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

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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 digestive functions. Our findings indicate that GLP-2 treatment during chronic TPN maintains intestinal structure and lactose digestive and hexose absorptive capacities, reduces intestinal hexose malabsorption, and may facilitate the transition to enteral feeding in TPN-fed infants.

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 enterocolitis (25) and osmotic imbalances (39). Additionally, 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 posttranslationally 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 days, and to determine whether GLP-2 enhances absorptive function in enterally refed piglets.
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, 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, 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 weighed daily, and feed intake was adjusted accordingly. The TPN solution consisted of an elemental diet of free amino acids, dextrose, lipid (Intralipid, Baxter Healthcare, Deerfield, IL), vitamins A, D, and E (Vital E, Schering-Plough Animal Health, Kenilworth, NJ), multivitamin solution (Multitrace-5, American Reagent Laboratories, Shirley, NY), B vitamins (Vedco, St. Joseph, MO), and folic acid (Vet. Med. Rx, Houston, TX) (8). The macronutrient and fluid intake of all three experimental groups was ~240 ml, 15 g protein, and 900 KJ kg−1 kg−1 day−1.

Enteral feeding and glucose tracer protocol. After 6 days of treatment (18 days age), all piglets were given a primed (10 ml/kg) continuous intraduodenal infusion (10 ml kg−1 h−1) of formula for 6 h. Administration of TPN and GLP-2 was stopped 6 h before intraduodenal infusion, and formula-fed piglets were fasted overnight. Paired carotid arterial and portal venous blood samples (3 ml) were collected the day before intraduodenal feeding and at 3, 4, 5, and 6 h postfeeding. The hematocrit, hemoglobin (Hemocue, Angelholm, Sweden) and blood O2 and CO2 (Chiron Diagnostics, Halstead, Essex, UK) were immediately analyzed, the remaining blood was centrifuged at 1,100 g and 4°C for 10 min, and was plasma removed. All samples were frozen in liquid nitrogen and stored at −80°C until analysis.

At the end of the 6-h intraduodenal feeding, piglets were euthanized by an intravenous barbiturate overdose (Beutana-D, Schering-Plough Animal Health). The small intestine from the ligament of Treitz to colon was removed via a midline laparotomy, then immediately placed on an ice-cold metal surgical pan and flushed with 50 ml ice-cold 0.9% NaCl. The undigested intestinal and stomach contents and saline flush were collected and weighed, and the intestine was cut in half. The proximal half (jejunum) and distal half (ileum) were measured and weighed. Ten-centimeter lengths of proximal jejum and ileum were removed for determination of active and passive glucose sequestration, and four small intestinal segments were fixed in 10% buffered formalin at 4°C for 24 h, then stored in 70% EtOH at 4°C for morphometric analysis. The remaining intestine was frozen for analysis of protein, DNA, SGLT-1, and GLUT-2 abundance and lactase activity.

Gas chromatography-mass spectrometry. Gas chromatography-mass spectrometry (GCMS) was performed on the pentaacetate derivative of glucose and pentfluorobenzyl 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 [3H]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 (Spectramax 190, Molecular Dynamics) using enzyme-based assays (ThermoDMA, Louisville, 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 homogenized in phosphate-buffered saline (pH 7.4) as described previously (4, 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 Axioskop microscope (Carl Zeiss, Göttingen, Germany and Scion image beta 4.0.2 software, National Institutes of Health, Bethesda, MD). Abundance of GLP-2 (Chemicon International) and SGLT-1 (Alpha Diagnostics) at 60 and 57 kDa, respectively, in whole mucosa, and isolated BBM extracts was determined via 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 MgCl2 at 2,400, 19,000, and 39,000 g, providing a minimum 10-fold enrichment of lactase activity (15, 54).

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 [3H]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 represented apical transport, were expressed as nanomoles of δ-glucose accumulated per minute per milligram of wet intestinal tissue (nmol mg−1 min−1) (3). Total intestinal lactase 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 (PBF) (1). In the calculations of portal glucose and lactate kinetics, PBF was converted
maximum rates of transport (nonlinear regression analysis (Enzfit, Biosoft, Elsevier) to estimate uptake at the different glucose concentrations were subjected to 0.05) as indicated by a post hoc Tukey’s test. Rates of apical glucose concentrations and IE of [13C]lactate and 13CO₂ production were calculated as per
\[ \text{IE}_{\text{port}} = \frac{\text{C}_{\text{port}}}{\text{C}_{\text{art}}} \]
for tracer infusion (\( R \)).

Tracer [13C]glucose uptake kinetics were determined by incorporating arterial and portal lactate concentrations.

\[ \text{NB} = \left( \frac{\text{C}_{\text{art}}}{\text{C}_{\text{port}}} \right) \times \text{PBF} \]

Intestinal O₂ uptake was calculated as per Ref. 2 by substituting arterio-portal difference (4). Intestinal [13C]lactate and 13CO₂ production were calculated as per Eq. 4, substituting to concentrations and IE of [13C]lactate and 13CO₂, respectively.

\[ ^{13}\text{C}_{\text{glucose}} = \left( \frac{\text{C}_{\text{art}}}{\text{C}_{\text{port}}} \right) \times \text{PBF} \]

\[ ^{2}\text{H}_{\text{glucose}} = \left( \frac{\text{C}_{\text{art}}}{\text{C}_{\text{port}}} \right) \times \text{PBF} \]

Whole body flux (\( Q \)) in mmol·kg⁻¹·h⁻¹ was calculated from the rate of tracer infusion (\( R \)) and enrichments of the infusate (\( \text{IE}_{\text{infusate}} \)) and plasma (\( \text{IE}_{\text{plasma}} \)).

\[ Q = R \times \left[ \frac{\text{IE}_{\text{infusate}}}{\text{IE}_{\text{plasma}}} - 1 \right] \]

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 (\( V_{\text{max}} \)) and apparent affinity constants (\( K_{\text{m}} \)).

RESULTS

As observed in previous experiments, TPN resulted in lower intestinal weight, jejunum and ileum 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, jejunum 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 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, 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></th>
<th>Enteral</th>
<th>TPN</th>
<th>TPN + GLP-2</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intestinal wt, g/kg body wt</td>
<td>50.9 ± 2.09</td>
<td>29.4 ± 1.48</td>
<td>35.6 ± 1.41</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Intestinal protein, mg/kg body wt</td>
<td>6.235 ± 369</td>
<td>2.99 ± 233</td>
<td>4.261 ± 235</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Intestinal DNA, mg/kg body wt</td>
<td>201 ± 13.6</td>
<td>141 ± 8.6</td>
<td>169 ± 8.6</td>
<td>0.004</td>
</tr>
<tr>
<td>Villus height, μm</td>
<td>Jejunum</td>
<td>943 ± 120</td>
<td>352 ± 90</td>
<td>722 ± 81</td>
</tr>
<tr>
<td>Ileum</td>
<td>1,456 ± 222</td>
<td>387 ± 157</td>
<td>1,057 ± 150</td>
<td>0.001</td>
</tr>
<tr>
<td>Lactase-specific activity, μmol·min⁻¹·g protein⁻¹</td>
<td>75.5 ± 12.1</td>
<td>36.6 ± 8.07</td>
<td>52.5 ± 7.49</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 (\( P < 0.05 \)).
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 increases in portal galactose concentrations, which did not differ from those of ENT piglets. The lowest portal galactose concentrations were measured in TPN-treated piglets (P < 0.05 compared with ENT and GLP-2 piglets). Galactose NPB was ∼55% of intake in ENT-fed piglets, considerably <93% of intake for glucose absorption. Although this may seem low, this is higher than 38% observed for 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 [13C]lactate kinetics.

Due to a small sample size and some sample error, it was not possible to calculate in vivo [13C]glucose and [2H]glucose tracer kinetics for ENT-fed piglets. Comparisons of in vivo [13C]glucose and [2H]glucose tracer kinetics are restricted to the TPN and GLP-2 treatments (Table 4). Treatment effects were not detected for arterial and portal [13C]glucose IE and concentrations, despite trends of lower portal IE and arterial concentrations in GLP-2-infused piglets. Likewise, [13C]glucose absorption, utilization, or whole blood flux did not differ between TPN and GLP-2 piglets. However, arterial and portal [13C]lactate enrichment and concentrations and net portal [13C]lactate production were significantly higher in TPN piglets (Table 5), suggesting TPN alone elevated intestinal glycolysis. As per intestinal CO2 production, 13CO2 production was not different between TPN and GLP-2. To discriminate between first pass metabolism of [13C]glucose and metabolism of arterial glucose by the PDV, intravenous coinfusion of [2H]glucose was performed (Table 5).

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 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><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 1-h⁻¹</td>
<td>3.86±0.417⁻</td>
<td>1.25±0.275ᵇ</td>
<td>2.29±0.305ᵇ</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>%Intake</td>
<td>93±10.6ᵃ</td>
<td>31±7.0ᵇ</td>
<td>58±7.7ᵇ</td>
<td></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 1-h⁻¹</td>
<td>2.24±0.34ᵃ</td>
<td>1.08±0.218ᵇ</td>
<td>2.03±0.248ᵇ</td>
<td>0.004</td>
</tr>
<tr>
<td>%Intake</td>
<td>55±8.5ᵃ</td>
<td>27±5.4ᵇ</td>
<td>52±6.1ᵇ</td>
<td></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 1-h⁻¹</td>
<td>0.33±0.120ᵇ</td>
<td>0.14±0.020ᵇ</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).

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 1-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>Portal [13C]glucose absorption mmol/kg⁻¹-h⁻¹</td>
<td>0.053±0.007</td>
<td>0.070±0.008</td>
<td>0.11</td>
</tr>
<tr>
<td>%of Intake</td>
<td>30±3.9</td>
<td>39±4.2</td>
<td></td>
</tr>
<tr>
<td>Whole body [2H]glucose flux (mmol/kg 1-h⁻¹)</td>
<td>3.51±0.211</td>
<td>4.74±0.233</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Arterial [2H]glucose enrichment (MPE)</td>
<td>5.66±0.251</td>
<td>3.65±0.286</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Portal [2H]glucose enrichment (MPE)</td>
<td>5.53±0.362</td>
<td>4.19±0.411</td>
<td>0.017</td>
</tr>
<tr>
<td>Portal [2H]glucose utilization, mmol/kg⁻¹-h⁻¹</td>
<td>0.13±0.035</td>
<td>0.05±0.044</td>
<td>0.19</td>
</tr>
<tr>
<td>Portal [2H]glucose extraction, % of Input</td>
<td>9.6±2.88</td>
<td>6.0±3.45</td>
<td>0.42</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).
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.

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 ENT (4), TPN (8), and TPN + GLP-2 (9) ($P < 0.05$).

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 ($P < 0.05$).
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).

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-

**DISCUSSION**

The functional and metabolic disturbances during chronic TPN and refeeding are associated with reduced intestinal glucose absorption, protein synthesis, and blood flow (9). This led us to postulate that reduced hexose absorption after chronic TPN occurred via a combination of either reduced hexose transporter abundance or increased mucosal glucose metabolism. The current study was designed to test whether a pharmacological dose of GLP-2, which prevents TPN-induced mucosal atrophy, translates into improved intestinal glucose absorption in piglets during refeeding. 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). Consistent with previous experiments, TPN induced mucosal atrophy and reduced intestinal weight, villus height, villus area, protein and DNA content, and lactose digestive capacity (5, 22, 29, 31). Our results also indicate that the protective effect of GLP-2 was demonstrated by improvement in all of these parameters compared with TPN-fed piglets. Our results indicate that GLP-2 treatment was able to partially maintain in vivo intestinal hexose absorptive function in TPN-fed piglets. Moreover, we show that the GLP-2-induced increase in hexose absorptive capacity occurred via increased villus surface area and upregulation of intestinal glucose transport and reduced intestinal glycolytic metabolism.

**Fig. 4.** Jejunal (A) and ileum (B) BBM GLUT-2 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).
GLP-2 increases trafficking of either SGLT-1 or GLUT-2 into the BBM. However, our observations indicate that GLUT-2 trafficking to the BBM requires a higher luminal glucose concentration than SGLT-1. Therefore, the improvement in glucose transport capacity of GLP-2 compared with TPN piglets is due to a combination of improved villus structure, increased mucosal absorptive surface area, and increased jejunum glucose transport, presumably due to increased abundance of mucosal glucose transporters.

Absorption of glucose across the BBM is dependent on two or more carriers. SGLT-1 is a low-capacity, high-affinity mucosal glucose and galactose transporter that is primarily responsible for apical glucose transport at low concentrations. GLUT-2 is a high-capacity, low-affinity hexose transporter in the basolateral membrane that is responsible for the transport of glucose out of the enterocyte into the blood. GLUT-2 is now recognized to be transiently recruited to the apical membrane where it can account for a significant proportion of apical glucose transport during the processing of meals (18, 19, 24).

Although the relative contributions of SGLT-1 and GLUT-2 to the measured rates of uptake were not determined, we observed an increase in the $V_{\text{max}}$ for mucosal glucose uptake with GLP-2 treatment. Furthermore, the different $V_{\text{max}}$ values were independent of changes in $K_m$. This suggests the treatment differences for rates of uptake were due to differences in the abundances of the apical membrane glucose transporters.

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 SGLT-1 abundance 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.

ACKNOWLEDGMENTS

The authors thank M. Riedijk for assistance during the experiment and X. Guan and B. Nichols for helpful discussions.

GRANTS

This work was supported by National Institutes of Health Grant HD-33920 (to D. G. Burrin) and by the USDA-ARS under Cooperative Agreement Number 58-6250-6-001.

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

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