Glucagon-like peptide-2 protects against TPN-induced intestinal hexose malabsorption in enterally refed piglets


1United States Department of Agriculture-Agricultural Research Service, Children’s Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, Houston, Texas; and 2Department of Biological Sciences, Mississippi State University, Starkville, Mississippi

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Cottrell, J. J., B. Stoll, R. K. Buddington, J. E. Stephens, L. Cui, X. Chang, and D. G. Burrin. Glucagon-like peptide-2 protects against TPN-induced intestinal hexose malabsorption in enterally refed piglets. Am J Physiol Gastrointest Liver Physiol 290: G293–G300, 2006. First published September 15, 2005; doi:10.1152/ajpgi.00275.2005.—Premature infants receiving chronic total parenteral nutrition (TPN) due to feeding intolerance develop intestinal atrophy and reduced nutrient absorption. Although providing the intestinal trophic hormone glucagon-like peptide-2 (GLP-2) during chronic TPN improves intestinal growth and morphology, it is uncertain whether GLP-2 enhances absorptive function. We placed catheters in the carotid artery, jugular and portal veins, duodenum, and a portal vein flow probe in piglets before providing either enteral formula (ENT), TPN or a coinfusion of TPN plus GLP-2 for 6 days. On postoperative day 7, all piglets were fed enterally and digestive functions were evaluated in vivo using dual infusion of enteral (13C) and intravenous (2H) glucose, in vitro by measuring mucosal digestive functions were evaluated in vivo using dual infusion of enteral (13C) and intravenous (2H) glucose, in vitro by measuring mucosal digestions and in vivo net glucose absorption in GLP-2 compared with TPN alone. These endpoints were similar in ENT and GLP-2 pigs except for a lower intestinal weight and net glucose absorption in GLP-2 compared with ENT pigs. The enhanced hexose absorption in GLP-2 compared with TPN pigs corresponded with higher lactose digestive and apical glucose transport capacities, increased abundance of sodium glucose transporter-1 (SGLT-1) and glucose transporter-2 (GLUT2). Both ENT and GLP-2 pigs had larger intestine weights, longer villi, and higher lactose digestive capacity and in vivo net glucose and galactose absorption compared with TPN alone. These increased intestinal abundance of sodium glucose transporter-1 (SGLT-1) in the brush-border membrane (BBM) (10).

TPN reduces intestinal blood flow and alters the metabolism of the intestinal mucosa, including decreased protein synthesis and increased glucose metabolism (5, 7, 14, 31). Given that most preterm infants receive some degree of TPN before commencement of enteral feeding, it is conceivable that TPN may compromise the transition to full enteral feeding.

Glucagon-like peptide-2 (GLP-2) is a gut hormone that is postranslationally processed from the proglucagon gene product localized in enteroendocrine L cells in response to enteral nutrition, especially carbohydrate and lipid (13, 20, 28, 36). A robust intestinal trophic response to GLP-2 treatment has been observed in many studies due in part to stimulation of epithelial cell survival, crypt cell proliferation, and protein synthesis (2, 6, 8, 12, 17, 40). GLP-2 may be useful in the clinical management of TPN-fed neonates, because it has been approved for treatment of adult short-bowel syndrome and many of its biological actions counteract the negative effects of TPN. Increased villus height after GLP-2 treatment is accompanied by increased intestinal disaccharidase and peptidase expression and activity (2). Moreover, transient increases in basolateral glucose net uptake have been observed in GLP-2-treated rodents and in TPN-fed piglets (17). The GLP-2-mediated stimulation of glucose uptake in rodents has been linked to increased intestinal abundance of sodium glucose transporter-1 (SGLT-1) in the brush-border membrane (BBM) (10).

It was previously shown (5) that chronic TPN induces hexose malabsorption in vivo in neonatal piglets and that this was associated with mucosal villus atrophy and reduced intestinal blood flow and lactase activity. We also observed that chronic TPN resulted in increased intestinal lactate release, indicative of increased mucosal glycolytic metabolism. Thus, given previous evidence of the intestinal trophic and vasoactive actions of GLP-2, we hypothesized that GLP-2 treatment of TPN-fed piglets would prevent mucosal atrophy and maintain normal intestinal lactase activity and hexose absorptive function, facilitating the transition from TPN to enteral nutrition. The dose of GLP-2 used in this study was selected based on previous evidence that it produced a robust intestinal trophic response and supraphysiological plasma GLP-2 concentration in TPN-fed piglets (6). Moreover, the current dose used also corresponds to the pharmacological GLP-2 dose used in a recently published clinical study with short-bowel patients (23). Therefore, the aim of this experiment was to quantify intestinal lactose digestion and hexose metabolism in piglets nourished on chronic TPN or TPN plus GLP-2 infusion for 6

TOTAL PARENTERAL NUTRITION (TPN) is widely used in the clinical management of infants with gastrointestinal disorders that are unable to tolerate enteral feeding. Whereas TPN is a life saving therapy, the provision of enteral nutrients is an important stimulus for intestinal growth, and prolonged TPN can result in intestinal atrophy (29), thereby reducing digestive capacity of the intestine through reductions in villus height and brush-border digestive enzymes (27). Because TPN has been observed to reduce intestinal lactase (9, 27) and glucose transport activity (21, 26), it has been suggested that TPN may increase the risk of malabsorption, resulting in necrotizing enterocolitides (25) and osmotic imbalances (39). Additionally, TPN-induced intestinal hexose malabsorption in vivo in neonatal piglets and that this was associated with mucosal villus atrophy and reduced intestinal blood flow and lactase activity. We also observed that chronic TPN resulted in increased intestinal lactate release, indicative of increased mucosal glycolytic metabolism. Thus, given previous evidence of the intestinal trophic and vasoactive actions of GLP-2, we hypothesized that GLP-2 treatment of TPN-fed piglets would prevent mucosal atrophy and maintain normal intestinal lactase activity and hexose absorptive function, facilitating the transition from TPN to enteral nutrition. The dose of GLP-2 used in this study was selected based on previous evidence that it produced a robust intestinal trophic response and supraphysiological plasma GLP-2 concentration in TPN-fed piglets (6). Moreover, the current dose used also corresponds to the pharmacological GLP-2 dose used in a recently published clinical study with short-bowel patients (23). Therefore, the aim of this experiment was to quantify intestinal lactose digestion and hexose metabolism in piglets nourished on chronic TPN or TPN plus GLP-2 infusion for 6

Address for reprint requests and other correspondence: D. G. Burrin, USDA-ARS, Children’s Nutrition Research Center, Dept. of Pediatrics, Baylor College of Medicine, Houston, TX 77030 (e-mail: dburrin@bcm.tmc.edu).

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days. To quantify the metabolic fate of intestinal glucose metabolism, we used a dual infusion of enteral ([13C]) and intravenous ([1H]) glucose, respectively, and further characterized the mucosal and cellular determinants of glucose transport, including SGLT-1 and glucose transporter-2 (GLUT-2) abundance.

MATERIALS AND METHODS

Animals and experimental design. Neonatal crossbred piglets (Large White × Hampshire × Duroc) were acquired from the Texas Department of Criminal Justice (Huntsville, TX) at 4 days of age. Piglets were fed enterally for 7 days with 50 g/kg body wt sow milk formula (Litter Life; Merrick, Middleton, WI), which consisted of the following: 527 g lactose, 100 g fat, and 250 g protein. The protocol was approved by the Animal Care and Use Committee of the Baylor College of Medicine and was conducted in accordance with the National Research Council’s Guide for the Care and Use of Laboratory Animals.

The surgical procedure used in this experiment has been described previously (5, 38). In summary, after overnight food withdrawal, catheters were surgically inserted into the carotid artery, jugular vein, portal vein, and duodenum. Additionally, ultrasonic flow probes (65-8S, Transonic, Ithaca, NY) were implanted on the portal vein at 11 days of age. All piglets received TPN for 24 h during surgical recovery, after which piglets were assigned to one of the following treatments: enteral formula (ENT; n = 4), continuous intravenous infusion of TPN via the jugular vein (TPN; 240 ml·kg⁻¹·day⁻¹, n = 10), or TPN plus coinfusion of GLP-2 (500 pmol·kg⁻¹·h⁻¹, n = 9) for 7 days. Human GLP-2 was mixed in sterile 0.9% NaCl with 0.1% human serum albumin vehicle (American Peptide). Piglets were fed enterally for 7 days with 50 g/kg body wt sow milk formula (Litter Life; Merrick, Middleton, WI), which consisted of the following: 527 g lactose, 100 g fat, and 250 g protein. The protocol was approved by the Animal Care and Use Committee of the Baylor College of Medicine and was conducted in accordance with the National Research Council’s Guide for the Care and Use of Laboratory Animals.

Gas chromatography-mass spectrometry. Gas chromatography-mass spectrometry (GCMS) was performed on the pentaaceted derivative of glucose and pentafluorobenzyl bromide derivative of lactate using a 5890 Series II gas chromatograph linked to a 5890 series quadrupole mass spectrometer (Hewlett Packard, Palo Alto, CA). The isotopic enrichment (IE) was determined using electron impact ionization for ions with a mass-to-charge ratio of 242–244 and 131–134 for [13C]glucose or [1H]glucose and [13C]lactate, respectively. 13CO2 was measured using continuous-flow gas flow coupled to an isotope ratio mass spectrometer (Gasbench II coupled to DELTAPlusXL, ThermoFinnigan).

Plasma and tissue analyses. Plasma glucose and lactate were measured spectrophotometrically (Spectrax 190, Molecular Dynamics) using enzyme-based assays (ThermoDMA, Louisvile, CO and Trinity Biotech, Wicklow, Ireland, respectively). Plasma galactose concentrations were measured using an electrochemical analyzer (2300 Stat Plus, Yellow Springs Instruments, Yellow Springs, OH).

Protein, DNA, and lactase activity were measured on tissue homogenates using previously established methods, after centrifugation in 10 mM MgCl2 at 2,400; 19,000; and 39,000 g, providing a minimum 10-fold enrichment of lactase activity (15, 30).

In vitro apical glucose uptake. The segments from each region were everted, and 1-cm sleeves were secured by silk ligatures on stainless steel rods (0.5-cm diameter). Throughout the process, the segments and mounted sleeves were kept in cold (2–4°C), aerated 95% O2 with 5% CO2 Ringer solution. Beginning 45 min after death, the sleeves were first incubated for 5 min in 37°C aerated ringers before they were transferred for 2 min in 37°C aerated ringers containing 0.2, 1, 5, 25, or 50 mM unlabeled glucose. After the incubation, the sleeves were rinsed for 20 s in cold, glucose-free Ringer solution.

Tracer concentrations of [14C]glucose and [1H]glucose (American Radiolabelled Chemicals) were added to the incubation solutions to respectively quantify the amount of α-glucose associated with the active tissues and to correct for δ-glucose associated with the adherent fluid, and they were passively absorbed independent of carriers. After the rinse, the sleeves were removed from the rods, weight recorded, solubilized (Solvable, Perkin Elmer, Boston, MA), and scintillant was added (Ultima Gold, Perkin Elmer) for determination of radioactivity by liquid-scintillation counting (TriCarb 2500, Perkin Elmer).

Rates of glucose uptake, which represent apical transport, were expressed as nanomoles of glucose accumulated per minute per milligram of wet intestinal tissue (mmol·mg⁻¹·min⁻¹) (3). Total intestinal lactose digestive capacity and apical glucose transport capacities were calculated as the product of lactase-specific activities and maximum rates of glucose uptake (Vmax) × intestinal mass normalized to body weight (g/kg).

Calculations. The net portal balance (NPB) of glucose and galactose was calculated using the difference in the portal (Cportal) and arterial (Cam) concentrations and portal blood flow (PBFB) (1). In the calculations of portal glucose and lactate kinetics, PBF was converted
maximum rates of transport (uptake at the different glucose concentrations were subjected to 0.05) as indicated by a post hoc Tukey’s test. Rates of apical glucose plasma (IEplasma) (5).

Tracer [13C]glucose uptake kinetics were determined by incorporating (Eq. 4) and enrichments of the infusate (IEinfusate) and arterial and portal lactate concentrations.

Whole body flux (Q) in mmol·kg⁻¹·h⁻¹ was calculated from the rate of tracer infusion (R) and enrichments of the infusate (IEinfusate) and plasma (IEplasma) (5).

Statistical analyses. All data were tested for significance using a general linear model ANOVA and a Kruskal-Wallis nonparametric analysis to confirm normality (Minitab 13, Minitab, PA). Means were considered significantly different at the <0.05 confidence interval, and superscripts, where present, indicate differences in means (P < 0.05) as indicated by a post hoc Tukey’s test. Rates of apical glucose uptake at the different glucose concentrations were subjected to nonlinear regression analysis (Enzfit, Biosoft, Elsevier) to estimate maximum rates of transport (Vₘₚₙ) and apparent affinity constants (Kₘₚₙ).

RESULTS

As observed in previous experiments, TPN resulted in lower intestinal weight, jejunal and ileal villus height, and protein and DNA concentration compared with ENT, and this remained evident even after 6 h of enteral refeeding (Table 1). In accordance with its trophic properties, administering GLP-2 resulted in higher intestinal mass, jejumum and ileum villus height, and protein and DNA content after 6 h of refeeding compared with TPN. However, the level of GLP-2-mediated protection against TPN did not represent a return to values observed in ENT-fed piglets. Lactase-specific activity was reduced by TPN, compared with ENT, whereas infusion of GLP-2 resulted in intermediate activity but not significantly different from ENT or TPN. Piglets receiving GLP-2 had higher total intestinal lactase digestive capacity than those receiving TPN alone, but activity was significantly less than for the ENT-fed piglets. The treatment differences were due mainly to the variation in intestinal mass rather than specific activity.

Fasted basal PBF was not different among treatments (3.42, 4.68, and 2.67 for ENT, TPN, and GLP-2, P = 0.15). The only difference for the degree of feeding hyperemia averaged across the 6-h refeeding period was the higher value for TPN compared with the GLP-2 piglets, but neither group was different from enteral (Table 2). Intestinal O₂ uptake was lower for piglets receiving TPN relative to ENT, irrespective of GLP-2 treatment but not significantly different to ENT or TPN. Piglets receiving GLP-2 had higher total intestinal lactose digestive capacity than those receiving TPN alone, but activity was significantly less than for the ENT-fed piglets. The treatment differences were due mainly to the variation in intestinal mass rather than specific activity.

The intraduodenal infusion of formula provided equivalent lactose and hence glucose intake to all treatment groups (3.95, 4.03, 3.95 mM; P = 0.58). After the 6-h enteral refeeding protocol, in all treatments, <1% of the lactose and glucose provided was recovered in the contents of the stomach and intestinal saline flush, but this does not include lactose and glucose undigested in the small intestine that entered the colon. Arterial and portal glucose concentrations measured hourly between 3 and 6 h after intraduodenal formula infusion were

### Table 1. Intestinal weight, protein and DNA contents, and lactase activities in neonatal piglets fed for 7 days with enteral, TPN, or TPN with GLP-2 and then fed enterally for 6 h

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Enteral</th>
<th>TPN</th>
<th>TPN + GLP-2</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intestinal wt, g/kg body wt</td>
<td>50.9 ± 2.69³</td>
<td>29.4 ± 1.48³</td>
<td>35.6 ± 1.41³</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Intestinal protein, mg/kg body wt</td>
<td>6.35 ± 0.36³</td>
<td>2.99 ± 0.23³</td>
<td>4.26 ± 0.23³</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Intestinal DNA, mg/kg body wt</td>
<td>201 ± 13.6³</td>
<td>141 ± 8.6³</td>
<td>169 ± 8.6³</td>
<td>0.004</td>
</tr>
<tr>
<td>Jejunum</td>
<td>943 ± 120³</td>
<td>352 ± 90³</td>
<td>722 ± 81³</td>
<td>0.002</td>
</tr>
<tr>
<td>Ileum</td>
<td>1,456 ± 22²</td>
<td>387 ± 157³</td>
<td>1,057 ± 150³</td>
<td>0.001</td>
</tr>
<tr>
<td>Lactase-specific activity, mmol·min⁻¹·g protein⁻¹</td>
<td>75.5 ± 12.1³</td>
<td>36.6 ± 8.0³</td>
<td>52.5 ± 7.4³</td>
<td>0.035</td>
</tr>
</tbody>
</table>

Values are means ± SE, the number of animal per group were enteral (6), total parenteral nutrition (TPN) (10), and TPN + glucoselike protein-2 (GLP-2) (9). Different superscripts indicate statistical differences between treatment for jejunum and ileum based on analysis of variance and Tukey’s test (P < 0.05).

### Table 2. Portal blood flow, oxygen uptake, and carbon dioxide production in neonatal piglets fed for 7 days with enteral, TPN, or TPN with GLP-2 for 7 days, then fed enterally for 6 h

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Enteral</th>
<th>TPN</th>
<th>TPN + GLP-2</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Portal blood flow, l/min/kg body wt</td>
<td>4.32 ± 0.53³</td>
<td>4.88 ± 0.34³</td>
<td>3.62 ± 0.38³</td>
<td>0.057</td>
</tr>
<tr>
<td>O₂ uptake, mmol·kg⁻¹·h⁻¹</td>
<td>4.37 ± 0.50³</td>
<td>2.85 ± 0.32³</td>
<td>2.90 ± 0.36³</td>
<td>0.032</td>
</tr>
<tr>
<td>CO₂ production, mmol·kg⁻¹·h⁻¹</td>
<td>9.27 ± 1.16³</td>
<td>4.90 ± 0.79²</td>
<td>7.15 ± 0.82³</td>
<td>0.008</td>
</tr>
<tr>
<td>Oxygen extraction ratio, %</td>
<td>18.5 ± 0.01³</td>
<td>11.7 ± 0.01³</td>
<td>18.5 ± 0.01³</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Respiratory quotient</td>
<td>2.2 ± 0.32</td>
<td>2.3 ± 0.17</td>
<td>2.4 ± 0.19</td>
<td>0.86</td>
</tr>
</tbody>
</table>

Values are means ± SE, the number of animal per group were enteral (4), TPN (10), and TPN + GLP-2 (9). Different superscripts indicate statistical differences between treatment for jejunum and ileum based on analysis of variance and Tukey’s test (P < 0.05).
not different among treatments (Table 3). However, glucose NPB was highest in ENT-fed piglets, comprising 93% of intake. Values for TPN and GLP-2 were lower (27% and 58% of intake, respectively), suggesting both groups had incomplete digestion and absorption of the administered lactose. Arterial galactose concentrations were increased by GLP-2 compared with ENT and TPN, and this was independent of concomitant intake, whereas GLP-2 improved galactose uptake approximately twofold (52%), which was comparable with ENT-fed piglets in a prior experiment (5). TPN reduced galactose NPB to 27% of dietary intake, whereas GLP-2 improved galactose uptake approximately twofold (52%), which was comparable with ENT-fed piglets.

TPN resulted in a higher arterial lactate concentration compared with ENT, whereas GLP-2-treated piglets were intermediate and did not differ from ENT and TPN piglets. Similarly portal vein plasma lactate concentrations were elevated in piglets receiving TPN but less so when GLP-2 was administered. Net portal release of lactate was lowest in ENT-fed piglets, highest in TPN-fed piglets, and intermediate for GLP-2 piglets. Thus mucosal glycolysis was elevated in TPN and, to a lesser degree, GLP-2 groups, which was confirmed by \(^{13}\)C-lactate kinetics.

Due to a small sample size and some sample error, it was not possible to calculate in vivo \(^{13}\)C-glucose and \(^2\)H-glucose tracer kinetics for ENT-fed piglets. Comparisons of in vivo \(^{13}\)C-glucose and \(^2\)H-glucose tracer kinetics are restricted to the TPN and GLP-2 treatments (Table 4). Treatment effects were not detected for arterial and portal \(^{13}\)C-glucose IE and concentrations, despite trends of lower portal IE and arterial concentrations in GLP-2-infused piglets. Likewise, \(^{13}\)C-glucose absorption, utilization, or whole body flux did not differ between TPN and GLP-2 piglets. However, arterial and portal \(^{13}\)C-lactate enrichment and concentrations and net portal \(^{13}\)C-lactate production were significantly higher in TPN piglets (Table 5), suggesting TPN alone elevated intestinal glycolysis. As per intestinal CO2 production, \(^13\)CO2 production was not different between TPN and GLP-2. To discriminate between first pass metabolism of \(^{13}\)C-glucose and metabolism of arterial glucose by the PDV, intravenous coinfusion of \(^{2}\)H-glucose was performed (Table 5). GLP-2 infusion in-
Tukey's test (13) between treatment for jejunum and ileum based on analysis of variance and TPN/H11001/H11006 glucagon-like peptide-2 (GLP-2) for 6 days and then refed enterally for 6 h.

Given enteral nutrition (ENT), total parenteral nutrition (TPN), or TPN/H11001/H11006 was considered (glucose highest for ENT piglets, but when total hexose intake was reduced by approximately two thirds after chronic TPN (Fig. 1). GLP-2 increased whole body [3H]glucose flux, which is consistent with the increased intestinal capacities to absorb glucose (see below). Second-pass glucose metabolism did not appear to be affected by administering GLP-2, because portal [3H]glucose utilization and extraction of intravenous [2H]glucose did not differ between TPN and GLP-2 piglets.

The \( V^{\text{max}} \) for apical glucose uptake in the jejunum was highest in ENT piglets, whereas values for TPN pigs were reduced by approximately two thirds after chronic TPN (Fig. 1). GLP-2 increased the \( V^{\text{max}} \) compared with TPN, but values remained lower than ENT. This pattern was also apparent in the ileum, with the exception that the protective effect of GLP-2 on \( V^{\text{max}} \) was not apparent. The treatment differences in \( V^{\text{max}} \) were independent of changes in the \( K_{\text{m}} \). Total intestinal lactose digestive capacities of ENT piglets (Fig. 2) exceeded the lactose intake by more than fivefold. The excess capacity was reduced in TPN piglets to about twofold, with GLP-2 piglets having an intermediate capacity relative to intake (3.5-fold). Similarly, maximum glucose absorptive capacities were highest for ENT piglets, but when total hexose intake was considered (glucose + galactose), the capacity exceeded intake by only 50%. Although glucose transport capacity of TPN piglets (4.05 mmol·kg\(^{-1}·h^{-1}\)) was equivalent to lactose intake (3.95 mmol·kg\(^{-1}·h^{-1}\)), it represented only half of the combined intake of hexoses (7.9 mmol·kg\(^{-1}·h^{-1}\)). Despite stimulating a higher glucose transport capacity relative to TPN

Table 5. Rates of portal [13C]lactate, and [13C]CO\(_2\) kinetics in neonatal piglets fed enterally, with TPN, or TPN with GLP-2 infusion for 7 days, then fed enterally for 6 h

<table>
<thead>
<tr>
<th></th>
<th>TPN</th>
<th>TPN + GLP-2</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial [13C]lactate enrichment (MPE)</td>
<td>1.33±0.057</td>
<td>1.17±0.066</td>
<td>0.068</td>
</tr>
<tr>
<td>Portal [13C]lactate enrichment (MPE)</td>
<td>1.41±0.060</td>
<td>1.20±0.068</td>
<td>0.021</td>
</tr>
<tr>
<td>Net portal [13C]lactate production, mmol·kg(^{-1}·h^{-1})</td>
<td>0.027±0.0041</td>
<td>0.014±0.0046</td>
<td>0.042</td>
</tr>
<tr>
<td>Arterial [13C]CO(_2) enrichment (MPE)</td>
<td>0.14±0.011</td>
<td>0.18±0.012</td>
<td>0.028</td>
</tr>
<tr>
<td>Portal [13C]CO(_2) enrichment (MPE)</td>
<td>0.15±0.009</td>
<td>0.18±0.011</td>
<td>0.038</td>
</tr>
<tr>
<td>Portal [13C]CO(_2) production, mmol·kg(^{-1}·h^{-1})</td>
<td>0.022±0.0067</td>
<td>0.008±0.0072</td>
<td>0.17</td>
</tr>
</tbody>
</table>

Values are means ± SE, the number of animals per group were TPN (10) and TPN+GLP-2 (9) (\( P < 0.05 \)).

Despite differences in \( V^{\text{max}} \) and glucose transport capacity, abundances of SGLT-1 detected by Western blotting in the jejunum BBM and mucosa did not differ between TPN and ENT piglets (Fig. 3). However, BBM and mucosa SGLT-1 abundance was higher in GLP-2 compared with TPN piglets. SGLT-1 was not reliably detected in mucosal homogenates from the ileum (data not shown). TPN significantly reduced ileum BBM SGLT-1 abundance compared with ENT-fed piglets, with an intermediate abundance for GLP-2 piglets. Jejunum and ileum mucosal GLUT-2 abundance did not differ among treatments (Fig. 4). Jejunum BBM prepared from TPN and GLP-2 piglets had 10-fold higher abundances of GLUT-2

![Fig. 1. In vitro maximal intestinal glucose transport activity (\( V^{\text{max}} \)) in piglets given enteral nutrition (ENT), total parenteral nutrition (TPN), or TPN + glucagon-like peptide-2 (GLP-2) for 6 days and then refed enterally for 6 h. Means ± SE, the nos. of animals per group were enteral (4), TPN (8), and TPN + GLP-2 (7). Different superscripts indicate statistical differences between treatment for jejunum and ileum based on analysis of variance and Tukey’s test (\( P < 0.05 \)).](http://ajpgi.physiology.org/)

![Fig. 2. Estimated mucosal lactose digestive and glucose transport capacities in piglets given ENT, TPN, or TPN + GLP-2 for 6 days and then refed enterally for 6 h. Means ± SE, the nos. of animals per group were ENT (4), TPN (8), and TPN + GLP-2 (7). Different superscripts indicate statistical differences between treatment for jejunum and ileum based on analysis of variance and Tukey’s test (\( P < 0.05 \)).](http://ajpgi.physiology.org/)

![Fig. 3. Jejunum (A) and ileum (B) brush-border membrane (BBM) and mucosal sodium glucose transporter-1 (SGLT-1) abundance in piglets given ENT, TPN, or TPN + GLP-2 for 6 days and then refed enterally for 6 h. Means ± SE, the nos. of animals per group were enteral (4), TPN (10), and TPN + GLP-2 (9). Different superscripts indicate statistical differences between treatment for BBM and mucosa based on analysis of variance and Tukey’s test (\( P < 0.05 \)). Differing superscripts are used to denote statistical treatment differences for BBM (a, b) and mucosa (x, y). Ileum mucosal SGLT-1 was not reliably detectable and is not shown.](http://ajpgi.physiology.org/)
Lactose hydrolysis in TPN-fed piglets was significantly lower than in ENT-fed piglets, suggesting that the TPN-induced mucosal atrophy causes functional defects in lactose digestion (5). GLP-2 treatment during TPN maintained lactase-specific activity and hence digestive capacity, consistent with other findings for lactase and other BBM disaccharides in piglets and mice (2, 33–35). Moreover, the GLP-2 treatment maintained lactose digestive capacity at a level twice that of TPN-fed piglets and approximately four times higher that the lactose intake during refeeding. The lactose digestive capacity was greatest for ENT piglets being approximately eight times higher than the lactose intake. Consistent with the estimated excess lactose digestive capacities, the lactose recovery from the stomach and intestine was <1\%, with similarly low values for GLP-2 and ENT piglets. Thus, although we did not account for lactose that could have passed into the colon or lost via mild diarrhea during the 6-h refeeding period, the low recovery of lactose is congruent with rapid digestion. Interestingly, lactase activity is considered to be the limiting factor for lactose digestion in adults (11, 16, 32), whereas our findings indicate lactase activity of pigs is in excess, even for those maintained by TPN. This likely reflects the developmentally high lactose digestive capacity in neonates (32).

One of the principal findings of this experiment was that GLP-2 resulted in higher absorption of glucose and galactose during the refeeding period compared with TPN. As observed previously (5), chronic TPN markedly reduced in vivo glucose absorption to ~30\% of intake; this was only one-third the rate (90\%) found in ENT-fed piglets. Infusion of GLP-2 partially maintained glucose uptake at ~58\% of intake, yet this was still less than ENT. The net rate of intestinal glucose absorption is determined by the combined processes of apical mucosal transport and mucosal metabolism. We previously reported that TPN increased intestinal glucose metabolism to lactate, reducing glucose appearance in the portal vein. In this study, the simultaneous infusion of enteral [\(^{13}\)C]glucose and intravenous [\(^{3}\)H]glucose isotopes during the refeeding period allowed us to determine that intestinal production of [\(^{13}\)C]lactate was halved in GLP-2-infused piglets compared with piglets receiving TPN alone. Furthermore, utilization of intravenous [\(^{3}\)H]glucose in second-pass metabolism was considerably less (<10\% of intake) compared with first-pass utilization of enteral [\(^{13}\)C]glucose (~66\% of intake). These findings indicate that enterally absorbed glucose was the principal source of glucose metabolized during the refeeding of the TPN and GLP-2 treatments. Although there was no difference in [\(^{13}\)C]glucose absorption, it is noteworthy that GLP-2 increased the [\(^{2}\)H]glucose whole body flux. This is most likely due to an increase in glucose absorption in GLP-2-treated piglets, rather than an increase in endogenous glucose release, because the increase in whole body [\(^{2}\)H]glucose flux with GLP-2 treatment (~1.2 mmol·kg\(^{-1}\)·h\(^{-1}\)) was largely accounted for by increased glucose absorption (~1.0 mmol·kg\(^{-1}\)·h\(^{-1}\)). Collectively, the NPB of glucose and increased [\(^{2}\)H]glucose flux indicate that GLP-2 treatment increased in intestinal glucose absorption and reduced intestinal glycolytic metabolism of glucose.

The findings for in vivo glucose absorption were consistent with apical glucose transport capacities calculated from in vitro measurements, with both showing that capacities were lowest for TPN, intermediate for GLP-2, and highest for ENT piglets. Apical glucose transport capacities measured in this exper-
GLP-2 IMPROVES GLUCOSE ABSORPTION AFTER TPN

The lower glucose uptake by the proximal small intestine of TPN compared with GLP-2 piglets coincided with a lower BBM abundance of SGLT-1, but not GLUT-2. A similar pattern was observed in the distal segment, except for the lower abundance of GLUT2 in the whole tissue of GLP-2 piglets. Although TPN resulted in lower BBM abundances of SGLT-1 in both intestinal regions compared with ENT piglets, GLUT-2 abundances were not affected. These findings suggest the abundances of SGLT-1 and GLUT-2 in the BBM are not regulated in parallel. Moreover, because GLUT-2 translocates to the BBM in response to high luminal glucose concentrations, it is conceivable that the relative differences in GLUT-2 abundance observed in this experiment are a result of the capacity of SGLT-1 to reduce luminal glucose concentrations (24). Assuming that increased BBM abundance equates to increased transport capacity, the increased BBM abundance of SGLT-1 in ENT piglets provided an increased capacity for removal of luminal glucose and thereby removed the trigger for GLUT-2 trafficking to the BBM. In contrast, in TPN-fed piglets, the inherently lower SGLT-1 abundance would result in lower glucose absorption and thereby lead to increased luminal glucose concentrations and provide a stimulus for GLUT-2 trafficking to the BBM. However, our observations with GLP-2 treatment do not fit this model, in that BBM abundance of both SGLT-1 and GLUT-2 increased. This can be explained in part by the observation in separate reports that GLP-2 increases trafficking of either SGLT-1 or GLUT-2 into the BBM, independent of luminal glucose concentrations (1, 10), but this is the first evidence that SGLT-1 and GLUT-2 translocation occurs concurrently.

In summary, the current study provides novel in vivo evidence that the intestinal trophic effects of GLP-2 treatment during TPN translate into improved intestinal function. We found that chronic GLP-2 treatment during 6 days TPN improved in vivo glucose and galactose absorption during 6 h of refeeding. This was attributed to the ability of GLP-2 to maintain intestinal villus surface area, increase lactose digestive and apical transport capacities of hexoses in addition to reduced intestinal glycolytic metabolism. Although poor gastric emptying and motor function contribute to feeding intolerance in premature infants, the transition to enteral feeding is limited by poor intestinal digestion and glucose absorption. Thus these findings provide support for future clinical studies in infants to assess whether GLP-2 treatment during TPN improves intestinal digestion and absorptive function, thereby accelerating the transition to enteral feeding and reducing the time to full feeding.

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DISCLOSURES

The contents of this publication do not necessarily reflect the views or policies of the U.S. Department of Agriculture, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

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