# Intestinal function in mice with small bowel growth induced by glucagon-like peptide-2

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**Brubaker, Patricia L., Angelo Izzo, Mary Hill, and Daniel J. Drucker.** Intestinal function in mice with small bowel growth induced by glucagon-like peptide-2. *Am. J. Physiol.* 272 (*Endocrinol. Metab.* 35): E1050–E1058, 1997.—Glucagon-like peptide-2 (GLP-2) stimulates small intestinal growth through induction of intestinal epithelial proliferation. To examine the physiology of GLP-2-induced bowel, mice were treated with GLP-2 (2.5 $\mu$g) or vehicle for 10 days. Small intestinal weight increased to 136 ± 2% of controls in GLP-2-treated mice, in parallel with 1.4 ± 0.1- and 1.9 ± 0.5-fold increments in duodenal RNA and protein content, respectively ($P < 0.05$–$0.001$). Similarly, the activities of duodenal maltase, sucrase, lactase, glutamyl transpeptidase, and dipeptidyl-peptidase IV (215 ± 28% of controls; $P < 0.001$) were increased by GLP-2. Oral or duodenal administration of glucose or maltose did not reveal any differences in the ability of GLP-2-treated mice to absorb these nutrients, possibly because of decreases in expression of the glucose transporters sodium-dependent glucose transporter-1 (SGLT-1) and GLUT-2. In contrast, absorption of leucine plus triolein was increased after duodenal administration in GLP-2-treated mice ($P < 0.01$–$0.001$). Finally, GLP-2 did not alter other markers of intestinal or pancreatic gene expression, including levels of mRNA transcripts for ornithine decarboxylase, multidrug resistance gene, amylase, proglucagon, proinsulin, and prosomatostatin. Thus induction of intestinal growth by GLP-2 in wild-type mice results in a normal-to-increased capacity for nutrient digestion and absorption in vivo.

intestinal growth; intestinal enzymes; nutrient digestion; nutrient absorption

THE PROGLUCAGON GENE is expressed in both the pancreatic A and intestinal L cells (12, 26). Tissue-specific posttranslational processing of proglucagon by the prohormone convertases gives rise to different proglucagon-derived peptides (PGDPs) in each tissue (8, 26). The most notable of the pancreatic PGDPs is glucagon, whereas the L cell produces several structurally related peptides, including the glucagon-like peptides GLP-1 and GLP-2, as well as glicentin and oxyntomodulin, which contain glucagon in their sequence. The function of GLP-1 as a glucose-dependent insulinotropic factor, as well as an enterogastrone, has been well established (27, 43). However, the physiological actions of the other intestinal PGDPs, and of GLP-2 in particular, have remained unclear.

A role for the intestinal PGDPs in bowel growth was strongly suggested by studies in two patients who were found to have PGDP-producing tumors in association with small intestinal villous hyperplasia and hypertrophy (1, 36). A large number of studies using experimental models of adaptation have subsequently provided correlative evidence for a relationship between increased production of the PGDPs and growth of the small intestine (2, 32, 39). We have very recently identified the intestinal PGDP GLP-2 as a potent stimulator of growth in the small intestine of mice (9). Treatment with 6.25–43.75 $\mu$g GLP-2 twice daily for 10 days leads to a 1.5- to 2-fold increase in small intestinal wet weight, predominantly due to a significant increment in villous height. Although the histological appearance of the small intestine is normal in GLP-2-treated mice, it remains to be established whether the newly grown small intestine is capable of normal physiological functions. These analyses are particularly important in view of a report by Lund et al. (24) suggesting that GLP-2 may stimulate differentiation rather than growth of intestinal epithelial IEC-6 cells in vitro. The results of the present study demonstrate that the growth-enhanced small intestines of GLP-2-treated mice have a normal-to-increased digestive capacity, likely due to increased activity of the brush-border enzymes.

## METHODS

Six-week-old female CD1 mice (Charles River Canada, St. Constant, QC, Canada) were injected twice daily with synthetic rat GLP-2 (2.5 $\mu$g sc) in phosphate-buffered saline (PBS) or with vehicle alone (PBS) for 8–10 days. Mice were then fasted overnight, and the small intestine was removed, rinsed with saline, and weighed. Duodenal sections (the first 5 cm) were collected for analysis of enzyme activities. Additional 5- to 10-cm segments from the duodenum, jejunum, ileum, colon, and pancreas were also collected for peptide and/or mRNA transcript analyses. All animal protocols were approved under Canadian Council of Animal Care Guidelines.

*In vitro enzyme assays.* Duodenal samples from vehicle- and GLP-2-treated mice were homogenized in 2.4 ml of sodium phosphate buffer, pH 7.0, at 4°C and stored at −70°C before analysis. Protein content was determined by the Lowry protein assay (22). All data are expressed as units per gram of protein, where 1 unit hydrolyzes 1 micromole of substrate per minute.

Maltase, sucrase, and lactase activities were determined by the method of Dahlqvist (7) by use of 28 mM maltose, sucrose, and lactose, respectively, as substrates. Briefly, 0.2–10 $\mu$l of intestinal homogenate were incubated with the appropriate disaccharide for 60 min at 37°C, followed by determination of free glucose levels by use of the glucose oxidase method. It has previously been established that these conditions are nonsaturating and within the linear range of the reaction curve (7). Production of glucose was determined by extrapolation from a glucose standard curve.

Assay for glutamyl transpeptidase (γ-GGT) activity was carried out according to the method of Tate and Meister (38). Briefly, 20–40 $\mu$l of intestinal homogenate were incubated with 5 mM L-γ-glutamyl-*p*-nitroanilide in tris(hydroxymethyl)-

aminomethane buffer at pH 8.0 and 37°C for 20 min. The optical density at 410 nm was read at 1-min intervals for 20 min, and the values obtained were averaged over the linear range of the curve. The concentration of the product, *p*-nitroaniline, was determined as the absorbance/8,800, where 8,800 $M^{-1} \cdot cm^{-1}$ is the $\epsilon$ for *p*-nitroaniline.

Dipeptidyl-peptidase IV (DPP IV) activity was ascertained by use of the method of Nagatsu et al. (29). Briefly, 2.5–5 $\mu$l of intestinal homogenate were incubated with 1.5 $\mu$mol of glycylproline *p*-nitroaniline in 0.1 M glycine, pH 8.7, for 30 min at 37°C; the reaction has been reported to be linear for 120 min (29). Enzymatic activity was halted by addition of 1 M sodium acetate, pH 4.2, and the absorbance at 385 nm was determined. Enzyme activity was calculated by comparison with the absorbance of 150 nmol of the product, *p*-nitroaniline.

*RNA analysis.* Total cellular RNA was extracted from the pancreas and from 10-cm segments of both proximal jejunum and distal ileum. Tissues were homogenized in guanidium isothiocyanate, and RNA was extracted using the phenol-acid precipitation procedure, as described previously (11). RNA was size-fractionated on a 1.0% (wt/vol) agarose-formaldehyde gel and stained with ethidium bromide to assess the migration and integrity of the RNA. The RNA was transferred onto a nylon membrane and hybridized with $\alpha$-$^{32}$P-labeled cDNA probes for mouse glucose transporter-2 (GLUT-2; a gift from Dr. B. Thorens, Université de Lausanne, Geneva, Switzerland), mouse sodium-dependent glucose transporter-1 (SGLT-1; a gift from Dr. D. Rhoads, MGH Cancer Center, Boston, MA), ornithine decarboxylase [ODC (31)], multidrug resistance gene (MDR; a gift from Dr. V. Ling, BC Cancer Research Centre, Vancouver, BC, Canada), and rat proglucagon, proinsulin, and prosomatostatin (11). Bands were detected by autoradiography, and 18S RNA was used as the loading control.

*In vivo assays.* Oral nutrient tolerance tests were administered to vehicle- and GLP-2-treated mice on *days 8–10* of treatment. Mice were fasted for 16–18 h, and nutrients were administered by gastric gavage in a final volume of 0.4–0.8 ml per mouse. The nutrients tested were D-glucose (1.5 mg/g), maltose (2.25 mg/g), or a mixture of L-[$^3$H]leucine [$2.1 \times 10^6$ disintegrations$\cdot$min$^{-1}$ (dpm)$\cdot$mouse$^{-1}$ (Amersham Life Science, Oakville, ON, Canada)] and [$^{14}$C]triolein [$5.2 \times 10^5$ dpm/mouse (Amersham Life Science), dried and reconstituted in 50 $\mu$l of 10% Intralipid (Kabi Pharmacia Canada, Baie d'Urfe, QC, Canada) and 450 $\mu$l of water]. Blood was collected by tail vein for direct determination of glucose concentrations (for oral glucose and maltose assays; Glucometer Elite, Bayer, Etobicoke, ON, Canada) or for determination of plasma dpm (for leucine and triolein assays) by scintillation counting (UltimaGold Cocktail; Packard Instrument, Meriden, CT).

Duodenal nutrient tolerance tests were carried out as for oral nutrients with the following modification: mice were anesthetized with acepromazine (Atravet)-ketamine (1:1; Ayerst Laboratories, Montreal, QC, Canada and MTC Pharmaceuticals, Cambridge, ON, Canada) and pentobarbital sodium (MTC Pharmaceuticals), after which the stomach and duodenum were exposed through a midline incision. D-Glucose (0.75 mg/g), maltose (1.13 mg/g), or a mixture of [$^3$H]leucine ($2.6 \times 10^6$ dpm/mouse) and [$^{14}$C]triolein ($1.7 \times 10^6$ dpm/mouse) was then injected into the lumen of the duodenum by use of a 30-gauge needle, and the tissues were returned to the abdominal cavity. Blood samples were collected and analyzed as described for the oral nutrient tolerance tests.

*Peptide analysis.* Duodenal and ileal sections, as well as colon and pancreas, from vehicle- and GLP-2-treated mice were homogenized twice in 5 ml of extraction medium [1 N HCl, 5% (vol/vol) formic acid, 1% (vol/vol) trifluoroacetic acid, and 1% (wt/vol) NaCl], as described in detail previously (11). Peptides and small proteins were collected by passage through a $C_{18}$ Sep-Pak (Waters Associates, Milford, MA) and were stored at −70°C before analysis. PGDP content was determined in two separate radioimmunoassays (RIAs): *1*) glucagon-like immunoreactivity (GLI) was detected using antiserum K4023 (BiosPacific, Emeryville, CA), which is directed toward the midsequence of glucagon and recognizes glicentin, oxyntomodulin, and glucagon (8, 11), and *2*) GLP-2 was detected using antiserum UTTH-7, which cross-reacts with the COOH-terminal end of GLP-2 and recognizes native GLP-2, as well as the pancreatic major proglucagon fragment in which the $NH_2$-terminal end of GLP-2 is linked to GLP-1 through an intervening peptide (unpublished data). Immunoreactive insulin (IRI) levels were determined by RIA with a kit from Linco Research (St. Charles, MO); protein content in the extracted samples was measured by the Lowry protein assay (22).

*Data analysis.* Three to ten mice were analyzed per group in each experiment. Statistical differences were determined by analysis of variance with n-1 post hoc custom hypotheses tests, using an SAS program (Statistical Analysis Systems, Cary NC) for IBM computers.

## RESULTS

The wet weight of the small intestine was significantly increased after twice daily administration of 2.5 $\mu$g GLP-2 for 10 days, to 136 ± 2% of vehicle controls ($n$ = 28–32; $P < 0.001$). This was paralleled by a 1.4 ± 0.1-fold increase in the duodenal RNA content ($P < 0.05$). Body weight was not significantly different between vehicle- and GLP-2-treated animals (Fig. 1).

Treatment of mice with GLP-2 for 10 days induced a significant increase in the activities of all of the brush-border enzymes tested, when expressed as units of enzyme per 5 cm of duodenum (as percent control: maltase, 189 ± 62; sucrase, 193 ± 44; lactase, 274 ± 103; $\gamma$-GGT, 204 ± 66; and DPP IV, 214 ± 66; Fig. 2). Taken together, there was an overall increase in the enzyme activities of the duodenum in GLP-2-treated mice, rising to 215 ± 28% of controls ($P < 0.001$). These

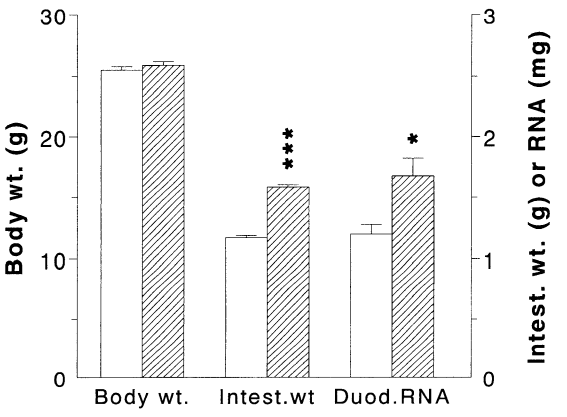



Fig. 1. Changes in body weight, total small intestinal wet weight, and total RNA content in 5 cm of duodenum in mice treated with vehicle (open bars) or 2.5 $\mu$g glucagon-like peptide-2 (GLP-2, hatched bars) twice daily for 10 days ($n$ = 3/group). *$P < 0.05$; ***$P < 0.001$.

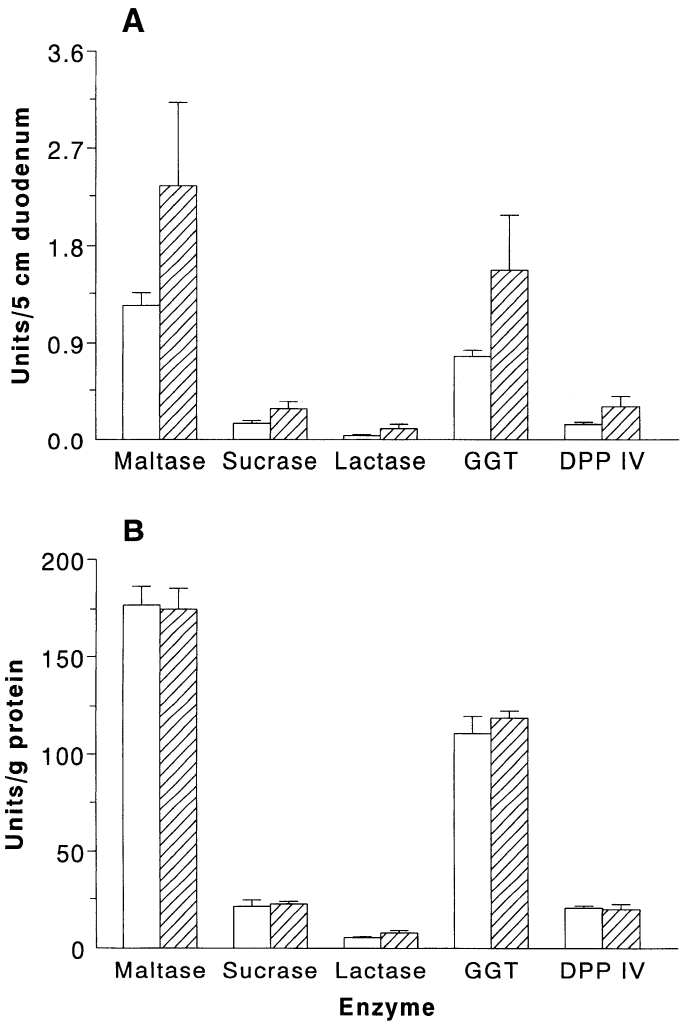



Fig. 2. Disaccharidase and peptidase activity per 5 cm of duodenum (*A*) or per g of protein in 5 cm of duodenum (*B*) from mice treated with vehicle (open bars) or 2.5 $\mu$g GLP-2 (hatched bars) twice daily for 10 days ($n$ = 3/group). GGT, glutamyl transpeptidase; DPP IV, dipeptidyl-peptidase IV. GLP-2 vs. vehicle: $P < 0.001$ (*A*); not significant (*B*).

changes occurred concomitantly with a similar increase in the total protein content of the duodenal segment, to 187 ± 54% of controls. Thus, when the data were normalized for the increased protein content (U enzyme activity per g of protein), no significant differences could be detected between vehicle- and GLP-2-treated mice for any of the enzymes assayed.

Analysis of total jejunal RNA from vehicle- and GLP-2-treated mice demonstrated that transcript levels for the luminal and basolateral facilitative glucose transporters (SGLT-1 and GLUT-2, respectively) were decreased in GLP-2-treated mice (Fig. 3*A*). Transcript levels in ileum were variable for SGLT-1 but were also reduced for GLUT-2. No marked changes were seen in either jejunum or ileum for expression of ODC, an enzyme involved in epithelial cell turnover (23). However, transcript levels for MDR, a marker of small bowel epithelium (14), were decreased in jejunum. Analysis of 18S RNA levels confirmed that loading of RNA onto each lane of the gel was equal. The results of densitometric scans of the blots are shown in Fig. 3*C*.

To assess the capacity of the intact intestine to digest a disaccharide, as well as to absorb glucose, oral glucose and maltose tolerance tests were conducted in conscious mice. Administration of either glucose or maltose was associated with a prompt rise in blood glucose levels, peaking at 20–30 min, followed by a slow fall toward basal levels over the subsequent 90 min (Fig. 4). No differences were seen between the glycemic profiles obtained for vehicle- and GLP-2-treated mice, although maltose administration was associated with a slightly faster rise in glycemia in mice receiving GLP-2 ($P < 0.01$ at $t = 10$ min). To assess the ability of the bowel to absorb amino acids, as well as to digest triglycerides and absorb the resultant fatty acids, similar studies were carried out using a mixture of [$^3$H]leucine and [$^{14}$C]triolein. Gastric gavage with this mixture resulted in a slow rise in plasma $^3$H levels, reaching a plateau at $t = 60$ min. In contrast, plasma $^{14}$C counts peaked at 45 min and then fell toward basal level over the ensuing 145 min. GLP-2 treatment did not significantly change the plasma profiles obtained in response to the oral leucine-triolein mixture.

GLP-1 is a known inhibitor of gastric emptying (43), a major determinant in the rate of flow of nutrients into the duodenum. To control for the possibility that GLP-2 treatment also affects transit from the stomach to the duodenum, we next studied the absorption of nutrients administered directly into the duodenum. The glycemic profiles obtained after duodenal administration of glucose or maltose were not different between vehicle- and GLP-2-treated mice (Fig. 5). In contrast, greater increments in the plasma levels of $^3$H and $^{14}$C were observed in GLP-2-treated mice ($P < 0.01$–$0.001$).

We also studied the effects of a single acute dose of GLP-2 on luminal nutrient absorption, because the results of a recent study suggested that intravenous infusion of GLP-2 rapidly increases the rate of epithelial cell basolateral D-glucose transport (5). Fasted mice were given a single subcutaneous injection of saline or GLP-2 (2.5 $\mu$g), followed by a duodenal glucose tolerance test 2 h later. No differences in glycemic profiles were detected between vehicle- and GLP-2-treated mice (Fig. 6).

We have previously observed that mice with PGDP-expressing tumors exhibit not only small bowel growth but also decreased expression of the intestinal and pancreatic PGDPs (10, 11). It is not known, however, whether chronic administration of GLP-2 alone has any effect on production of the endogenous PGDPs by proglucagon-expressing L or A cells. Therefore, sections of duodenum, ileum, colon, and pancreas were examined for their levels of the endogenous PGDPs by use of RIAs for both GLI and GLP-2 (Fig. 7). As anticipated from the known distribution of L cells in the mouse intestine (12), GLI and GLP-2 levels were lowest in duodenum and highest in colon. GLP-2 treatment did not affect the concentrations of the PDGPs in any of the tissues examined. Examination of the levels of proglucagon mRNA transcripts in intestine and pancreas similarly revealed a lack of effect of GLP-2 treatment

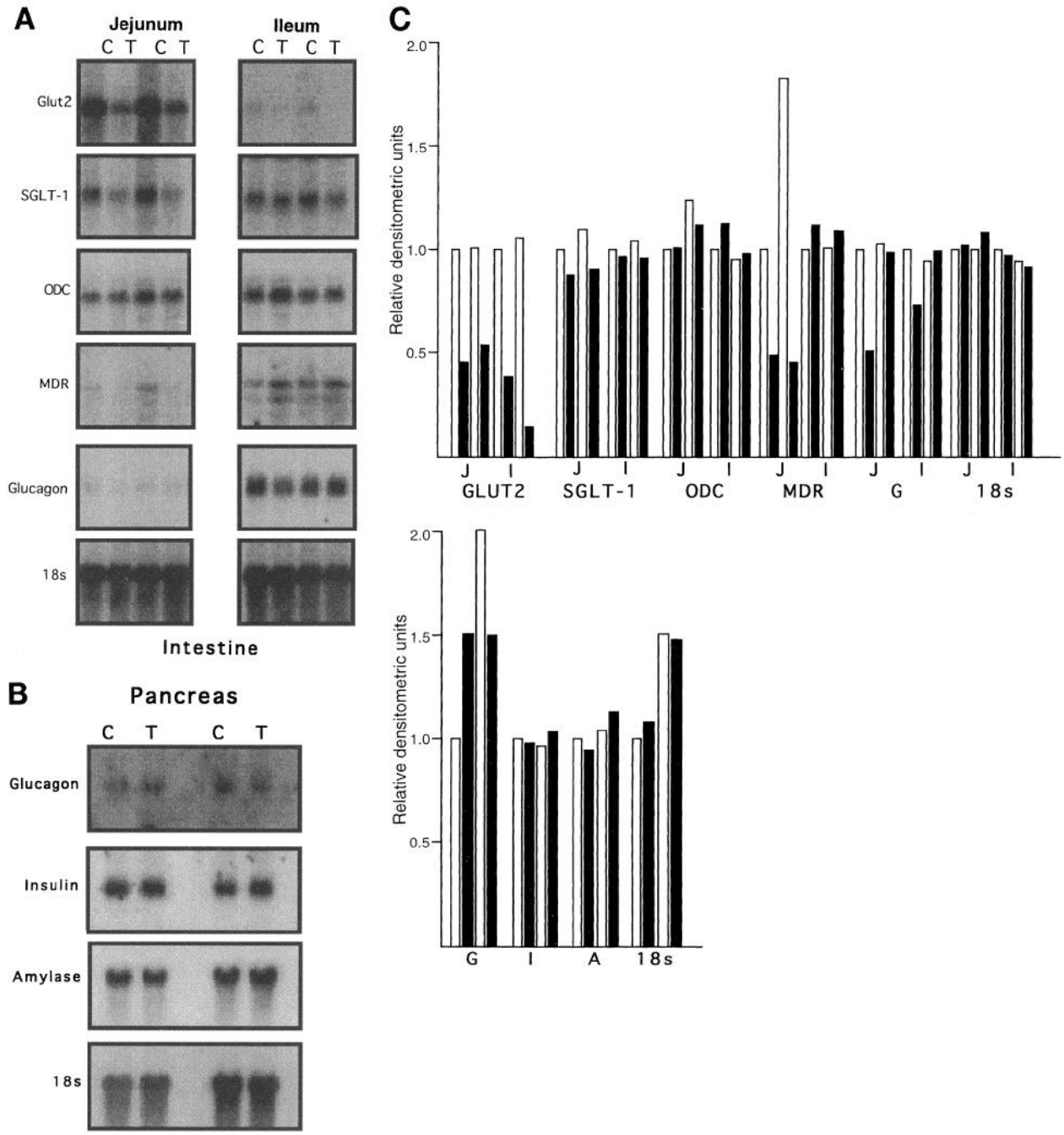



Fig. 3. Northern blot analysis of 10 $\mu$g total RNA in segments of proximal jejunum and distal ileum (*A*) and pancreas (*B*) from mice treated with vehicle (control, C) or 2.5 $\mu$g GLP-2 (treated, T) twice daily for 10 days. *C*: laser densitometry scanning of Northern blots depicted in *A* and *B*. *Top*: data from intestinal blots (*A*); *bottom*: data from pancreas (*B*). For each cDNA probe, the first control mRNA transcript was arbitrarily assigned a relative value of 1. J, jejunum; I, ileum; G, glucagon; I, insulin; A, amylase; SGLT-1, sodium-dependent glucose transporter-1; ODC, ornithine decarboxylase; MDR, multidrug resistance gene. Open bars, control mice; solid bars, GLP-2-treated mice.

on the L and A cell, respectively (Fig. 3). These observations suggest that GLP-2 administration is not associated with inhibition of endogenous proglucagon gene expression in vivo. As an additional indicator of pancreatic gene expression, immunoreactive insulin concentrations (Fig. 7) and mRNA transcript levels for proinsulin, amylase (Fig. 3, *B* and *C*) and prosomatostatin (not shown) were also assessed and found to be normal after 10 days of GLP-2 administration.

## DISCUSSION

Treatment of mice for 10 days with 2.5 $\mu$g GLP-2 twice daily resulted in a 36% increase in small bowel wet weight. This change is consistent with the results of a previous study, in which 1.5- to 2-fold increments in murine small intestinal weight were observed after 10 days of treatment with higher concentrations of GLP-2 (9). Although increased nutrient intake by itself can

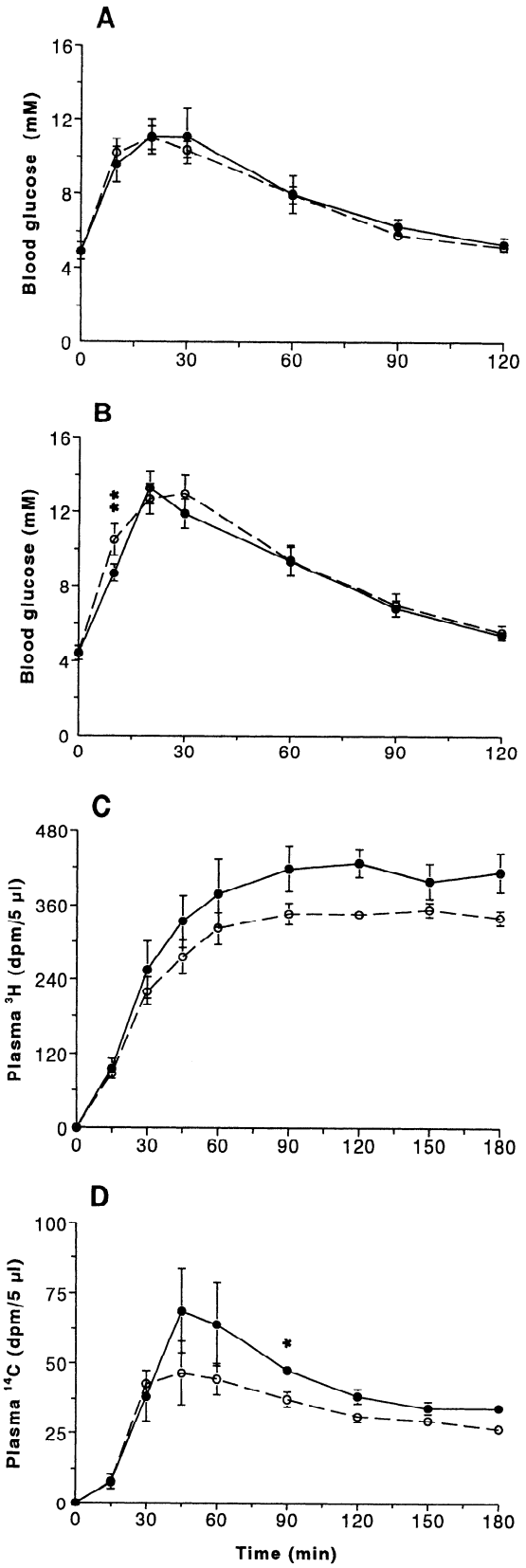


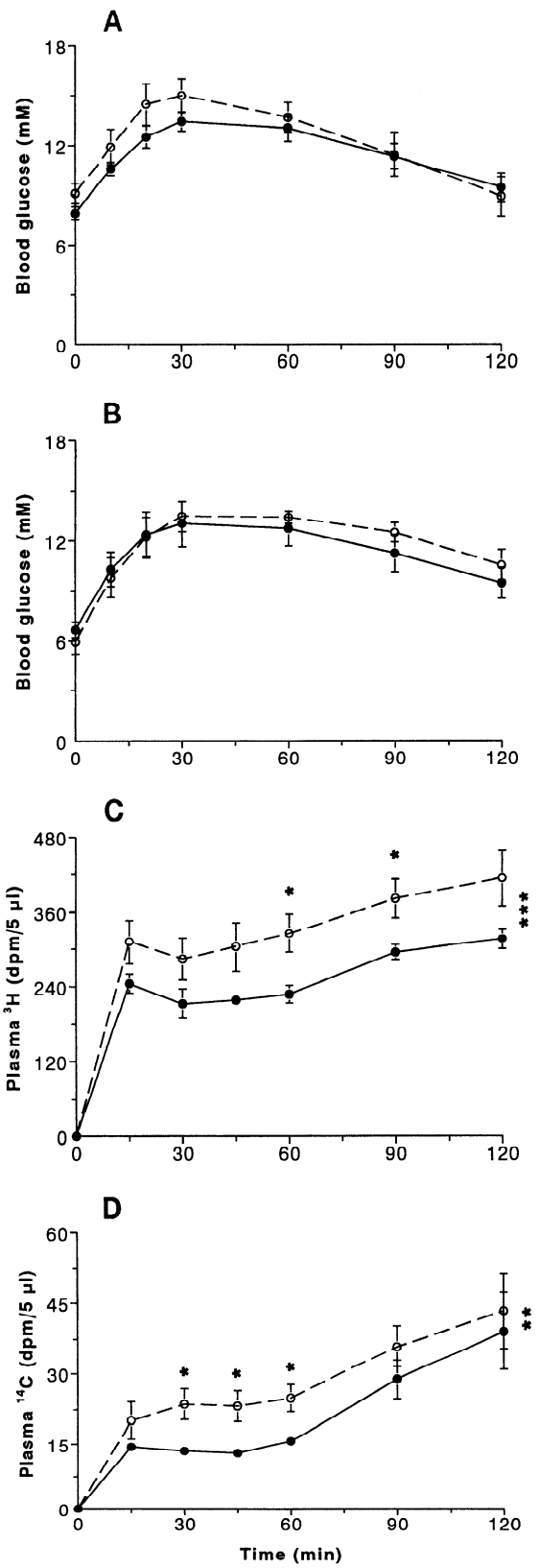

Fig. 4. Oral nutrient tolerance tests in mice treated twice a day for 10 days with vehicle (●; $n = 4$–5) or GLP-2 (2.5 μg; ○; $n = 5$–9). Nutrients [glucose (1.5 mg/g, *A*), maltose (2.25 mg/g, *B*), or [$^3$H]leucine [$2.1 \times 10^6$ disintegrations·min$^{-1}$ (dpm)·mouse$^{-1}$, *C*] plus [$^{14}$C]triolein ($5.2 \times 10^5$ dpm per mouse, *D*)] were administered by gastric gavage, followed by tail vein sampling for determination of blood glucose concentrations or plasma levels of $^3$H and $^{14}$C, as appropriate. $^*P < 0.05$, $^{**}P < 0.01$.

stimulate intestinal growth (2), the growth-promoting effects of GLP-2 are not due to a stimulation of food intake after GLP-2 administration (Brubaker and Drucker, unpublished data) and occur in the absence of any changes in body weight. Consistent with the observed increase in intestinal weight, both total protein and total RNA were also increased in the duodenum of GLP-2-treated mice. Morphological analyses have demonstrated that GLP-2 increases intestinal villous height through stimulation of crypt cell proliferation but has no effect on crypt depth or muscle thickness (9). Thus the results of the present study confirm the previous report of the intestinal growth-enhancing properties of GLP-2 and extend these findings by the analysis of macromolecule expression and enzymatic function in the small bowel of GLP-2-treated mice.

The activities of the brush-border disaccharidases and peptidases determined for control mouse duodenum were comparable to those reported by others (6, 13, 28). Interestingly, the twofold increment in enzyme activity observed in GLP-2-treated mice occurred in parallel with intestinal growth; hence the increased mucosal epithelium induced by GLP-2-treatment exhibited normal enzyme concentrations. Similar to the effects of GLP-2 administration alone, intestinal growth after massive small bowel resection is associated with up to 110% increases in the activities of maltase, sucrase, and lactase in young rats (44). It must be noted, however, that increased dietary substrate loading can lead to induction of maltase, sucrase, DPP IV, and γ-GGT, but not of lactase, in mouse intestine (6, 13, 34). Similarly, the activities of maltase, sucrase, lactase, and DPP IV in human intestine are markedly decreased by total parenteral nutrition and are restored by refeeding (15). However, the GLP-2-induced increase in enzyme activity occurred in the absence of changes in nutrient intake (Brubaker and Drucker, unpublished data), suggesting a direct effect of GLP-2 on the intestinal mucosa. The mechanisms whereby the effects of GLP-2 are exerted are not known, because the GLP-2 receptor has not yet been identified. Nonetheless, the effects of GLP-2 appear to be relatively specific for intestinal enzymes, as pancreatic amylase levels were not affected.

GLP-2 is not the only growth factor known to stimulate small intestinal growth. Elevation of growth hormone levels in transgenic mice leads to intestinal hyperplasia in association with enhanced production of insulin-like growth factor I (IGF-I) in the intestine (41). Interestingly, although duodenal sucrase activity is increased in the transgenic mice, lactase levels remain normal. Treatment with IGF-I alone, however, does not affect the activity of any of the duodenal disaccharidases (42). Furthermore, systemic administration of cortisol, a steroid hormone important for intestinal development (28), leads to increases in the activities of maltase, sucrase, lactase and γ-GGT (20), whereas a luminal growth factor, pentagastrin, has no effect on any of the disaccharidases (16), and induction of epithelial hyperplasia with either bombesin or triiodothyronine is associated with decreased lactase expression

(18). A different pattern is again seen with administration of epidermal growth factor, which increases maltase activity and stimulates intestinal growth in resected rats (37). Thus, although many different factors



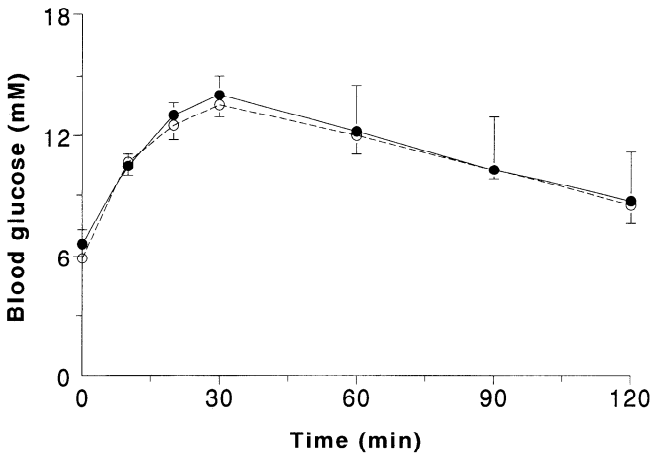




Fig. 6. Duodenal glucose tolerance test in mice pretreated with vehicle (●; $n$ = 3) or GLP-2 (2.5 μg; ○; $n$ = 4) 2 h before testing. Glucose (0.75 mg/g) was administered by injection into duodenum of anesthetized mice, followed by tail vein sampling for determination of blood glucose concentrations.

stimulate growth of the small intestine, including GLP-2, the effects of each factor on intestinal brush-border enzyme activity appear to be unique.

Despite the increased capacity of the small intestine to digest disaccharides after chronic treatment with GLP-2, no marked changes were observed in the glycemic responses to orally administered maltose. Similarly, glucose absorption alone was not affected after either chronic or acute administration of GLP-2. Assimilation of maltose across the intestine requires cleavage by maltase into two units of glucose, followed by absorption of the glucose across the enterocyte through the actions of both SGLT-1 and GLUT-2 (17, 40). Unexpectedly, the mRNA transcript levels for both of these facilitative glucose transporters were decreased in the jejunum of GLP-2-treated mice, and GLUT-2 transcript levels were also decreased in the ileum. Thus, despite the increased capacity of the small intestine to cleave maltose after GLP-2 treatment, this enhanced digestive activity may be uncoupled from subsequent absorption of the glucose product. Interestingly, mRNA transcript levels for both GLUT-2 and SGLT-1 are known to undergo adaptive increases in times of perceived glucose demand, such as in insulin-deficient diabetes mellitus (4, 17), whereas basolateral glucose transport is increased by hyperglycemia (25). The results of the present study therefore suggest that, in the absence of an increased requirement for glucose, SGLT-1 and GLUT-2 expression does not undergo parallel adaptation with that of the disaccharidases. In recent experiments with rats, acute (4-h infusion) administration of GLP-2 increased the maximum veloc-

Fig. 5. Duodenal nutrient tolerance tests in mice treated twice a day for 10 days with vehicle (●; $n$ = 6–8) or GLP-2 (2.5 μg; ○; $n$ = 5–7). Nutrients [glucose (0.75 mg/g, $A$), maltose (1.13 mg/g, $B$), or [$^3$H]leucine (2.6 × $10^6$ dpm/mouse, $C$) plus [$^{14}$C]triolein (1.7 × $10^6$ dpm/mouse)] were administered by injection into the duodenum of anesthetized mice, followed by tail vein sampling for determination of blood glucose concentrations or plasma levels of $^3$H and $^{14}$C, as appropriate. *$P < 0.05$ vs. same time point for vehicle-treated mice; **$P < 0.01$, ***$P < 0.001$ for GLP-2 vs. vehicle treatment over all time points.

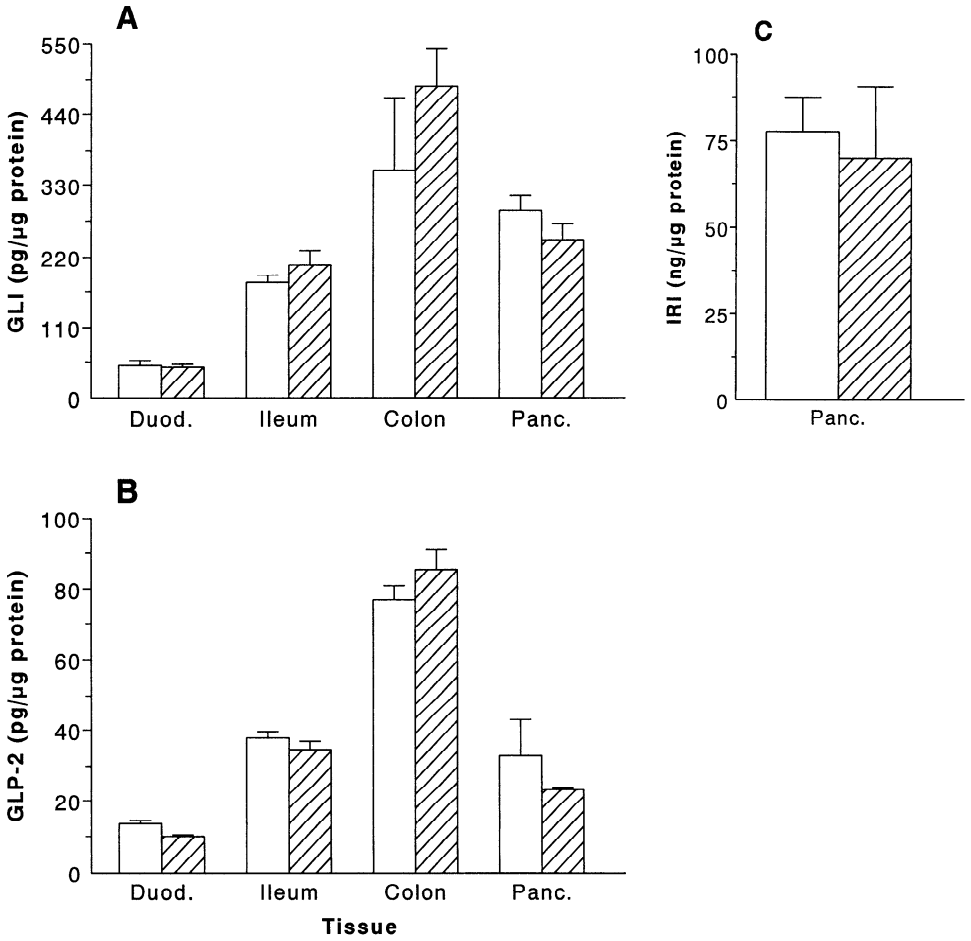



Fig. 7. Concentrations of glucagon-like immunoreactivity (GLI, *A*), GLP-2 (*B*), and immunoreactive insulin (IRI, *C*) in 5 cm of duodenum, ileum, colon, and/or pancreas of mice treated with vehicle (open bars) or 2.5 μg GLP-2 (hatched bars) twice daily for 10 days ($n$ = 3/group).

ity for D-glucose transport in jejunal basolateral membrane vesicles (5). How these findings relate to the present observations made in vivo remains to be determined; however, we did not observe changes in glucose absorption after a single injection of GLP-2. Finally, it must be noted that the normal glycemic profiles obtained for GLP-2-treated mice in response to luminal glucose or maltose strongly suggest that the enteroinsular axis is not affected by chronic administration of GLP-2. Consistent with this suggestion, the levels of glucagon and insulin, as well as of the mRNA transcripts for the pancreatic hormones, were normal after treatment with GLP-2.

In contrast to the lack of effect of GLP-2 on carbohydrate handling by the intestine, absorption of leucine and triolein by the duodenum was increased. This phenomenon was not observed after oral administration of these nutrients, presumably because of the ability of the stomach to control the rate of duodenal nutrient delivery. Whether GLP-2 has any effect on gastric emptying, as described for GLP-1 (43), remains to be determined. Leucine absorption by the gut occurs through the actions of branched-chain amino acid transporters on the luminal and basolateral sides of the enterocyte (35). In contrast to the relatively simple pattern for amino acids, triolein must first be digested by pancreatic lipase and the fatty acids absorbed into mixed micelles. Fatty acid diffusion across the lipid bilayer of the enterocyte is then facilitated by a fatty acid-binding protein, followed by reesterification into triglycerides, packaging into chylomicrons, and progressive movement through the lymphatic system into the circulation (21). It is therefore difficult at the present time to ascertain at which level(s) the stimulatory effects of GLP-2 on uptake of leucine and triolein were exerted.

Synthesis of GLP-2 by the L cell occurs concomitantly with that of the other PGDPs contained within the sequence of proglucagon, including glicentin, oxyntomodulin, and GLP-1 (8, 26). Oxyntomodulin is known to exert inhibitory effects on gastric acid secretion (33), whereas GLP-1 is both an incretin and enterogastrone (27, 43); GLP-1 also inhibits pancreatic glucagon release (19, 43). It was therefore important to establish whether chronic administration of GLP-2 results in either stimulation or inhibition of the intestinal L cell, leading to changes in production of these bioactive peptides. Analysis of proglucagon gene expression, as well as of intestinal GLI (glicentin and oxyntomodulin) and GLP-2 content, did not reveal any effect of GLP-2 on the intestinal L cell. Similarly, GLP-2 did not affect proglucagon mRNA transcript levels or PGDP content in the other major site of proglucagon gene expression, the pancreatic A cell. These findings are consistent with those of previous reports showing no effect of GLP-2 on secretion of glicentin and oxyntomodulin from fetal rat

intestinal L cells in primary culture (3) or on glucagon release from the perfused rat pancreas (19). This finding of a lack of effect of GLP-2 on either the L or A cell may be important for future considerations of GLP-2 as a potential therapeutic agent.

Although no information is available in the literature regarding circulating concentrations of GLP-2 in mice, fasting plasma levels in humans have been reported to be 600 pg/ml (30). Preliminary analyses in our laboratory have indicated that the plasma GLP-2 concentration in fasting mice is ~320 pg/ml and that this is raised 150-fold 2 h after administration of 43.75 $\mu$g of GLP-2 (Brubaker, unpublished data). In the present studies, therefore, treatment of mice with 2.5 $\mu$g of GLP-2 was predicted to raise GLP-2 levels by approximately ninefold. It must be noted, however, that these values reflect the circulating levels of immunoreactive peptide only, and determinations of increments in biologically active GLP-2 levels await detailed studies on the metabolism of this peptide.

In summary, the results of the present study demonstrate that administration of GLP-2 stimulates duodenal brush-border disaccharidase and peptidase activity in association with growth of the small intestine. These findings suggest that GLP-2 induction of small bowel growth results in proliferation of intestinal epithelium that appears to retain the functional capacity for normal nutrient absorption in vivo.

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