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

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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 by measuring mucosal digestive functions were evaluated in vivo using dual infusion of enteral (13C) and intravenous (2H) glucose, in vitro by measuring mucosal transport capacities, increased abundance of SGLT-1, but not GLUT-2, intestinal weight and net glucose absorption in GLP-2 compared with endpoints were similar in ENT and GLP-2 pigs except for a lower glucose and galactose absorption compared with TPN alone. These findings indicate that GLP-2 treatment during chronic TPN maintains intestinal lactose digestion and hexose metabolism in 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 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 lactase activity and rates of apical glucose transport, and by assessing the abundances 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 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).
days. To quantify the metabolic fate of intestinal glucose metabolism, we used a dual infusion of enteral \(^{(13)C}\) and intravenous \(^{(2H)}\) 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, Transonics, 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\(^{-1}\)), or TPN plus coinfusion of GLP-2 (500 pmol kg\(^{-1}\) day\(^{-1}\)).

Treatment and experimental design. Piglets were entered into one of the following three dietary treatments: enteral formula (ENT; \(n = 4\)), TPN (TPN; 240 ml kg\(^{-1}\) day\(^{-1}\)), or TPN plus coinfusion of GLP-2 (500 pmol kg\(^{-1}\) day\(^{-1}\)).

**Gas chromatography-mass spectrometry.** Gas chromatography-mass spectrometry (GCMS) was performed on the pentaacetate 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 \(^{[13]C}\)glucose or \(^{[2H]}\)glucose and \(^{[13]C}\)lactate, respectively. \(^{13}CO_2\) 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 (Spectramax 190, Molecular Dynamics) using enzyme-based assays (ThermoDMA, Louisville, CO). Protein, DNA, and lactase activity were measured on tissue homogenates (2300 Stat Plus, Yellow Springs Instruments, Yellow Springs, OH). Protein, DNA, and lactase activity were measured on tissue homogenates (2300 Stat Plus, Yellow Springs Instruments, Yellow Springs, OH). Protein, DNA, and lactase activity were measured on tissue homogenates (2300 Stat Plus, Yellow Springs Instruments, Yellow Springs, OH). Protein, DNA, and lactase activity were measured on tissue homogenates (2300 Stat Plus, Yellow Springs Instruments, Yellow Springs, OH). Protein, DNA, and lactase activity were measured on tissue homogenates (2300 Stat Plus, Yellow Springs Instruments, Yellow Springs, OH). Protein, DNA, and lactase activity were measured on tissue homogenates (2300 Stat Plus, Yellow Springs Instruments, Yellow Springs, OH). Protein, DNA, and lactase activity were measured on tissue homogenates (2300 Stat Plus, Yellow Springs Instruments, Yellow Springs, OH).

**In situ jejunal 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% O\(_2\) with 5% CO\(_2\)) Ringer solution. Beginning 45 min after death, the sleeves were first incubated for 5 min in 37°C aerated ringer before they were transferred for 2 min in 37°C aerated ringers containing 0.2, 1, 5, 25, or 50 mM unlabeled lactate. After the incubation, the sleeves were rinsed for 20 s in cold, glucose-free Ringer solution. Tracer concentrations of \(^{[14]C}\)glucose and \(^{[2H]}\)lactate (American Radiolabeled Chemicals) were added to the incubation solutions to respectively quantify the amount of 

\[ \text{G} + \text{H} \rightarrow \text{G} + \text{H} \]  

In these calculations of portal glucose and lactate transport kinetics, PBF was converted to a rate constant as described previously (6, 14). Morphometric analyses of formalin-fixed, paraffin-embedded sections were performed after hematoxylin and eosin staining. Villus height, area, crypt depth, and muscularis thickness were measured using an Axioptot microscope (Carl Zeiss, Göttingen, Germany and Scion image beta 4.0 software, National Institutes of Health, Bethesda, MD). Abundance of GLUT-2 (Chemicon International) and SGLT-1 (Alpha Diagnostics) at ~60 and 57 kDa, respectively, in whole mucosa, and isolated BBM extracts was determined using Western blot analysis and subsequent densitometric analysis (Personal Densitometer SI, Molecular Dynamics, and PDSI Scanner Control v5.03 software, Amersham Biosciences, Buckinghamshire, UK). Western blot analysis inlays presented in Figs. 3 and 4 were generated by scanning films from each Western blot (Expression 636, Epson, Nagano, Japan) and cropping bands from ENT, TPN, and GLP-2 piglets that were representative of the treatment mean (Photoshop version 7, Adobe Systems). BBM tissue was prepared as per previously established methods, after centrifugation in 10 mM MgCl\(_2\); at 2,400; 19,000; and 39,000 g, providing a minimum 10-fold enrichment of lactase activity (15, 50).

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to plasma flow by correcting for the hematocrit. The NPB represents the net intestinal absorption of glucose and galactose into the portal blood. Intestinal O2 uptake was calculated as per Ref. 2. Intestinal lactate production was also calculated as per Ref. 2 by substituting lactate and 13CO2 production were calculated as per Eq. 3.

\[ \text{Eq. 3} \]

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></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 ± 2.09a</td>
<td>29.4 ± 1.48b</td>
<td>35.6 ± 1.41c</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Intestinal protein, mg/kg body wt</td>
<td>6.23 ± 0.36a</td>
<td>2.99 ± 0.23b</td>
<td>4.26 ± 0.23ab</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Intestinal DNA, mg/kg body wt</td>
<td>201 ± 13.6a</td>
<td>141 ± 8.6b</td>
<td>169 ± 8.6c</td>
<td>0.004</td>
</tr>
<tr>
<td>Jejunum Villus height, μm</td>
<td>943 ± 120a</td>
<td>352 ± 90b</td>
<td>722 ± 81c</td>
<td>0.002</td>
</tr>
<tr>
<td>Ileum Lactase-specific activity, μmol·min⁻¹·g protein⁻¹</td>
<td>1.456 ± 0.22a</td>
<td>387 ± 15b</td>
<td>1.057 ± 0.15ab</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>75.5 ± 12.1a</td>
<td>36.6 ± 8.07ab</td>
<td>52.5 ± 7.49abc</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).

RESULTS

As observed in previous experiments, TPN resulted in lower oxygen extraction and CO2 production compared with enteral feeding. The intraduodenal infusion of formula provided equivalent lactose and hence glucose intake to all treatment groups (3.95, 4.68, and 2.67 for ENT, TPN, and GLP-2, respectively). 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. 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.

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 infusion. Intestinal CO2 production was lowest in TPN piglets and highest for ENT piglets. Values were intermediate for GLP-2 treatment but not significantly different to ENT or TPN.

The intraduodenal infusion of formula provided equivalent lactose and hence glucose intake to all treatment groups (3.95, 4.03, and 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
GLP-2 IMPROVES GLUCOSE ABSORPTION AFTER TPN

Table 3. Plasma concentrations and net portal balances of glucose, galactose and lactate in neonatal piglets fed enterally, with TPN, or TPN with GLP-2 infusion for 7 days, then fed enterally for 6 hours

<table>
<thead>
<tr>
<th></th>
<th>Enteral</th>
<th>TPN</th>
<th>TPN + GLP-2</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Glucose</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial, mM</td>
<td>6.81±0.215</td>
<td>7.02±0.140</td>
<td>6.84±0.155</td>
<td>0.60</td>
</tr>
<tr>
<td>Portal, mM</td>
<td>7.99±0.277</td>
<td>7.61±0.175</td>
<td>7.65±0.199</td>
<td>0.50</td>
</tr>
<tr>
<td>Net portal balance, mmol/kg h⁻¹ %Intake</td>
<td>3.86±0.417a</td>
<td>1.25±0.275b</td>
<td>2.29±0.305c</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Galactose</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial, mM</td>
<td>0.45±0.044a</td>
<td>0.36±0.028b</td>
<td>0.64±0.032b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Portal, mM</td>
<td>1.15±0.085a</td>
<td>0.66±0.054b</td>
<td>1.31±0.061b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Net portal balance, mmol/kg h⁻¹ %Intake</td>
<td>2.24±0.34a</td>
<td>1.08±0.218b</td>
<td>2.03±0.248a</td>
<td>0.004</td>
</tr>
<tr>
<td><strong>Lactate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial mM</td>
<td>0.81±0.117a</td>
<td>1.17±0.074b</td>
<td>0.96±0.086b</td>
<td>0.022</td>
</tr>
<tr>
<td>Portal mM</td>
<td>0.90±0.110b</td>
<td>1.53±0.081b</td>
<td>1.27±0.081b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Net portal release, mmol/kg h⁻¹</td>
<td>0.33±0.320a</td>
<td>1.44±0.202b</td>
<td>0.92±0.235b</td>
<td>0.013</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).

The table shows the plasma concentrations and net portal balances of glucose, galactose, and lactate in neonatal piglets fed enterally, with TPN, or TPN with GLP-2 infusion for 7 days, then fed enterally for 6 hours. The values are given as means ± standard error (SE). The number of animals per group is as follows: enteral (4), TPN (10), and TPN+GLP-2 (9). Significant differences are indicated by different superscripts, based on analysis of variance and Tukey’s test (P < 0.05).

Table 4. Rates of [13C]glucose and [2H]glucose whole body flux and gut absorption and utilization kinetics in neonatal piglets fed either enterally, TPN, or TPN with GLP-2 infusion for 7 days and 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>Whole body [13C]glucose flux, mmol/kg h⁻¹</td>
<td>4.81±0.204</td>
<td>4.91±0.231</td>
<td>0.75</td>
</tr>
<tr>
<td>Arterial [13C]glucose enrichment (MPE)</td>
<td>3.82±0.157</td>
<td>3.52±0.170</td>
<td>0.21</td>
</tr>
<tr>
<td>Portal [13C]glucose enrichment (MPE)</td>
<td>3.85±0.168</td>
<td>3.42±0.179</td>
<td>0.086</td>
</tr>
<tr>
<td>Whole body [2H]glucose flux (mmol/kg h⁻¹)</td>
<td>0.053±0.007</td>
<td>0.070±0.008</td>
<td>0.11</td>
</tr>
<tr>
<td>Arterial [2H]glucose enrichment (MPE)</td>
<td>30±3.9</td>
<td>39±4.2</td>
<td></td>
</tr>
<tr>
<td>Portal [2H]glucose enrichment (MPE)</td>
<td>0.132±0.0074</td>
<td>0.115±0.0074</td>
<td>0.13</td>
</tr>
<tr>
<td>Whole body [2H]glucose utilization, mmol/kg h⁻¹</td>
<td>73±7.1</td>
<td>64±4.5</td>
<td></td>
</tr>
<tr>
<td>Arterial [2H]glucose utilization</td>
<td>3.51±0.211</td>
<td>4.74±0.233</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Portal [2H]glucose utilization</td>
<td>5.66±0.251</td>
<td>3.65±0.286</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Whole body [2H]glucose extraction, % of Input</td>
<td>0.13±0.035</td>
<td>0.05±0.044</td>
<td>0.19</td>
</tr>
</tbody>
</table>

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

The table shows the rates of [13C]glucose and [2H]glucose whole body flux and gut absorption and utilization kinetics in neonatal piglets fed either enterally, TPN, or TPN with GLP-2 infusion for 7 days and then fed enterally for 6 h. The values are given as means ± standard error (SE). The number of animals per group is as follows: TPN (10) and TPN+GLP-2 (9) (P < 0.05).
creased 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 [3H]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 exceeding total hexose intake.

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 piglets, providing GLP-2 did not result in capacities that exceeded total hexose intake.

![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).](image)

![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).](image)

![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.](image)
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 lactase 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 [13C] and intravenous [2H] glucose isotopes during the refeeding period allowed us to determine that intestinal production of [13C]lactate was halved in GLP-2-infused piglets compared with piglets receiving TPN alone. Furthermore, utilization of intravenous [3H]glucose in second-pass metabolism was considerably less (<10% of intake) compared with first-pass utilization of enteral [13C]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 [13C]glucose absorption, it is noteworthy that GLP-2 increased the [3H]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 [3H]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 [3H]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 experi-
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.

ACKNOWLEDGMENTS

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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.

REFERENCES

21. Inoue Y, Espat NJ, Frohnapple DJ, Epstein H, Copeland EM, and Burrin DG.
11. Drucker DJ, Erlich P, Asa SL, and Brubaker PL.
10. Cheeseman CI.