Synergistic effect of supplemental enteral nutrients and exogenous glucagon-like peptide 2 on intestinal adaptation in a rat model of short bowel syndrome\textsuperscript{1–4}

Xiaowen Liu, David W Nelson, Jens J Holst, and Denise M Ney

ABSTRACT

Background: Short bowel syndrome (SBS) can lead to intestinal failure and require total or supplemental parenteral nutrition (TPN or PN, respectively). Glucagon-like peptide 2 (GLP-2) is a nutrient-dependent, proglucagon-derived gut hormone that stimulates intestinal adaptation.

Objective: Our objective was to determine whether supplemental enteral nutrients (SEN) modulate the intestinotrophic response to a low dose of GLP-2 coinfused with PN in a rat model of SBS (60% jejunoileal resection plus cecectomy).

Design: Rats were randomly assigned to 8 treatments by using a 2 × 2 factorial design and maintained with either TPN or PN for 7 d. The 3 main treatment effects were the following: transection or resection (TPN alone), ± SEN (days 4–6), and ± GLP-2 (100 μg · kg body wt\textsuperscript{-1} · d\textsuperscript{-1}).

Results: The treatments induced differential growth of duodenal and jejunal mucosa. Significant differences in villus height, crypt depth, dry mass, and concentrations of protein and DNA were observed between the treatments and TPN alone (SEN: 15–59% increase; GLP-2: 14–84% increase; and SEN + GLP-2: 63–160% increase). Plasma concentrations of bioactive GLP-2 were significantly greater with GLP-2 infusion (TPN alone: 25 ± 9 pmol/L; SEN: 29 ± 10 pmol/L; GLP-2: 59 ± 31 pmol/L; SEN + GLP-2: 246 ± 40 pmol/L) and correlated with mucosal growth. Jejunal succrease activity (in U/cm) was significantly greater with SEN than without SEN. SEN + GLP-2 induced dramatic mucosal growth and greater plasma concentration of GLP-2 (SEN × GLP-2 interaction, \(P < 0.0001\)). Resection significantly increased expression of proglucagon mRNA in colon.

Conclusions: Combination treatment with SEN and GLP-2 induced a synergistic response resulting in greater mucosal cellularity and digestive capacity in parenterally fed rats with SBS. This shows that SEN improve the intestinotrophic response to exogenous GLP-2, possibly by stimulating enterocyte proliferation and differentiation. \textit{Am J Clin Nutr} 2006;84:1142–50.

KEY WORDS Jejunoileal resection, cecectomy, parenteral nutrition, intestinal adaptation, proglucagon, glucagon-like peptide 2, GLP-2

INTRODUCTION

Short bowel syndrome (SBS) in humans is characterized by severe loss of intestinal absorptive capacity with resulting malabsorption, fluid and electrolyte losses, and wasting of lean body mass that often result in intestinal failure (1). Many persons with SBS, in particular those from whom large amounts of ileum and colon have been removed, show limited intestinal adaptation and require long-term total or supplemental parenteral nutrition (TPN or PN, respectively) (2–4). The absence of significant intestinal adaptation with SBS may reflect limited enteral nutrition, a known stimulus for intestinal growth (5–7), and a deficiency of endogenous intestinal growth factors due to resection of large areas of the intestine that produce the hormonal growth factors (8, 9). Enteral nutrients are thought to stimulate intestinal adaptation by acting directly to provide energy and protein for the mucosa or indirectly by increasing pancreaticobiliary secretions and intestinal blood flow and triggering the release of hormones such as gastrin and glucagon-like peptides (10).

Glucagon-like peptide 2 (GLP-2) is a 33-amino acid intestinotrophic hormone derived from posttranslational processing of proglucagon in the enteroendocrine L cells of the ileum and colon (11). The primary stimulus for secretion of GLP-2 is enteral nutrient intake, in particular intake of lipid and carbohydrate in humans (10, 12). TPN is associated with reduced circulating concentrations of GLP-2 compared with enteral feeding, and administration of GLP-2 attenuates the mucosal hypoplasia induced by TPN in animal models (6, 13, 14). Supplementation of TPN with short-chain fatty acids, products of fiber fermentation that provide an energy source for the colon, increases enterocyte proliferation, mucosal growth, and plasma concentration of GLP-2 (15). In addition to nutrient stimulation of GLP-2 secretion, the mucosal growth induced by intestinal resection is associated with increased plasma concentrations of GLP-2 in models where residual ileum is present (6, 16–18). In summary, GLP-2

\textsuperscript{1} From the Department of Nutritional Sciences, University of Wisconsin-Madison, Madison, WI (XL, DWN, and DMN), and the Department of Medical Physiology, The Panum Institute, University of Copenhagen, Copenhagen, Denmark (JJH).

\textsuperscript{2} This manuscript (in abstract form) was one of five finalists in the American Society for Nutrition Young Investigator Award Competition at Experimental Biology, San Francisco, CA, April 2006.

\textsuperscript{3} Supported by NIDDK R01-42835 and T32-07665 and by funds from the College of Agricultural and Life Science (project no. 4672), University of Wisconsin-Madison.

\textsuperscript{4} Reprints not available. Address correspondence to DM Ney, University of Wisconsin-Madison, Department of Nutritional Sciences, Madison, WI 53706. E-mail: ney@nutrisci.wisc.edu.

Received February 22, 2006. Accepted for publication June 23, 2006.
were fed a low-residue, semielemental liquid diet that contained a semipurified diet ad libitum. Three days before surgery, the rats were adapted to the facility for 3 d while being fed stainless steel cages with unlimited access to water in a room and surgery. Male Sprague-Dawley rats (Harlan, Madison, WI) were maintained at full strength infusion of 2.5 mL/h for the following: TPN alone, resection (TPN alone), transection or resection, + SEN, + GLP-2, and resection + SEN + GLP-2. Rats in these 8 groups were maintained with TPN or PN for 7 d. The 3 main treatment effects were the following: transection or resection, ± SEN (days 4–6), and ± GLP-2. The dose of GLP-2 was 100 μg · kg body wt⁻¹ · d⁻¹. The final sample size in the transection groups were the following: TPN alone, n = 7; SEN, n = 6; GLP-2, n = 5; and SEN + GLP-2, n = 6. The final sample size in the resection groups were the following: TPN alone, n = 6; SEN, n = 6; GLP-2, n = 8; and SEN + GLP-2, n = 8. A nonsurgical group of rats fed the semipurified diet ad libitum was included for reference (oral reference group; n = 12).

On the day of surgery, rats were anesthetized by inhalation of isoflurane (IsoFlo, Abbott Laboratories, North Chicago, IL) via an anesthesia machine. After anesthesia, rats underwent a 60% jejunoileal resection + cecectomy with an end-to-end jejunoileal anastomosis.

FIGURE 1. Timeline of the experimental protocol. A 2 × 2 × 2 factorial design was used to measure the effects of transection or resection, exogenous glucagon-like peptide-2 (GLP-2), and supplemental enteral nutrients (SEN) on intestinal growth in rats maintained with total parental nutrition (TPN) or parenteral nutrition (PN) + SEN for 7 d. Vital is a low-residue, semielemental liquid diet that contains hydrolyzed protein and medium-chain triacylglycerols (Vital; donated by Ross Products Division, Abbott Laboratories, Columbus, OH) ad libitum to clean out the bowel before surgery. The same semielemental liquid diet was used to provide SEN on days 4–6; this diet provides 1 kcal/mL with 16.7% energy from protein, 9.5% energy from fat, and 73.8% energy from carbohydrate. This formulation has been shown to protect the intestinal mucosa from injury induced by radiation (30, 31).

Rats were randomly assigned with the use of a 2 × 2 × 2 factorial design to the following treatment groups (n = 8 for each group): transection (TPN alone), resection (TPN alone), transection + supplemental enteral nutrient (SEN), resection + SEN, transection + GLP-2, resection + GLP-2, transection + SEN + GLP-2, and resection + SEN + GLP-2. Rats in these 8 groups were maintained with TPN or PN for 7 d. The 3 main treatment effects were the following: transection or resection, ± SEN (days 4–6), and ± GLP-2. The dose of GLP-2 was 100 μg · kg body wt⁻¹ · d⁻¹. The final sample size in the transection groups were the following: TPN alone, n = 7; SEN, n = 6; GLP-2, n = 5; and SEN + GLP-2, n = 6. The final sample size in the resection groups were the following: TPN alone, n = 6; SEN, n = 6; GLP-2, n = 8; and SEN + GLP-2, n = 8. A nonsurgical group of rats fed the semipurified diet ad libitum was included for reference (oral reference group; n = 12).

On the day of surgery, rats were anesthetized by inhalation of isoflurane (IsoFlo, Abbott Laboratories, North Chicago, IL) via an anesthesia machine. After anesthesia, rats underwent a 60% jejunoileal resection + cecectomy with an end-to-end jejunoileal anastomosis or transection surgery and placement of a TPN catheter in the superior vena cava, as previously described (27). Resected rats had bowel 40 cm distal to the ligament of Treitz to 1 cm distal to the cecum removed. Transected rats received cuts 40 cm distal to the ligament of Treitz and 1 cm distal to the cecum followed by restoration of bowel continuity. All rats received oxymorphone hydrochloride for pain management and prophylactic ampicillin up to 48 h after surgery (27). Infusion of TPN solution was initiated with the use of a Harvard syringe pump (Harvard apparatus Inc, Holliston, MA) at 1.0 mL/h immediately after surgery (day 0), advanced to 1.67 mL/h on day 1, and maintained at full strength infusion of 2.5 mL/h (60 mL/d) for the groups not receiving SEN for days 2–6. Rats given TPN on days 2–6 received the following daily nutrient intake: 64 kcal, 2.6 g protein (16.5% of energy), 1.7 g fat (24% of energy), and 11 g dextrose (59% of energy). The composition and preparation of the nutritionally complete TPN solution was previously reported (5).

The treatment groups given oral SEN received 2.5 mL TPN solution/h for days 2–4 and then the infusion was gradually decreased to 1.67 mL/h on day 5 and 1.0 mL/h on day 6. SEN was offered ad libitum in graduated feeding tubes on days 4–6. Thus, ≈50% of energy needs were provided by PN during the last 2 d of the study. Nutrient intake for the 4 treatment groups receiving SEN for days 4–6 is summarized in Table 1.

Rats infused with GLP-2 received 100 μg human GLP-2 · kg body wt⁻¹ · d⁻¹ concurrent with continuous intravenous infusion of TPN solution for days 1–7. Vehicle was infused in rats not given GLP-2. The dose of GLP-2 was chosen based on a preliminary dose-response study conducted in TPN rats as within the lowest range of GLP-2 (50–100 μg · kg body wt⁻¹ · d⁻¹) that would attenuate TPN-induced mucosal atrophy and restore plasma GLP-2 concentrations to the concentrations noted in
orally fed rats. Human GLP-2 (preproglucagon 126–158; CA Peptide Research Inc, Napa, CA) was diluted in phosphate buffered saline (pH = 7.4) 1 d before surgery and added to the TPN solution daily.

Body weights, the volume of TPN solution infused, and the amount of SEN consumed were recorded daily. Urine was collected into containers with 0.1% boric acid for determination of nitrogen balance (27). After 7 d of TPN or PN + SEN, the rats were anesthetized with isofluorane and killed by exsanguination.

**Intestinal composition and histology**

At the time of the kill, the entire small and large bowel, liver and kidneys were removed for analysis. The intestine adjacent to the anastomosis (1 cm on either side) was discarded. The residual bowel was sectioned into duodenum, pylorus to ligament of Treitz; jejunum, between the ligament of Treitz and anastomosis; and colon, distal to anastomosis. All segments were immediately flushed with ice-cold saline and put on a chilled glass plate to be sectioned. The first 2 or 3 cm of duodenum and colon and the midsection of jejunum were used for determining wet and dry mucosal or intact mass. The next 1 cm was fixed in 10% buffered formalin for histology, and the next 3 cm were collected for determination of protein (bicinchoninic acid protein assay, Pierce Chemicals, Rockford, IL), DNA (32), and sucrase activity (33). The remaining tissue from each region of bowel was snap frozen intact in liquid nitrogen and stored at −70 °C for RNA extraction. Fixed tissue for histology was paraffin embedded, cut into 5-μm sections, and stained with hematoxylin and eosin for histomorphology (27).

**TABLE 1**

<table>
<thead>
<tr>
<th>Day</th>
<th>Transection Group</th>
<th>Resection Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 4</td>
<td>PN + SEN 91 ± 4²</td>
<td>PN + SEN + GLP-2 95 ± 6</td>
</tr>
<tr>
<td></td>
<td>Total kcal</td>
<td>kcal (% of total)</td>
</tr>
<tr>
<td></td>
<td>3.7 ± 0.2</td>
<td>1.1 ± 0.2 (30)</td>
</tr>
<tr>
<td></td>
<td>0.30 ± 0.04</td>
<td>5.0 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>Carbohydrate from SEN</td>
<td>g</td>
</tr>
<tr>
<td></td>
<td>0.2 (30) 0.30</td>
<td>0.2 (33) 0.34</td>
</tr>
<tr>
<td></td>
<td>0.2 (33) 0.37</td>
<td>0.3 (41) 0.37</td>
</tr>
<tr>
<td></td>
<td>0.04 5.0</td>
<td>0.06 5.7</td>
</tr>
<tr>
<td></td>
<td>0.3 (63) 0.51</td>
<td>0.2 (63) 0.44</td>
</tr>
<tr>
<td></td>
<td>0.02 7.4</td>
<td>0.03 8.5</td>
</tr>
</tbody>
</table>

¹ n = 6–8 per group. Rats received 60 mL of PN solution on day 4, 40 mL on day 5, and 24 mL on day 6. The differences in intake of SEN between the treatment groups on days 4–6 were not significant.

² x ± SE (all such values).

**Plasma bioactive GLP-2**

Plasma bioactive GLP-2 was quantified by RIA with the use of an antibody specific to the N-terminus of GLP-2 (34). Blood was collected into chilled tubes containing a final concentration of 1 mg EDTA/mL, 0.1 mmol Diprotin A/L (MP Biomedicals, Aurora, OH), and 0.01 mmol aprotinin/L (Calbiochem, La Jolla, CA).

**Proglucagon mRNA**

Total RNA was extracted from intact colon by using TRizol reagent (Gibco BRL Life Technologies, Grand Island, NY). Quantification of proglucagon mRNA was done with the use of a Northern Max kit (Ambion, Austin, TX) (6). All bands were quantified by light densitometry with the use of OPTIQUANT version 03.00 software package (Packard Instruments, Meriden, CT).

**Statistical analyses**

SAS version 8.2 (SAS Institute, Cary, NC) and R (downloaded from the following website: http://www.r-project.org) were used for the statistical analysis. Outliers (Bonferroni P ≤ 0.05) were excluded from analysis. The differences between treatment groups were examined by one-way analysis of variance (ANOVA) followed by the protected least-significant-differences technique. General linear models (GLMs) were used to analyze the main effects of the 3 treatments and their interactions. Changes in body weight were assessed by repeated-measures analysis. Data from gels were analyzed by using the linear mixed-effects model. All values are presented as means ± SE, P ≤ 0.05 was considered statistically significant.
RESULTS

Body weight and energy intake

Changes in daily body weight are shown in Figure 2. No significant differences in body weights were observed between the groups before surgery, and on the day of surgery body weights ranged from 231–240 g. During the period after surgery and before SEN treatment (day 0 to day 4; P < 0.025), Resected rats showed a lower body weight than did transected rats. Combination treatment with SEN or GLP-2 resulted in significant growth of duodenal and jejunal mucosa; however, a differential pattern of response was noted as follows: SEN < GLP-2 < SEN + GLP-2. SEN induced greater duodenum and jejunum mucosal dry mass and concentrations of protein and DNA by 15–59% than did TPN alone in both transected and resected rats (Figure 3). GLP-2 induced greater duodenum and jejunum mucosal dry mass and concentrations of protein and DNA by 14–84% than did TPN alone in both transected and resected rats. Combination treatment with SEN + GLP-2 induced significantly greater mucosal mass and concentrations of protein and DNA by 108–160% in both duodenum and jejunum than did TPN alone in both transected and resected rats. Indexes of mucosal adaptive growth showed significant effects for the 3 treatments and their interactions.

Intestinal adaptive growth

Rats given resection alone exhibited minimal adaptive growth of the residual duodenum and jejunum compared with rats given transection alone (Figure 3 and Figure 4), but they showed dramatic adaptive growth in the residual colon as discussed in the next section. These data confirm the differential jejunal and colonic adaptive growth induced by 60% jejunoileal resection plus cecectomy as previously reported in this model (27, 28, 35, 36).

The 3 treatments induced significant growth of duodenal and jejunal mucosa; however, a differential pattern of response was noted as follows: SEN < GLP-2 < SEN + GLP-2. SEN induced greater duodenum and jejunum mucosal dry mass and concentrations of protein and DNA by 15–59% than did TPN alone in both transected and resected rats (Figure 3). GLP-2 induced greater duodenum and jejunum mucosal dry mass and concentrations of protein and DNA by 14–84% than did TPN alone in both transected and resected rats. Combination treatment with SEN + GLP-2 induced significantly greater mucosal mass and concentrations of protein and DNA by 108–160% in both duodenum and jejunum than did TPN alone in both transected and resected rats. Indexes of mucosal adaptive growth showed significant interaction (SEN × GLP-2, P < 0.0001), which indicated a synergistic effect of treatment with SEN + GLP-2 to enhance mucosal adaptive growth beyond that expected by additive effects of the 2 treatments. Treatment with SEN + GLP-2 did not significantly alter the ratio of protein to DNA in jejunal mucosa, which suggests that the greater mass of jejunum was due to a greater cell number. In contrast, individual treatment with SEN or GLP-2 resulted in a significantly greater (P < 0.01) ratio of protein to DNA than that observed with TPN alone, which indicates greater cell number and cell size.

Individual treatment with SEN or GLP-2 resulted in significantly higher jejunum villus height in both transected and resected rats than with TPN alone (P < 0.0001; Figure 4). Individual treatment with GLP-2 but not SEN resulted in a significantly greater jejunum crypt depth than with TPN alone in both transected and resected rats (P = 0.0067). The synergistic effect of combination treatment with SEN + GLP-2 was noted for jejunum villus height (P = 0.0477) but not for crypt depth.
Treatment with SEN, GLP-2, or both did not significantly induce colonic growth in either transected or resected rats based on colon mass and concentrations of protein and DNA in colon. Resection was the only treatment to induce growth of the residual colon. Colon data are as follows: mean colon dry mass from intact sections was 20 ± 0.9 mg/cm for the transection group and 22 ± 0.8 mg/cm for the resection group (P = 0.04), mean colon protein was 7.1 ± 0.2 mg/cm for the transection group and 8.7 ± 0.2 mg/cm for the resection group (P < 0.0001), and mean colon DNA was 1.3 ± 0.1 mg/cm for the transection group and 1.6 ± 0.1 mg/cm for the resection group (P = 0.01).

Sucrase activity

Individual treatment with SEN or GLP-2 resulted in significantly greater jejunal sucrase segmental activity than with TPN alone (Figure 5). However, the increment in sucrase activity was greater with SEN than with GLP-2 treatment (P for SEN < 0.001; P for GLP-2 = 0.0237). Combination treatment with SEN and GLP-2 induced significantly greater sucrase segmental activity than with either treatment alone (P for SEN × GLP-2 interaction = 0.0432).

When sucrase activity was expressed as specific activity, individual treatment with SEN or combination treatment with SEN and GLP-2 induced significantly greater sucrase activity. However, individual treatment with GLP-2 did not significantly alter sucrase specific activity compared with TPN alone. The mean sucrase specific activity was 0.11 ± 0.02 units/mg protein for TPN alone, 0.16 ± 0.01 units/mg protein for SEN, 0.10 ± 0.01 units/mg protein for GLP-2, and 0.15 ± 0.01 units/mg protein for SEN + GLP-2 (P < 0.05).

Bioactive plasma GLP-2 and its correlation to growth

Individual treatment with GLP-2 induced a significantly (100%) greater concentration of bioactive GLP-2 in plasma than did individual treatment with SEN or TPN alone (Figure 6). Individual treatment with SEN did not significantly alter the concentration of bioactive GLP-2 in plasma. A significant interaction (P < 0.0001) was observed between treatment with SEN and GLP-2, such that the plasma concentration of bioactive GLP-2 due to combination treatment with SEN and GLP-2 was 4-fold that with GLP-2 alone (Figure 6). Resection alone did not significantly alter the plasma concentrations of GLP-2, which is consistent with the lack of resection-induced mucosal growth in small intestine. The mean values for plasma concentration of bioactive GLP-2 were 25 ± 9 pmol/L for TPN alone, 29 ± 10 pmol/L for SEN, 59 ± 31 pmol/L for GLP-2, and 246 ± 40 pmol/L for SEN + GLP-2 (P < 0.05). Additionally, as shown in Figure 6C, concentrations of bioactive GLP-2 in plasma showed a significant positive correlation with variables of duodenum and jejenum growth, ie, with mucosal dry mass and concentrations of protein and DNA (r² = 0.5676–0.6812, P < 0.0001).

Expression of proglucagon mRNA

Bowel resection induced significantly greater expression (by almost 4-fold) of proglucagon mRNA in colon than that seen with transection control surgery (main effect of surgery, P < 0.0001) (Figure 7). Individual treatment with SEN did not significantly alter proglucagon mRNA in either resected or transected rats. Interestingly, individual treatment with GLP-2 or combination treatment with SEN and GLP-2 induced a significant increase in colon proglucagon mRNA in resected rats, but this was not observed in transected rats. A significant positive correlation (P < 0.0001, r² = 0.6519) was observed between the abundance of proglucagon mRNA and plasma concentration of GLP-2 in resected rats given TPN alone, PN + SEN, and TPN + GLP-2.
DISCUSSION

Intestinal adaptation after bowel resection is a poorly understood, multifactorial process that results in growth and improved function of the residual intestine (3, 4). The presence of luminal nutrients (5–7, 13) and intestinal growth factors such as GLP-2 (7, 8, 11, 12, 19, 20, 22, 25, 26) are key stimulators of intestinal adaptation. We investigated how treatment with SEN, GLP-2, and SEN/L1151 GLP-2 affected intestinal adaptation in parenterally fed rats with SBS. Our results confirm previous findings of the intestinotrophic effects of luminal nutrients and GLP-2 administration after bowel resection (6, 20, 25, 26) and of the positive correlation between mucosal growth and plasma concentration of GLP-2 (6, 18). We showed for the first time that combination treatment with SEN and a low dose of GLP-2 induced a synergistic response to enhance mucosal adaptive growth, plasma concentration of bioactive GLP-2, and sucrase activity in a rat model of SBS with intestinal failure.

The synergistic growth response as indicated by the significant statistical interaction between SEN and GLP-2 treatment reflects a greater villus height and greater mucosal concentrations of protein and DNA in jejunum, ie increased enterocyte cellularity, than that expected from the additive effects of treatment with either SEN or GLP-2 alone. This observation suggests that the mechanisms of the intestinotrophic actions of SEN and GLP-2 are likely interrelated. Interestingly, the synergistic growth response to SEN and GLP-2 was noted in both the transection and the resection groups maintained with TPN, which suggests that the mechanism of the response is independent of the adaptive process induced by resection.

Adaptive intestinal growth or an increase in mucosal cellularity reflects the balance between enterocyte production, loss by apoptosis, or both (37). Provision of GLP-2 or enteral nutrients both increase enterocyte cellularity by increasing enterocyte proliferation and decreasing enterocyte apoptosis in parenterally fed rats or piglets (5, 7, 13). We previously noted that a large dose of another intestinal growth factor, insulin-like growth factor-I (IGF-I), stimulated enterocyte proliferation and crypt depth in rats to a greater extent than did enteral nutrition (5). Likewise in the current study, a low dose of GLP-2 and not SEN increased crypt depth consistent with an increase in enterocyte proliferation. In summary, the observed synergistic mucosal growth response most likely reflects the net effects of both SEN and GLP-2 in increasing enterocyte proliferation and decreasing apoptosis (5, 7).

Greater sucrase activity suggests that the enterocyte growth induced by SEN and GLP-2 resulted in improved intestinal digestive capacity. However, differential changes in segmental and specific sucrase activity due to treatment with either SEN or GLP-2 suggest that these treatments have varying effects on enterocyte functional activity. SEN appear to provide a greater stimulus for maturation and differentiation of the enterocyte than does GLP-2, which appears to provide a greater stimulus for enterocyte proliferation based on a significant increase in crypt depth. Two observations provide support for this view. First, the magnitude of the effect on sucrase segmental activity was greater

FIGURE 4. Histologic tests of the jejunum, showing mucosal growth due to supplemental enteral nutrients (SEN) + glucagon-like peptide 2 (GLP-2) treatment in transected and resected rats. Light micrographs of jejunum stained with hematoxylin and eosin (A) and morphometric indication of jejunum villus height (B) and crypt depth (C). TPN, total parenteral nutrition. Values are means (±SEs). Means with different letters are significantly different, P < 0.05. The differences between treatment groups were examined by one-way ANOVA and then by the protected least-significant-differences technique. General linear models were used to analyze main effects of the 3 treatments and their interactions.
with SEN than with GLP-2, an increase of 117% compared with 43%, respectively, over values obtained with TPN alone. Secondly, unlike SEN, GLP-2 did not induce significantly greater sucrase specific activity than did TPN alone. This suggests that when sucrase activity is normalized to protein concentration instead of unit length, the presence of immature, less differentiated enterocytes becomes apparent, consistent with a strong proliferative effect of GLP-2. Immature, less well-differentiated enterocytes are known to have lower expression of disaccharidases than do mature cells (38). In summary, SEN appear to provide a greater stimulus for differentiation of enterocytes than does GLP-2, whereas GLP-2 provides a greater stimulus for proliferation of enterocytes than does SEN. The combination of SEN + GLP-2 induces a synergistic increase in sucrase segmental activity.

Resection of the ileum and cecum with creation of a jejuno-colic anastomosis has a dramatic effect to induce growth of the residual colon in rats fed enterally or parenterally as confirmed in the current study (27, 28, 35, 36). This response is associated with the combination of ileal and cecal resection as removal of just the cecum or just the distal small bowel results in mild adaptive growth in residual colon (29). Interestingly, infusion with GLP-2 in the present study or with IGF-I in our earlier work (27, 28) did not induce further growth of residual colon beyond the effects of resection alone, possibly because expression of IGF-I (28) and proglucagon mRNA are already upregulated due to the resection. However, Kripke et al (35) noted that intragastric feeding of an elemental diet supplemented with short-chain triacylglycerols, but not medium-chain triacylglycerols, further stimulates colonic growth beyond that observed with resection in this model. Humans with SBS with colonic continuity have decreased diarrhea and up-regulation of the peptide transporter PepT1, which is consistent with colonic adaptation (39). Current recommendations to stimulate colonic adaptation include a diet low in fat and high in complex carbohydrates that provides short-chain fatty acids as the primary fuel for colonocytes (15, 40). Thus, it is not surprising that provision of ∼50% of energy from SEN did not stimulate colonic growth in the present study, given that the formula was low in residue and contained medium-chain triacylglycerols. Overall, modifications in enteral nutrients may have more potential to stimulate colonic growth than do exogenous IGF-I or GLP-2 in a rat SBS model with a jejuno-colic anastomosis.

A surprising observation was that GLP-2 infusion resulted in less loss of body weight in resected rats during the first 3 d after surgery. The GLP-2 receptor has only been identified in tissues found in the brain and the gastrointestinal tract (11). Moreover, a large number of in vivo studies have shown that GLP-2 treatment does not induce changes in body weight or organ weight other than the intestine, which suggests that GLP-2 is an
Glucagon-like peptide 2 and short bowel syndrome

Enteral nutrition provided, however, SEN alone had a modest intestinotrophic effect and did not increase plasma concentration of GLP-2; how-

The exact mechanisms by which GLP-2 promotes epithelial growth are unknown. Paracrine and neural pathways are thought to act as downstream mediators of GLP-2 action because the GLP-2 receptor is not expressed on epithelial cells, which are the known target cells of GLP-2 action (11). Our finding that enteral nutrients were needed to optimize the intestinotrophic response to exogenous GLP-2 in parenterally fed rats with SBS support the hypothesis that GLP-2 action is dependent on downstream mediators. Enteral nutrients may optimize GLP-2 action by directly or indirectly stimulating downstream mediators such as intestinal blood flow (12), neural activity, and other gut hormones that will enhance the ability of GLP-2 to increase epithelial proliferation and decrease apoptosis. In conclusion, we showed for the first time that combination treatment with SEN + GLP-2 induced a synergistic growth response resulting in greater concentrations of bioactive GLP-2 in plasma and greater mucosal cellularity and digestive capacity in parenterally fed rats with SBS that do not otherwise show resection-induced growth of the small intestine. Additional studies are needed to determine the mechanisms underlying this synergistic mucosal growth response. Our findings have clinical implications for treatment with a combination of GLP-2 and enteral nutrients in humans with intestinal failure.

We thank Sangita G Murali, Michael J Grahm, and Angela K Draxler for their expert technical assistance. We acknowledge Ross Products Division, Abbott Laboratories (Columbus, OH) for their generous donation of the Vital used to provide supplemental enteral nutrition in this study.

XL, DWN, and DMN were involved in the design and implementation of the study, analysis of the data, and drafting of the manuscript; JH contributed to the analysis of plasma GLP-2 and interpretation of the data. All authors contributed to the final version of the manuscript. The authors had no conflict of interest.

REFERENCES


7. Burrin DG, Stoll B, Guan X, Cui L, Chang X, Holst JJ. Glucagon-like peptide 2 is thought to be a key hormonal mediator of resection-induced adaptive growth (6–9, 11, 17, 18, 20, 22, 25, 26). In support of this view, we observed a significant positive correlation between mucosal growth and plasma concentration of bioactive GLP-2 (18). Moreover, there was a synergistic effect of SEN + GLP-2 to increase plasma concentration of GLP-2; however, SEN alone had a modest intestinotrophic effect and did not increase plasma concentration of GLP-2 (10, 12). This may reflect the amount of energy provided, ≈50% of energy needs during the last 2 d of the study, whereas neonatal pigs require 60% of energy requirements from enteral nutrition to sustain normal plasma concentration of GLP-2 and mucosal growth (10).

GLP-2 is a proglucagon-derived gut hormone that is synthesized in ileum and colon and cosecreted with GLP-1, oxyntomodulin, and glicentin after posttranslational processing (11). In the present study, resected rats showed greater concentrations of colon proglucagon mRNA than did transected rats. This observation may reflect that ileum is the primary source of proglucagon mRNA in transected rats, whereas the entire ileum is removed in the resected rats and the colon becomes the primary site of GLP-2 production (6). Thus, resected rats with SBS express more proglucagon mRNA in the colon than do transected rats, and this is significantly correlated with higher circulating concentrations of bioactive GLP-2 suggesting transcriptional regulation. However, resected rats given SEN + GLP-2 showed a concentration of plasma GLP-2 4-fold that observed with GLP-2 alone, but the same concentration of colon proglucagon mRNA. This suggests that translational or posttranslational regulation of GLP-2 synthesis also exists with combination treatment of SEN + GLP-2 (11). In summary, resection induces greater expression of proglucagon mRNA in association with greater plasma concentrations of bioactive GLP-2 than transaction and a low dose of GLP-2 does not down-regulate endogenous proglucagon expression in the colon of rats with SBS.

 scrolls through lipid microdroplets. This hypothesis that GLP-2 action is dependent on downstream mediators such as intestinal blood flow, neural activity, and other gut hormones that may enhance the ability of GLP-2 to increase epithelial proliferation and decrease apoptosis.

FIGURE 7. Representative bands from Northern blot analysis of proglucagon mRNA (A) and mean (±SE) ratios of the abundance of proglucagon mRNA to 18S rRNA (B) in the colon of rats subjected to transection or resection surgery and treatment with supplemental enteral nutrients (SEN), glucagon-like peptide 2 (GLP-2), or both. A nonsurgical group of rats fed the semipurified diet ad libitum was included for reference (oral group). Means with different letters are significantly different, P < 0.05. Data from gels were analyzed by using the linear mixed-effects model.

A

Oral group

Transsection group

Resection group

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN

PN

TPN