Nutrient-stimulated GLP-2 release and crypt cell proliferation in experimental short bowel syndrome

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Submitted 1 June 2004; accepted in final form 16 September 2004


Martin G. R., L. E. Wallace, B. Hartmann, J. J. Holst, L. Demchysyn, K. Toney, and D. L. Sigalet. Nutrient-stimulated GLP-2 release and crypt cell proliferation in experimental short bowel syndrome. Am J Physiol Gastrointest Liver Physiol 288: G431–G438, 2005. First published September 23, 2004; doi:10.1152/ajpgi.00242.2004.—Glucagon-like peptide-2 (GLP-2) is an enteroendocrine peptide that is released in response to luminal nutrients and has unique trophic actions in the gastrointestinal tract. These features suggest GLP-2 may be important in controlling intestinal adaptation. We examined the relationship over time of GLP-2 production and adaptation to intestinal resection, the effects of resection-induced malabsorption on GLP-2 production, and the correlation of endogenous serum GLP-2 levels with adaptation as measured by crypt-cell proliferation (CCP). We initially examined the effect of nutrient malabsorption, induced by a 90% resection of the proximal intestine studied on day 4, on the time course and levels of GLP-2 release. Secondly, the degree of malabsorption was varied by performing intestinal transection or 50, 75, or 90% resection of proximal small intestine. Finally, the relationship of GLP-2 levels over time with adaptation to a 90% resection was examined by determining GLP-2 levels on days 7, 14, and 28, and correlating this with intestinal adaptation, as assessed by morphology and CCP rate. A 90% resection significantly increased basal and postprandial GLP-2 levels, with a net increase in nutrient-stimulated exposure over 90 min; GLP-2 exposure (integrated levels vs. time) increased 12.7-fold in resected animals (P < 0.001). Basal and postprandial GLP-2 levels significantly correlated with the magnitude of intestinal resection (r² = 0.71; P < 0.001), CCP (r² = 0.48; P < 0.005), and nutrient malabsorption (protein, P < 0.001; fat, P < 0.005). The increase in CCP was maintained to 28 days after small bowel resection and was associated with an ongoing elevation in GLP-2 release. These findings suggest that GLP-2 is important in initiating and maintaining the small intestinal adaptive response to resection.

nutrient malabsorption; intestinal adaptation; intestinal resection; intestinal morphology; nutrition

GLUCAGON-LIKE PEPTIDE-2 (GLP-2) is distinct among enteric hormones in its trophic effects in the gastrointestinal tract (13, 48). GLP-2 is produced in the hindgut and released in response to nutrients in the intestinal lumen (12, 13, 22). This has led to the suggestion that GLP-2 may be important in regulating intestinal adaptation in response to variations in nutrient availability resulting from dietary modification, mucosal disease, or surgical resection (26, 37). GLP-2 administration enhances intestinal crypt-cell proliferation (CCP) and villus height (17, 29, 35, 42), increases the expression of the glucose transporters Na+/glucose cotransporter-1 (SGLT-1) and GLUT-2 (5, 14, 29), reduces the severity of NSAID-induced enteritis (7), and is protective of intestinal cells after exposure to radiation and chemotherapeutic agents (8, 33). These observations suggest that GLP-2 would be useful for the treatment of gastrointestinal disorders associated with insufficient mucosal function.

Nevertheless, the physiological role of GLP-2 in the intact animal is not well understood. GLP-2 is a 33-amino acid peptide member of the pituitary adenylate cyclase activating peptide/glucagon superfamily produced in the enteroendocrine L cells that are localized to the distal small bowel and colon (10, 16, 24, 36). The effects of GLP-2 appear to be transduced by a specific G protein-coupled receptor (30, 32) localized to the enteric nervous system and select brain regions, including the hippocampus, the dorsal medial hypothalamus, and areas of the brainstem (6, 27, 43). Actions of GLP-2 in the gastrointestinal tract appear to be mediated primarily through enteric neuronal signaling, although this remains controversial (6, 40, 44).

The physiological effects of GLP-2 are difficult to isolate in vivo, because after a meal, GLP-2 is released together with GLP-1, and peptide YY (PYY) (21, 25). With these hindgut hormones, GLP-2 has been shown to alter both gastric motility and aspects of small intestinal peristalsis (9, 25, 47). However, GLP-2 is unique among these hindgut hormones in its ability to increase the nutrient absorptive capacity of the intestinal mucosa. GLP-2 increases the expression of SGLT-1 activity acutely (14), increases intestinal blood flow (19), and, over the longer term, is trophic to the intestinal mucosa (17, 29).

We propose that the primary physiological role of GLP-2 is to regulate the nutrient absorptive capacity of the bowel; undigested nutrients in the distal bowel induce GLP-2 release from the L cells into the blood, thereby increasing local and proximal intestinal nutrient absorption. These characteristics are likely important as the small intestine responds to variations in dietary nutrient availability. However, they become critically important in controlling the response to loss of intestinal absorptive area after resection. We hypothesized that GLP-2 release would be increased by nutrient malabsorption after resection and that these elevated GLP-2 levels would be predictive of the adaptive response of remnant intestine.

These studies examined the time course of GLP-2 release after intestinal resection, both after an individual meal and over time as intestinal adaptation occurred. We also assessed the degree of resection-induced nutrient malabsorption and its effects on circulating GLP-2 levels, nutrient absorption, and intestinal adaptation. We show that there is a direct correlation between increased GLP-2 levels and intestinal CCP after resection.

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MATERIALS AND METHODS

Experimental design. The animal studies were conducted under the guidelines established by the Canadian Council of Animal Care following the approval of the Animal Care Committee at the University of Calgary. To provide maximal stimulation of the remnant L cells in the distal ileum and colon (43), the proximal small intestine was surgically resected to induce nutrient malabsorption. After surgery, we monitored the subsequent meal-stimulated GLP-2 release, as well as the adaptive response of the remnant bowel. Effects of massive resection on GLP-2 release were first investigated in an early time-course study in which the control (transected and reanastomosed) or 90% proximal small intestinal resected animals \( (n = 12) \) were assessed after 4 days for their fasting and postprandial GLP-2 levels (15, 30, 45, 60, 90 min postprandial).

To establish the relationship between nutrient malabsorption and GLP-2 production, a second study was performed. Controls again received an intestinal transection and reanastomosis, whereas the resected groups underwent either a 50, 75, or 90% proximal intestinal resection \( (n = 8 \text{ in each group}) \). The rats underwent nutrient absorption studies from days 5-7, and then on day 7 fasted and postprandial GLP-2 levels were assessed. To assess intestinal adaptation changes over a longer time period, a third group received either an intestinal transection or a 90% resection. These groups of animals were examined on postsurgical days 7, 14, and 28 \( (n = 7-8 \text{ in each group, at each time point}) \). Endpoints determined were gross and microscopic intestinal morphology, GLP-2 levels, and CCP rates.

Surgical and maintenance methods. Male Sprague-Dawley (SD) rats weighing 250–275 g were housed in metabolic caging and allowed to acclimatize to their environment for 10 days before experimental manipulation. Rats were fasted overnight before surgery, anesthetized with halothane \((1–2\% \text{ inhalation by mask})\) and, under sterile conditions, opened with a midline laparotomy. Intestinal length was measured in a standardized fashion, and resections or transections of the intestine were then performed. Transections were done 10 cm above the ileocecal junction. With the aid of an operating microscope, interrupted sutures of 6-0 silk \((\text{Ethicon, Toronto, ON, Canada})\) were used for the bowel anastomosis. In cases of intestinal resection bowel length was measured from ligaments of Treitz to the ileal cecal valve, and proximal bowel was resected beginning 1 cm distal to the ileal cecal valve. Animals in this weight range typically have a small bowel length of 100 cm, and accordingly, residual distal length measured from the ileal cecal valve proximally was 50, 25, or 10 cm corresponding to the 50, 75, or 90% resection groups. After transection or resection, the abdomen was closed with interrupted sutures of 4-0 absorbable polygylcyn suture \((\text{Ethicon})\). Animals were allowed water immediately after surgery and their food was returned on the second postoperative day. All rats were pair fed throughout the study, restricting all animal groups to a daily food limit of 18 g, which all animals ingested avidly \((\text{Prolab RMH 2500}; \text{PMI Nutritional, Brentwood, MO})\).

Serum GLP-2 quantification. Basal GLP-2 levels ascertained from peripheral blood samples were drawn between 0900 and 1100 after an overnight fast. In the initial time-course study, samples were drawn at time 0 (basal) and then 15, 30, 45, 60, and 90 min after gavaging the animals with 2 ml of a liquid meal \((\text{Ensure plus}; \text{Abbott Laboratories})\). Subsequent studies were done by sampling at basal, 15, and 60 min postgavage. Blood was drawn into heparinized syringes, transferred into chilled EDTA tubes, and centrifuged \((\text{in } < 10 \text{ min from original draw})\) at 2,500 g for 10 min at 4°C. Plasma was collected and placed into cryovials containing 1 mM PMSF in 90% ethanol, quickly frozen on dry ice, and stored at –70°C until analysis. GLP-2 levels were measured by an ELISA with the use of a rabbit polyclonal capture antibody raised against the first 18 residues of native human GLP-2 \((\text{ALE 0303}; \text{NPS Pharmaceuticals, Mississauga, ON, Canada})\). The antibodies to the NH\(_2\)- and COOH-terminal sequences of the GLP-2 were raised in rabbits. The immunogen ALE-0303 \([\text{native NH}_{2}-\text{terminal GLP-2-(1–17)}]\) was conjugated to KLH via a sulphydryl group incorporated to the COOH end of the peptide fragment. Rabbit anti-ALE0303 antibodies were affinity purified by using an affinity column made of immobilized ALE-0303 to a gel matrix. These purified antibodies react to the front end of the NH\(_2\)-terminal sequences of native GLP-2 and some GLP-2 analogs. They did not cross react with COOH-terminal GLP-2-(18–33), glucagon or GLP-1 \((\text{neither human nor anglerfish})\). The specificity of these antibodies was confirmed by competition assay \((\text{Fig. 1})\) and Western blot analysis \((\text{K. Toney and L. Demchynshyn, unpublished data})\). This antibody recognizes GLP-2-(1–33), and may react with GLP-2-(3–33) or the pancreatic major proglucagon fragment.

To further confirm the validity of the GLP-2 ELISA, samples from a preliminary study in which the intact controls \((\text{fasted, } n = 6; 1 \text{ h postprandial, } n = 6)\), 90% resection \((\text{fasted, } n = 6; 1 \text{ h postprandial, } n = 8)\), parenterally maintained no GLP-2 \((n = 4)\), parenterally maintained plus GLP-2 administered intravenously \((n = 6)\) groups had their blood sampled and plasma extracted on day 7 after intestinal surgery. The plasma extracts were then assessed for GLP-2 levels by both ELISA assay and by the current benchmark, a radioimmunobassay that utilizes a rabbit GLP-2 antiserum against the NH\(_2\)-terminal

![Fig. 1. Glucagon-like peptide-2 (GLP-2; 1–33, 3–33) ELISA validation. A: detected GLP-2 levels using anti-ALE-0303 ELISA. Results show the specificity of binding in the presence of exogenous glucagon, glucagon-like peptide-1, the native GLP-2 NH\(_2\)-fragment, and synthetic GLP-2 (Gly2GLP2). B: correlation of GLP-2 levels using anti-ALE-0303 ELISA vs. GLP-2-(1–33) specific antibody (92160) radioimmunoassay (RIA). The correlation was highly significant \((r^2 = 0.944)\) with a 95% confidence interval of 0.944 to 0.985; number of x- and y-axis pairs = 36.](http://ajpgi.physiology.org/Downloadedfromhttp://ajpgi.physiology.org/36/2/32060609/32060609)
fragment of human GLP-2 (Code no. 92160) (22). The values were compared, and as seen in Fig. 1, the levels as determined by the two methods were significantly correlated ($r^2 = 0.944$; 95% confidence interval of 0.944 to 0.985). Levels reported in this study were those from the ELISA assay.

In vivo nutrient absorption. In the nutrient absorption vs. GLP-2 release study, the absorption of dietary nutrients was quantified. Animals were maintained in metabolic caging. On day 5 postoperation, the animals underwent a 48-h balance study in which total food intake was measured by giving a measured quantity of food daily and subtracting any uneaten food from the daily total. Stool output was assessed by daily fecal pellet collections. As previously described, the total energy of the food intake and stool output was measured by bomb calorimetry (PARR 1261 Oxygen Bomb Calorimeter), fat analysis was measured by modified Folch’s technique, and protein was analyzed by the Lowry assay (28). The value was reported as the percentage of absorbed nutrient/energy from the diet. The mineral content of the diet is <2% and considered insignificant. Macronutrient absorption was calculated directly from the measured intake and output (28).

Gross morphology and histology. After collection of blood for GLP-2 levels, animals received an intraperitoneal injection of 50 mg/kg of 5-bromo-2′-deoxyuridine (BrdU; Sigma-Aldrich, St. Louis, MO). One hour later they were euthanized with an overdose of pentobarbital sodium (50 mg/kg iv; Huntington Laboratories, Toronto, Canada). Intestinal morphology was assessed in all groups as previously described (31, 39). Briefly, bowel length was measured along the antimesenteric border in a standardized fashion, and the proximal and distal intestinal circumference was measured 2 cm below the ligament of Treitz and 2 cm above the ileocecal valve. Samples for microscopic morphology were harvested 2 cm below the distal anastomosis, in 10% formalin for 48 h, dehydrated, and embedded in paraffin. Sections cut at 6-μm thickness were stained with hematoxylin and eosin, and then an observer, blinded to the origin of the tissue, assessed morphology. For each sample slide, microscopic measurements of villus height, crypt depth, and number of villi per 100 μm, was recorded from a minimum of 10 well-oriented villi per crypt units (39). Total mucosal surface area from each animal’s remnant intestine was also calculated to perform correlation comparisons with the serum GLP-2 levels. The serosal and villus surface area was used to calculate intestinal surface area using methods previously described (31, 39).

CCP. CCP was assessed in days 7, 14, and 28 rats by quantification of the incorporation of the S-phase marker BrdU in intestinal crypts (15). Preparations of the tissue were sectioned (6 μm) and denatured with 1 M HCl for 1 h at 60°C, and then blocked for 2 h with a PBS solution containing 10% normal goat serum and 1% BSA. Ileal tissue was incubated in a 1:200 dilution of monoclonal mouse BrdU primary antibody (Sigma) in the same blocking solution at 4°C overnight. Crypt cells incorporating BrdU were detected by immunofluorescence with a Cy3-conjugated anti-mouse IgG secondary antibody (Jackson Immunoresearch Laboratories, West Grove, PA). Ten consecutive well-oriented crypts per slide were reviewed by an observer blinded to tissue source and reported as the number of BrdU staining cells per crypt.

Statistical methods. Statistical analysis was done by using GraphPad Prism, Version 3.0 software (GraphPad, San Francisco, CA). Means were reported as ± SD vs. control animals at parallel time points.

RESULTS

In general, all animals tolerated and survived the surgery (>95% survival rate). In the initial time-course study, GLP-2 levels were increased both at baseline and after meal stimulation after a 90% resection (Fig. 2). If the serum concentration of GLP-2 vs. time is integrated to give the area under the curve (AUC) as a measure of exposure to the peptide, then over 90 min, the AUC of the 90% resected animals is 171.1 vs. 15.8 for nonresected animals ($P < 0.001$).

Nutrient absorption and GLP-2 levels. In the second study, varying the extent of resection affected the degree of malabsorption (Fig. 3). Rats that received >75% resection had increased water content in their stool, which resulted in decreased consistency and mild diarrhea (data not shown). As the degree of resection was increased, there was a trend to lose weight; this reached significance in the 90% resection animals (Fig. 3). Balance studies demonstrated that in parallel to the degree of intestinal resection, there was an increase in nutrient and energy malabsorption. The animals subjected to a 90% resection absorbed significantly less energy, fat, and protein compared with the transected controls (Fig. 3). Resection of 50% or greater of the small bowel resulted in significant increases in protein malabsorption (Fig. 3). There was a progressive increase in the 1-h postprandial GLP-2 levels with further degrees of resection (Fig. 4). There was a significant inverse correlation between fat and protein absorption and postprandial GLP-2 levels (Fig. 5A) and also between calculated intestinal surface area and GLP-2 levels (Fig. 5B).

GLP-2 and intestinal adaptation over time. The 90% resected rats had significant reductions in weight gain up to day 7 when compared with controls. This was improved by day 14 and normalized by day 28 (Table 1). After 90% intestinal resection, there were sustained increases in both the fasting, and postprandial levels of GLP-2 (Fig. 6). The postprandial rise in GLP-2 was maximal at days 4–7, but at all time points, the resected animals had significantly greater GLP-2 levels. Intestinal morphology showed the expected increase in gross and microscopic morphology as the weight, length, and the diameter of the remnant intestine was significantly increased by day 7 postsurgery and remained elevated up to day 28 (Table 1). Indicative of a specific intestinal tropic response, small intestinal weight as a percentage of body weight was increased at all time points (Table 1). When compared with controls, intestinal resection significantly increased villus height by day 7 and continued to stay significantly elevated out to 28 days (Table 1).
Fig. 7). There was no change in kidney or liver weights at any of the time points tested (Table 1). CCP was significantly increased in resected animals at all time points measured (Table 1). We correlated GLP-2 levels with CCP from all rats tested over the 28-day study and found that the levels significantly correlated with increased CCP ($r^2 = 0.48; P < 0.01$ Fig. 8).

**DISCUSSION**

This study shows that intestinal resection is associated with a sustained increase in fasting and postprandial-stimulated GLP-2 levels. The extent of resection, the resultant nutrient malabsorption, and the rate of CCP correlated with the rise in GLP-2 levels. These results corroborate previous studies (26), which have shown that intestinal resection does increase GLP-2 levels. However, the investigators recorded the GLP-2 levels 1–3 h postprandially (assuming a normal nocturnal feeding pattern for the animals). Results from the present study demonstrate important elements in the time course of nutrient-stimulated GLP-2 release, because both the basal and postprandial levels of GLP-2 were increased after a major resection. The detailed time-course study suggests that the release of GLP-2 is controlled by a combination of an early neuronal/humoral stimulus as well as luminal stimulants. Clearly, the neuronal or humoral components are responsible for the elevation of GLP-2 in the early postoperative period, because the 15-min postprandial rise in GLP-2 levels occurs before the gavaged meal comes in contact with the L cell population in the ileum. This confirms previous studies done by using surrogate markers of GLP-1 and -2 release in an anesthetized animal model (34).

Previous studies (2, 41) have suggested that after resection or transposition, the L cell population of remnant ileum does not increase, nor does the tissue content of GLP-2. This suggests that the remnant L cell population is more sensitive to proximal neuronal or increased humoral signaling factors, e.g., glucose-dependent insulmotropic polypeptide (GIP) or CCK (stimulators of hindgut hormone release), which are upregulated after bowel resection (1). Basal and postprandial levels of gastrin, CCK, GIP, PYY, and enteroglucagon are reported to be elevated at 1 mo after small bowel resection, though only enteroglucagon, PYY, and GIP showed sustained elevations after enterectomy; the gastrin and CCK changes were transient (1). After the paradigms established for GLP-1 and PYY, the nutrient-stimulated release of the foregut hormones GIP and CCK may provide the stimulus for the early release of GLP-2 and the other hindgut hormones (4, 34).
The present study also showed that there is a consistent increase in the fasting or basal levels of GLP-2 after intestinal resection. Although the basal levels were sampled from fasted animals, there was still a large amount of nutrient-rich material in the distal bowel. Therefore, the increase in basal GLP-2 levels in the resected animals could be the result of the increased presence of undigested nutrients not normally found in the distal bowel (primarily cecum and colon). This hypothesis is supported by the nutrient balance studies in which the rats that had the greatest degree of intestinal resection had decreased nutrient absorption and elevated GLP-2 levels (Figs. 3 and 4). This may be augmented by the presence of pancreaticobiliary secretions not normally present in the distal bowel. Previous studies (3, 18, 45) have shown that bile and pancreatic secretions diverted into the ileal lumen can stimulate mucosal growth and ileal adaptation. As noted in the era before the discovery of GLP-2, bile and pancreatic secretions may stimulate the intestinal segment containing the “enteroglucagon” containing cells to release peptide (3). Therefore, in our study in which we removed 90% of the proximal intestine, the delivery of the pancreaticobiliary secretions/chyme mixture could stimulate the distal L cells, thereby increasing the production of GLP-2.

The increase in GLP-2 was shown to correlate directly both with the degree of intestinal resection and with nutrient malabsorption. As hypothesized, GLP-2 levels were inversely correlated with both intestinal nutrient absorption and surface area (Fig. 5, A and B). This suggests a dose-response relationship between nutrient delivery into the distal small intestine and colon that is responsible for stimulating enteroendocrine L cell release of GLP-2. However, as noted in the time-course experiments, this may involve a direct effect of luminal nutrients on the L cell or an alteration in the neuronally mediated signaling mechanisms or perhaps both (4, 11, 34).

The third phase of the study demonstrated a significant correlation between serum GLP-2 levels and CCP (Table 1, Fig. 8). Increased CCP rates after the administration of exogenous GLP-2 have been previously reported (29), but an association between endogenous GLP-2 levels and CCP has not been shown. Though correlative, these results are supportive of a role for GLP-2 in the initiation of intestinal adaptation. Increases in intestinal CCP rates appear to be the fundamental mechanism underlying nonspecific intestinal adaptation to increased nutrient availability (46, 46a). Thus a role for GLP-2 in

![Graph A: Correlation of nutrient absorption with serum GLP-2 levels.](image)

![Graph B: Correlation of fat absorption with serum GLP-2 levels.](image)

![Graph C: Correlation of mucosal surface area with serum GLP-2 levels.](image)

**Table 1. Ileal crypt cell proliferation and morphological changes**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>C7</th>
<th>R7</th>
<th>C14</th>
<th>R14</th>
<th>C28</th>
<th>R28</th>
</tr>
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<tr>
<td>Body weight, %</td>
<td>0.04±2.8</td>
<td>-12.3±9*</td>
<td>3.5±3.7</td>
<td>0.05±5.4</td>
<td>15.7±3.6</td>
<td>13.1±4.4</td>
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<tr>
<td>Villus height, μm</td>
<td>512±52</td>
<td>658±36*</td>
<td>525±62</td>
<td>706±74*</td>
<td>509±61</td>
<td>707±68*</td>
</tr>
<tr>
<td>CCP†</td>
<td>13.9±2.7</td>
<td>25.3±4.1*</td>
<td>14.2±2.9</td>
<td>23.6±4.3*</td>
<td>14.3±5.2</td>
<td>25.7±8*</td>
</tr>
<tr>
<td>Bowel weight, %</td>
<td>0.47±0.12</td>
<td>1.09±0.2*</td>
<td>0.4±0.2</td>
<td>0.93±0.1*</td>
<td>0.5±0.06</td>
<td>0.8±0.1*</td>
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<tr>
<td>Bowel length, cm</td>
<td>10.2±0.5</td>
<td>12.3±1.2*</td>
<td>11.1±1.2</td>
<td>13.1±0.2*</td>
<td>12.0±0.9</td>
<td>14.0±4*</td>
</tr>
<tr>
<td>Bowel width, cm</td>
<td>0.46±0.05</td>
<td>0.9±0.13*</td>
<td>0.48±0.04</td>
<td>1.0±0.12*</td>
<td>0.5±0.05</td>
<td>1.1±0.1*</td>
</tr>
<tr>
<td>Colon weight, g</td>
<td>0.99±0.2</td>
<td>1.24±0.1*</td>
<td>0.97±0.2</td>
<td>1.02±0.06</td>
<td>1.1±0.2</td>
<td>1.29±0.3</td>
</tr>
<tr>
<td>Kidney weight, g</td>
<td>1.19±0.08</td>
<td>1.13±0.1</td>
<td>1.14±0.1</td>
<td>1.06±0.06</td>
<td>1.2±0.1</td>
<td>1.1±0.05</td>
</tr>
<tr>
<td>Liver weight, g</td>
<td>8.92±0.5</td>
<td>8.66±1.4</td>
<td>8.83±0.9</td>
<td>8.23±0.9</td>
<td>9.2±0.7</td>
<td>8.9±0.6</td>
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Data are expressed as means ± SD. All harvested tissue values are reported as wet weight. Intestinal morphology showed the expected increase in gross and microscopic morphology. Length, diameter, weight, and villus height of the remnant ileum was significantly increased at all time points following 90% intestinal resection. Crypt cell proliferation (CCP) was significantly increased by day 7 and remained elevated out to 28 days. Body weight is represented as % of preoperational weight. Bowel weight is reported as bowel wet weight as a percentage of final body weight. C, control; R, 90% resection; 7, 14, and 28, 7, 14, and 28 days, respectively. †CCP, 5-bromo-2′-deoxyuridine-positive cells per crypt. *P < 0.05 vs. controls at similar time point.
controlling this response would allow for manipulation of these events in a variety of pathophysiological states.

If GLP-2 is the fundamental regulator of CCP and adaptation, then an understanding of the control of GLP-2 release would become critical in predicting the response to such diseases as Crohn’s disease or to surgical resection of the small intestine. For example, adults with short bowel syndrome without a colon in continuity had a significant reduction in their postprandial GLP-2 levels compared with subjects with a colon (24). In addition, the response of the intestine may vary with age. We have recently reported that short bowel syndrome infants with jejunum and colon intact but no ileum, have severely reduced GLP-2 levels compared with similar adult patients (23, 38). This area requires further exploration, because it appears there may be ontological differences in the

![Fig. 6. Serum GLP-2 level changes at basal, 15 min, and 60 min after either transection and reanastomosis or 90% resection of the small intestine. Post-surgery animals were gavaged with 2 ml of a liquid replacement meal followed by blood draws at the above time points. All time points including basal (prefeeding) were significantly elevated after intestinal resection. Data reported as means ± SD; *P < 0.05.](image)

![Fig. 7. Small intestinal morphology and crypt cell proliferation on day 14 in control (C14) and 90% resection (R14) animals. Intestinal resection (B, D) induced significant increases in villus height and crypt cell proliferation at all time points tested when compared with control animals (A, C) (see Table 1; *P < 0.05).](image)

![Fig. 8. Serum GLP-2 levels correlate with crypt cell proliferation. Crypt cell proliferation was assessed by the 1-h incorporation and detection of the S-phase marker, 5-bromo-2’-deoxyuridine (BrdU). The 1-h (IR) postprandial serum GLP-2 levels correlated significantly with crypt cell proliferation (*r* = 0.482).](image)
GLP-2 producing L cells between infant and adult populations. The present studies do not allow us to comment on the mechanisms by which GLP-2 may be exerting these trophic effects, or the specific levels required to induce adaptation. The possibilities could include a relationship between the CCP and the basal level of GLP-2 or the peak postprandial stimulated level or both. These areas will require further study.

The site of intestinal resection must also be considered, because absorption and transport differences have been demonstrated when comparing the jejunum and ileum. We (29) have shown previously that the administration of GLP-2 to parenterally maintained rats (i.e., no luminal nutrients) that have all but 10 cm of ileum removed results in significant increases in SGLT-1 protein expression. Comparative studies, in which we parenterally fed cecotomized rats that had only 20 cm of remnant jejunum, showed that GLP-2 administration did not increase SGLT-1 expression in the jejunal remnant (unpublished results). Additionally, in the ileum of parenterally maintained rats, the administration of GLP-2 had no effect on apoptosis; whereas in the jejunum, GLP-2 increased apoptosis. In both of these studies, GLP-2 increased morphological parameters of adaptation such as villus height, bowel weight, and CCP, and yet there were differential effects on both the expression of nutrient transporters and apoptosis. This suggests that whereas GLP-2 is important in controlling adaptation, there are spatial or regional systems in place that use varying pathways. This area requires further exploration.

In summary, we found that the degree of intestinal resection and the resulting nutrient malabsorption was significantly correlated with increased GLP-2 levels. The resection-induced malabsorption and increase in GLP-2 levels also correlated with significant increases in intestinal villus height and CCP. Postprandial and basal GLP-2 levels, as well as nutrient malabsorption after resection, were significantly increased, indicating that luminal nutrients stimulate GLP-2 production. The importance of the time-course study revealed an increase in GLP-2 levels much earlier than would be predicted by the nutrient stimulation of the L cells directly, because nutrients delivered proximally could not have progressed down the small bowel in this short time frame. Therefore, this suggests that there may be a primary neuronal or humoral mechanism that is capable of stimulating the early release of GLP-2 followed by a second mechanism involving nutrients that can directly stimulate the release of GLP-2 from the L cells. The significant increase in nutrient-stimulated GLP-2 secretion and CCP out to 28 days suggests that GLP-2 is involved in not only the initiation, but also is involved in maintaining the ongoing adaptive process. These increases in mucosal proliferation that are temporally associated with a maintained GLP-2 release suggest that GLP-2 is important in initiating and maintaining the small intestinal adaptive response to resection.

REFERENCES


