding. We have recently shown that nonresected rats maintained with TPN have a 3.5- and 13-fold increase in apoptosis in the crypt and villus, respectively, compared with orally fed animals (7). Thus during TPN, resection decreased the level of apoptosis compared with the heightened state of apoptosis in animals with intact bowel, similar to the ability of exogenous IGF-I to inhibit crypt cell apoptosis during TPN (7). The significant resection-induced inhibition of apoptosis during TPN compared with parenterally fed transection controls was observed in the crypt but not the villus, suggesting independent regulation of apoptosis in the two compartments. Even more crucial to establishing the hyperplastic state in the mucosa, resection suppressed apoptosis near the base of the crypt in the stem cell zone. Decreases in apoptosis in the crypt, especially near the base, should have more pronounced and lasting effects to increase enterocyte mucosal cellularity than in the villi due to the multiple cell divisions in the crypt, because a single crypt stem cell may give rise to 60–120 enterocytes (35, 36).

Contrary to our observations of no significant difference in apoptosis between orally fed resected and transected rats, resection significantly increased apoptosis in both the crypt and villus in orally fed rabbits (43) and mice (17, 18). This resection-induced increase in apoptosis was explained as part of the homeostatic process in which the new steady state of enhanced cell birth balances increased cell death (17). This discrepancy between increased apoptosis observed previously and our observation of no change in apoptosis must be interpreted cautiously for several reasons. First, we speculate that differences in methodology for detecting apoptosis may help explain the divergent results because the TUNEL method, used in previous reports (17, 18, 43), is associated with nonspecific staining (22, 54) and shows substantial variability in intestinal sections (54). Second, inherent differences in rates of apoptosis between species may account for the disparity. Third, it is possible that increased apoptosis in the ileum of rabbits and mice and no difference in the jejunum of rats after resection can be explained by differences in how the jejunum and ileum increase mass after resection (56). Lastly, the location of the bowel resected or the transection cut and/or the proximity of the histology segment to the resection or transection cut(s) may account for the variation because cutting and suturing of the bowel results in transient hyperplastic changes (49, 52).

Our data suggest that the ileum may be contributing to the resection-induced increase in plasma bioactive GLP-2 in orally fed rats on the basis of a significant positive correlation between plasma bioactive GLP-2 and ileum proglucagon mRNA. Rountree et al. (37), Taylor et al. (42), and Lund et al. (26) reported similar approximately twofold increases in ileal proglucagon mRNA ~1 wk after bowel resection in orally fed rats, which was likely attributable to an increase in the amount of transcript per L-cell instead of an increase in L-cell number in the ileum (11). However, prior reports have not correlated proglucagon message with circulating bioactive GLP-2 during intestinal adaptation (25, 37, 42, 45). Thus this is the first report to demonstrate significant resection-induced intestinal adaptive growth and concomitant increases in both plasma bioactive GLP-2 and ileal proglucagon mRNA. It has been suggested that the increased luminal contents that reach the distal bowel after resection are responsible for stimulating proglucagon expression and GLP-2 release (47).

Despite evidence that enteral feeding stimulates GLP-2 secretion (3, 8), we show for the first time that resection induced increases in plasma bioactive GLP-2 at 12, 24, and 48 h after resection in TPN rats. The transient, resection-induced increases in proglucagon mRNA we observed in the colon in the absence of luminal nutrients due to TPN are consistent with a previous report in which proglucagon mRNA was rapidly and transiently increased in the ileum at 12 h after bowel resection in the
absence of luminal nutrients due to a 36-h fast (37). The significant intestinal adaptive growth in TPN rats was associated with a significant positive correlation between plasma bioactive GLP-2 and colon proglucagon mRNA, suggesting the colon may be contributing to the resection-induced increase in plasma bioactive GLP-2 in parenterally fed rats. Colonic GLP-2 synthesis is plausible given previous observations in which ileal-resected rats (45) and humans (21) with colon in continuity have elevated plasma bioactive GLP-2 levels. Although it is unclear why the increases in proglucagon mRNA occurred in different bowel segments, i.e., ileum with oral feeding and colon with TPN, it is clear that there is a rapid increase in proglucagon expression after resection that is independent of luminal nutrients. This is further supported by the observation that peak GLP-2 levels and the greatest adaptive growth in orally fed animals occurred in the first week after resection when food intake was at a minimum (25), suggesting that luminal nutrients may not be the primary stimulus for GLP-2 secretion after resection. Herein, we show that parenteral nutrients alone are not sufficient to maintain the increases in proglucagon mRNA after resection. It appears that luminal nutrients are required for sustained increases in proglucagon mRNA (26, 37) and circulating bioactive GLP-2, because orally fed rats showed elevated plasma bioactive GLP-2 at both 72 h and 7 days after resection, whereas the early increases in GLP-2 peptide and proglucagon mRNA in TPN resected rats were transient.

The mechanism by which GLP-2 mediates its intestinotrophic effects is unknown. The GLP-2 receptor has been localized to enteroendocrine cells in humans (55) and enteric neurons in mice (1). However, the lack of identification of the receptor on multiple immortalized intestinal epithelial cell lines (55) indicates that the GLP-2 receptor is not on enterocytes. This suggests that GLP-2 does not directly stimulate gut growth but acts indirectly by stimulating the release of (an) unidentified downstream mediator(s) that, in turn, mediate(s) GLP-2’s actions (55). Thus the early transient increase in plasma bioactive GLP-2 at 12, 24, and 48 h after resection in TPN animals, in the presence of a significant adaptive growth response that remained 5 days later, is consistent with current understanding of (a) downstream mediator(s). Moreover, exogenous GLP-2 increases enterocyte proliferation and decreases apoptosis (4, 46), the same response we noted in TPN rats in association with increases in endogenous plasma bioactive GLP-2. This supports a role for endogenous GLP-2 in resection-induced intestinal adaptation during TPN. Further studies are needed regarding the regulation of GLP-2 secretion during resection and TPN.

In conclusion, the results of these studies demonstrate that luminal nutrients are not essential for resection-induced adaptive growth when residual ileum and colon are present and that GLP-2 may mediate this intestinal adaptation in both parenterally and enterally fed rats. Although luminal nutrients are not essential for resection-induced adaptation in parenterally fed rats, enteral nutrition enhances the adaptive growth (10), which is most evident in the considerable resection-induced increase in enterocyte proliferation observed in orally fed animals. Thus maximal adaptation to bowel resection most likely involves the factors stimulated by the endogenous response to resection that are independent of exogenous luminal nutrients and the direct and/or indirect effects of luminal nutrients. Further elucidation of the factor(s) regulating intestinal adaptation to resection in rodent models may lead to improved treatments for patients with short bowel syndrome who are dependent on parenteral nutrition.

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