Glucagon-like peptide-2 acutely increases proximal small intestinal blood flow in TPN-fed neonatal piglets

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Glucagon-like peptide-2 acutely increases proximal small intestinal blood flow in TPN-fed neonatal piglets. Am J Physiol Regul Integr Comp Physiol 290: R283–R289, 2006. First published September 15, 2005; doi:10.1152/ajpregu.00588.2005.—Glucagon-like peptide-2 (GLP-2) is a gut hormone that is secreted in response to enteral feeding and stimulates small intestinal mucosal growth. We have previously shown that GLP-2 infusion acutely increases portal venous blood flow in TPN-fed piglets. The aim of this study was to localize the vasoactive effect of GLP-2 within the gastrointestinal tissues and other visceral organs in TPN-fed piglets. Tissue blood flow rates were quantified using fluorescent microsphere deposition in anesthetized TPN-fed piglets given intravenous infusion of GLP-2 at either 500 pmol·kg⁻¹·h⁻¹ (low GLP-2, n = 7 pigs) or 2,000 pmol·kg⁻¹·h⁻¹ (high GLP-2, n = 8 pigs) for 2 h. Compared with baseline, the low and the high GLP-2 treatment significantly increased the blood flow rate in the duodenum (+77%) and jejunum (+40% and 80%), respectively, but blood flow to the distal small intestine and colon (−15%) was unchanged or slightly decreased. Baseline mucosal blood flow was five-fold higher than serosal blood flow; however, high GLP-2 treatment increased serosal (+140%) to a larger degree than mucosal blood flow (+73%). The high GLP-2 dose increased pancreatic flow (+34%) but decreased blood flow in the kidneys (−14%) and stomach (−12%), whereas the spleen and brain were unaffected. These findings suggest that the acute GLP-2-mediated stimulation of portal blood flow in TPN-fed pigs occurs principally via increased blood flow through the superior mesenteric artery to the proximal small intestine, a tissue region where the GLP-2R mRNA abundance and trophic GLP-2 effects are greatest.

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**MATERIALS AND METHODS**

**Animals and surgical protocol.** The study protocol was approved by the Animal Care and Use Committee of Baylor College of Medicine and was conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* [DHHS publication no. (NIH) 85–23, revised 1985, Office of Science and Health Reports, DRR/NIH, Bethesda, MD 20205]. Neonatal, 2-day-old, sow-fed, female crossbred (Large White × Hampshire × Duroc, the Texas Department of Criminal Justice, Huntsville, TX) piglets were transported to the Children’s Nutrition Research Center’s animal facility and fasted until surgery within 6–8 h of arrival. Piglets were housed at a temperature of 32–34°C with a 12:12-h light-dark cycle. At 2 days of age, piglets began receiving total parenteral nutrition (TPN) via the jugular vein at half-rate intake during the first day after surgery and thereafter were maintained at full-rate daily intake of 240 ml fluid, 13 g protein, and 900 kJ energy per kg body wt for 3 days before the microsphere infusion protocol. TPN solution consisted of glucose (104 g/l), lipid (21 g/l), Intralipid, Baxter Healthcare, Deerfield, IL), a complete amino acid mixture (55 g/l; Ajinomoto, Tokyo, Japan), electrolytes, trace minerals, and vitamins to meet the requirements for neonatal pigs (6); piglets were not given any enteral feeds during this study.

Piglets were fasted on the morning before surgery and surgically implanted with catheters in the right external jugular vein (silicone tubing, 1.65 mm outside diameter) and umbilical artery [3.5-Fr single-lumen catheter (Sherwood Medical, St. Louis, MO)] under general anesthesia with isoflurane. The jugular catheter was externalized between the scapulas, and both catheters were filled with heparinized saline (20 U/ml) and placed in a protective animal jacket. Preoperatively, piglets received an intramuscular antibiotic (20 mg/kg enrofloxacine, Bayer, Shawnee Mission, KS) and intramuscular analgesic (20 U/ml) saline and placed in a protective animal jacket. Preoperatively, piglets received an intramuscular antibiotic (20 mg/kg enrofloxacine, Bayer, Shawnee Mission, KS) and intramuscular analgesic (20 U/ml) saline and placed in a protective animal jacket. Preoperatively, piglets received an intramuscular antibiotic (20 mg/kg enrofloxacine, Bayer, Shawnee Mission, KS) and intramuscular analgesic (0.1 mg/kg butorphenol tartrate, Fort Dodge Labs, Fort Dodge, IA) immediately after surgery.

**Microsphere infusion protocol.** Four days after initial surgery (pigs ~4–5 days old), piglets were placed under general anesthesia with isoflurane (2–3%), and TPN was continuously administered, while vitals (heart rate, blood pressure, oxygen saturation, and temperature) were continuously monitored every 15 min throughout the entire experiment using LW-6000 Life Window vitals monitor (Digicare Biomedical Technology, Boynton Beach, FL). A right common caecal catheter [3.5-Fr single-lumen catheter (Sherwood Medical)] was inserted, and the tip was advanced into the left ventricle; placement was verified by fluoroscopy. After piglets received their ventricular catheter, TPN was continued for another 20 min before the microsphere infusion, and GLP-2 treatment was begun. GLP-2 infusions were stopped, and the piglet was euthanized immediately after surgery.

**Microsphere analysis.** The use and technical aspects of the FMS [FluoSpheres Blood Flow Determination Kits #2 (F-8891), Molecular Probes, Eugene, OR] have been previously described (25, 27, 30). In short, FMS were injected directly into the heart via the carotid catheter to ensure adequate mixing. Blood flow was determined on the basis of the ratio between the measured fluorescence of the tissue sample and the arterial reference blood sample obtained via the umbilical catheter at a constant withdrawal rate of 2 ml/min using a Harvard PHD 4400 pump (Harvard Apparatus, Holliston, MA). FMS were recovered via the sedimentation method (32). Briefly, the 1-gram samples were stored at room temperature for 2 wk and then dissolved with ethanolic KOH (Sigma-Aldrich, Milwaukee, WI); FMS was removed by centrifugation at 2,000 g for 15 min. The polystyrene FMS casing was then dissolved with 2-ethoxethyl acetate (Sigma-Aldrich), releasing the fluorophores. The intensities of the respective fluorophores were determined using a 96-well plate reader (SpectraMax Gemini XS spectrofluorometer, Molecular Devices, Sunnyvale, CA).

**GIP-2 radioimmunoassay.** At the end of the 120-min infusion period, blood samples were collected in EDTA tubes, centrifuged at 3,000 g at 4°C, and plasma was frozen immediately in liquid nitrogen. Plasma GIP-2 (1–33) concentrations were quantified by radioimmunoassay as described previously (5, 6). This assay recognizes the biologically active forms of the human and porcine GIP-2 (1–33) peptides.

**Calculations.** The regional blood perfusion (Qi) rate of a piece of tissue (~1 g) was calculated by dividing the reference blood sample fluorescence (fref) by the fluorescence of the tissue sample (fi), multiplied by the rate of withdrawal of the reference blood sample (R):

\[
Q_i (\text{ml/min}) = \frac{f_{\text{ref}}}{f_i} \times R (\text{ml/min})
\]

The Qi flow was corrected for the amount of tissue used to extract the beads. The absolute blood flow rate to the organ or tissue bed of interest was calculated as the product of the tissue perfusion rate (Qi) per gram of tissue and the organ weight (g). Using these values, we estimated the superior mesenteric artery (SMA) blood flow as the sum of blood flows to the PJ, DJ, PI, DI, and PC, whereas the celiac blood flow was calculated by summing the flows to the stomach, duodenum, pancreas, spleen, and liver. For each piglet, we also calculated the mean organ blood flow rates across the four time points (15, 30, 45, and 120 min); this value was expressed as an absolute rate (ml/min) and relative to the time 0 baseline flow (%baseline).

**Statistical analyses.** All data were tested for significance using a general linear model ANOVA (Minitab 13, Minitab, PA). The effect of GIP-2 on small intestinal blood flow was analyzed using several different statistical models. The differences in absolute blood flow rates were first tested using a three-way ANOVA with treatment (low vs. high GIP-2), time (0, 15, 30, 45, and 120 min) and intestinal segment (PJ, DJ, PI, DI) as main effects (Fig. 1). Differences among segments were tested using a post hoc Tukey’s test. We then tested for differences in the absolute and relative blood flow rates between the baseline and the mean GIP-2-treated value estimated from all four time-points (15, 30, 45, and 120 min) (Table 1, Fig. 2). In this model, we tested for differences in the absolute and relative intestinal blood flow rates using three-way ANOVA with treatment (low and high GIP-2), segment (PJ, DJ, PI, DI) and subsection (mucosal and proximal jejunum (PJ), distal jejunum (DJ), proximal ileum (PI), and distal ileum (DI). Of these individual sections, up to five 1-g pieces were used for whole gut analysis, and two separate pieces of the section were divided into mucosa and serosa by blunt dissection using a microscope slide. The duodenum is defined as the distal to the pylorus to ligament of treitz and is not analyzed as part of the small intestine for this study but as its own GI segment. One-gramp samples of tissue were collected from each of the following organs: brain, left and right kidneys, liver, spleen, pancreas, proximal colon (PC) (the spiral portion of the colon), distal colon (DC), stomach, and duodenum. All tissues were placed into individual preweighed 15-ml polypropylene tubes and stored at room temperature.
serosal) as main effects. Differences between the saline baseline and the respective low and high GLP-2 treatment were determined using a paired *t*-test. Finally, we tested for differences in absolute and relative blood flow rates of the remaining organs (Table 2, Fig. 3) and for the celiac and SMA totals (Fig. 4) using ANOVA with treatment as a main effect. Data are expressed as means ± SE, and means were considered significantly different at *P* < 0.05.

**RESULTS**

*Body weight, vital statistics, and plasma GLP-2.* There was no significant difference in the mean body between either the low or high GLP-2 groups (1.95 ± 0.17 kg). Animals had their vital statistics measured during the experiment under general anesthesia. There was no significant difference (*P* > 0.05) in the mean values between the two treatment groups in heart rate (167 ± 23 beats/min), mean arterial blood pressure (41 ± 5

![Fig. 1. Absolute blood flow rates (ml/min) at baseline (T-0) and at various times (T-15, 30, 45, and 120 min) after starting GLP-2 infusions at either the low (500 pmol·kg⁻¹·h⁻¹, n = 7) (A) or the high (2,000 pmol·kg⁻¹·h⁻¹, n = 8) rates (B). In the low GLP-2 group, there was no significant difference between the GLP-2 infusion time points (open bars) and the baseline blood flow rates for any of the small intestinal segments. Values are presented as means ± SE. There were significant treatment × time (*P* < 0.05) and treatment × segment interactions based on ANOVA.](http://ajpregu.physiology.org/)

![Fig. 2. Mean values for relative (% baseline) blood flow in mucosal, serosal, and whole intestinal sections in piglets infused with either the low GLP-2 (500 pmol·kg⁻¹·h⁻¹, n = 7) (solid bars) or the high GLP-2 (2,000 pmol·kg⁻¹·h⁻¹, n = 8) (open bars) infusion rates. Significantly different from saline baseline (dashed line at 100%) based on paired *t*-test (*P* < 0.05). Significant effects of treatment (*P* < 0.05; high > low GLP-2), segment (*P* < 0.05; jejunum > ileum), section (*P* < 0.05; serosa > mucosa) and treatment × section interaction (*P* < 0.05), and treatment × section interaction (*P* < 0.05) were observed and are described in detail in the RESULTS section. Values are presented as means ± SE.](http://ajpregu.physiology.org/)

**Table 1. Mean absolute blood flow in mucosal and serosal intestinal subsections estimated at baseline and during low and high GLP-2 infusion rates**

<table>
<thead>
<tr>
<th>Segment</th>
<th>Baseline Low</th>
<th>Low GLP-2</th>
<th>Baseline High</th>
<th>High GLP-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proximal jejunum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mucosa</td>
<td>22.3 ± 3.2</td>
<td>26.5 ± 4.1</td>
<td>20.9 ± 2.9</td>
<td>35.5 ± 5.0</td>
</tr>
<tr>
<td>Serosa</td>
<td>3.45 ± 0.59</td>
<td>6.65 ± 1.56</td>
<td>4.05 ± 0.69</td>
<td>8.94 ± 0.96</td>
</tr>
<tr>
<td>Distal jejunum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mucosa</td>
<td>19.2 ± 3.5</td>
<td>18.5 ± 4.1</td>
<td>22.0 ± 3.1</td>
<td>31.1 ± 5.5</td>
</tr>
<tr>
<td>Serosa</td>
<td>3.67 ± 0.73</td>
<td>5.79 ± 1.49</td>
<td>3.42 ± 0.35</td>
<td>7.18 ± 0.65</td>
</tr>
<tr>
<td>Proximal ileum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mucosa</td>
<td>13.8 ± 1.5</td>
<td>12.5 ± 2.3</td>
<td>18.4 ± 3.4</td>
<td>22.5 ± 4.1</td>
</tr>
<tr>
<td>Serosa</td>
<td>3.49 ± 0.68</td>
<td>5.24 ± 1.30</td>
<td>2.90 ± 0.35</td>
<td>5.24 ± 0.43</td>
</tr>
<tr>
<td>Distal ileum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mucosa</td>
<td>13.7 ± 3.0</td>
<td>10.6 ± 2.2</td>
<td>13.4 ± 2.2</td>
<td>15.7 ± 3.0</td>
</tr>
<tr>
<td>Serosa</td>
<td>2.69 ± 0.43</td>
<td>3.23 ± 0.53</td>
<td>2.57 ± 0.44</td>
<td>3.61 ± 0.45</td>
</tr>
</tbody>
</table>

Values are presented as means ± SE. GLP-2 infusion rates are low (500 pmol·kg⁻¹·h⁻¹, n = 7) and high (2,000 pmol·kg⁻¹·h⁻¹, n = 8). All values for mean absolute blood flow are given in milliliters per minute. Significantly different from baseline based on paired *t*-test (*P* < 0.05). Significant effect of treatment (*P* < 0.05; high > low GLP-2), segment (*P* < 0.05; PI, DI) and treatment × segment interaction (*P* < 0.05), and treatment × section interaction (*P* < 0.05). Details of interactions are described in RESULTS section. PJ, proximal jejunum; DI, distal jejunum; PI, proximal ileum; DI, distal ileum.
Table 2. Mean absolute blood flow of gastrointestinal tissues, visceral organs, and brain measured at baseline and during low and high GLP-2 infusion rates

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Baseline Low</th>
<th>Low GLP-2</th>
<th>Baseline High</th>
<th>High GLP-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach</td>
<td>6.09±0.98</td>
<td>4.84±1.10</td>
<td>7.05±1.41</td>
<td>5.84±1.02</td>
</tr>
<tr>
<td>Duodenum</td>
<td>3.78±1.11</td>
<td>4.41±1.45</td>
<td>3.56±0.71</td>
<td>6.00±1.09</td>
</tr>
<tr>
<td>Proximal colon</td>
<td>15.6±3.31</td>
<td>13.2±3.18</td>
<td>18.2±5.7</td>
<td>14.3±4.18</td>
</tr>
<tr>
<td>Distal colon</td>
<td>2.72±0.62</td>
<td>2.45±0.38</td>
<td>2.86±0.95</td>
<td>2.61±0.73</td>
</tr>
<tr>
<td>Spleen</td>
<td>29.1±6.6</td>
<td>19.6±3.9</td>
<td>24.2±4.30</td>
<td>18.4±1.8</td>
</tr>
<tr>
<td>Pancreas</td>
<td>1.37±0.31</td>
<td>1.44±0.35</td>
<td>1.07±0.16</td>
<td>1.38±0.19</td>
</tr>
<tr>
<td>Left kidney</td>
<td>18.3±3.0</td>
<td>13.5±1.2</td>
<td>18.4±1.9</td>
<td>15.9±2.0</td>
</tr>
<tr>
<td>Right kidney</td>
<td>17.5±2.3</td>
<td>12.9±1.1</td>
<td>17.9±2.1</td>
<td>15.4±2.0</td>
</tr>
<tr>
<td>Brain</td>
<td>23.9±2.3</td>
<td>23.2±4.7</td>
<td>25.0±3.9</td>
<td>30.3±6.7</td>
</tr>
</tbody>
</table>

Values are presented as means ± SE. All values for mean absolute blood flow are given in milliliters per minute. GLP-2 infusion rates are low (500 pmol·kg⁻¹·h⁻¹, n = 7) and high (2,000 pmol·kg⁻¹·h⁻¹, n = 8).

mmHg), oxygen saturation (96.1 ± 3.0%), or core body temperature (37.9 ± 1.3°C). The plasma concentrations of the biologically active GLP-2 (1–33) peptide were 400 ± 24 pmol/l and 1,834 ± 88 pmol/l in the low and high GLP-2 infusion groups, respectively.

Small intestinal blood flow. The changes in absolute blood flow rates to each small intestinal segment in the two GLP-2 groups are shown in Fig. 1. The changes in proximal and distal jejunum (PJ, DJ) and ileum (PI, DI) absolute blood flow rates after low and high GLP-2 infusions are shown in Fig. 1. There was a significant main effect of time in the high GLP-2 but not the low GLP-2 infusion group, indicating that the absolute flow rates in response to low GLP-2 were not statistically different from the baseline. Moreover, the absolute blood flow in the high GLP-2 infusion group was greater than the low GLP-2 infusion (treatment × time, P < 0.05). In both the high and low GLP-2 infusion groups, there was a main effect of segment, indicating that blood flow was higher in the PJ and DJ than in the DI. In addition, the effect of high GLP-2 was higher in the PJ and DJ, than in the PI and DI segments (treatment × segment, P < 0.05).

We also quantified the absolute (ml/min) and relative (%baseline) (Figs. 2B and 3) blood flow rates in the whole intestine for the baseline and the mean GLP-2-treated value estimated from all four time-points (15, 30, 45, and 120 min). The absolute blood flow was increased by the high GLP-2 infusion rate but not the low GLP-2 infusion (treatment, P < 0.05) and only in the PJ and DJ but not the PI and DI segments (segment, P < 0.05) (data not shown). However, the relative blood flow in the low GLP-2 group in the PI (149%) was significantly (P < 0.05) higher than the baseline values. Similarly, the relative blood flow rates in the high GLP-2 group in the PJ (189%) and DJ (170%) were higher (P < 0.05) than baseline.

Mucosal vs. serosal small intestinal blood flow. In addition to analysis of whole tissue blood flow in the four small intestinal segments, we estimated the absolute (ml/min) (Table 1) and relative (%baseline) (Fig. 2) blood flow rates to the mucosa vs. serosa sections. As expected, the absolute blood flow to the mucosa was 5- to 6-fold higher (section, P < 0.05) than the serosal section throughout all of the small intestinal segments. Moreover, the high GLP-2 infusion increased blood flow to both mucosa and serosa above the value seen in either the baseline or low GLP-2 group (treatment, P < 0.05). However, the high GLP-2-mediated increase in blood flow was proportionally greater in the serosa than the mucosal fraction (treatment × section, P < 0.05). We observed higher absolute rates of mucosal and serosal blood flow in the high GLP-2 group in the PJ and DJ compared with the PI and DI (treatment × segment, P < 0.05). The mean relative estimates in the PJ and DI segments indicated that the serosa blood flow was...
240% and 155%, whereas the mucosa blood flow was 173% and 114%, respectively. In both the low and high GLP-2 groups, the mucosal flow rates in the PJ segment were higher than in any of the other segments. However, the serosal flow rates in both GLP-2 groups were similar in all four small intestinal segments.

**Nonsmall intestinal tissues.** We quantified the absolute (Table 2) and relative (Fig. 3) blood flow rates of the remaining GI tract tissues, other abdominal organs, and brain. There were no significant changes in blood flow in any tissue or organ in the low GLP-2 group compared with the baseline. However, the duodenal blood flow was significantly higher (177%) in the high GLP-2 group than saline baseline (100%). Interestingly, blood flow in both the stomach and the proximal colon decreased ($P < 0.05$) from baseline by $-12\%$ and $-15\%$, respectively. The distal colon was the only section of the GI tract that was unchanged from baseline in response to GLP-2 treatment.

The only nonintestinal organ in which the blood flow was increased by GLP-2 was the pancreas. The relative pancreatic blood flow tended to be higher ($P > 0.05$) $(121\%)$ at the low GLP-2 rate but was significantly increased $134\%$ with the high GLP-2 rate compared with baseline $(100\%)$. The renal blood flow rate was similar in the right and left kidney and was decreased ($P < 0.05$) by $-15\%$ at the high, but not low, GLP-2 rate. Similarly, the splenic blood flow tended to be reduced ($-15\%$) by GLP-2 infusion, but this was not statistically significant. Blood flow to the brain was not affected by GLP-2 treatment.

**Calculated SMA and celiac artery flows.** On the basis of the known anatomy of the organs perfused by the celiac artery and the SMA, the actual flows for each artery were calculated. Figure 4 shows the calculated blood flow of the celiac and superior mesenteric artery in the low and high GLP-2 groups expressed relative to baseline. For the celiac artery, both GLP-2 infusion rates tended to decrease the blood flow to $81\%$–$87\%$ of the baseline value, but neither was statistically significant. In contrast, the SMA flow tended to be higher $(121\%)$ ($P > 0.05$) in the low GLP-2 group but was statistically higher $(141\%)$ in the high GLP-2 group than in the baseline group $(100\%)$.

**DISCUSSION**

The primary aim of this study was to ascertain whether the stimulatory effect of GLP-2 on portal blood flow that we previously observed in TPN-fed piglets is localized to the small intestine. Two main aspects of GLP-2 function form the basis for this aim. First, the trophic actions of GLP-2 are localized to the mucosal epithelium of the small intestine, and secondly, the GLP-2R mRNA is localized mainly in the stomach, small and large intestine, and not other organs drained by the portal vein. We examined the brain as well, given the report that the GLP-2R mRNA is localized in the hypothalamus, and central GLP-2 administration affects specific feeding behaviors (20, 36). A key finding of this study was that GLP-2 acutely increased proximal small intestinal and pancreatic blood flow, but the distal small intestine, colon, and stomach were largely unaffected or slightly reduced. These findings suggest that the acute GLP-2-mediated stimulation of portal blood flow in TPN-fed piglets is dominated by increased blood flow in the proximal small intestine, specifically the duodenum and jejunum, a tissue region where the GLP-2R mRNA abundance and trophic GLP-2 effects are greatest.

The finding that GLP-2 increased the rate of blood flow compared with baseline in the proximal intestine to a greater extent than the distal small intestine and colon is generally consistent with the relative abundance of GLP-2R mRNA in the neonatal pig (24). Despite the relatively low GLP-2R mRNA abundance and the absence of an acute blood flow response, previous reports indicated that chronic GLP-2 treatment induces a modest trophic effect in the ileum and colon (5, 24). Thus, although the GLP-2R mRNA is expressed in the stomach, small intestine, and colon, it is possible that GLP-2 differentially affects blood flow acutely in a tissue-specific manner, because blood flow in the stomach and colon decreased. However, we also observed that the absolute blood flow rates in the baseline period were significantly higher in the PJ than the DI segment; this is consistent with evidence that the vascular supply is greater in the proximal than distal intestine. Thus the inherent anatomical difference in blood flow resulted in increased vascular delivery of exogenous GLP-2 peptide in the proximal compared with distal small intestine. Although the GLP-2 trophic actions have consistently been observed in the small intestinal mucosa, the cellular location of the GLP-2R is controversial (i.e., enteroendocrine, enteric neurons, and subepithelial myofibroblasts) (2, 22, 36). This issue is of particular relevance in light of the differences in GLP-2-mediated increase in mucosal vs. serosal blood flow. We found that the high GLP-2 treatment increased mucosal blood flow in all intestinal segments, except the distal ileum. In contrast, high GLP-2 treatment increased serosal blood flow above baseline in all small intestinal segments, even when expressed as absolute flow. Moreover, although the absolute flow to the serosa is significantly lower than the mucosa, the relative increase in GLP-2-induced blood flow was consistently greater in the serosa than the mucosa. These findings may implicate a more immediate and robust activation of GLP-2 receptors in the submucosal and myenteric plexus regions of the small intestinal wall, since other possible GLP-2R-containing cells (e.g., enteroendocrine and subepithelial myofibroblasts) would be represented in the mucosal fraction.

The GLP-2-induced stimulation of proximal small intestinal blood flow was reflected in the significant increase in calculated SMA flow rate but not the celiac flow rate. This observation is consistent with our recent findings based on ultrasonic flow probe measurements in TPN-fed piglets (unpublished observations). This finding can be explained by the fact that 90% of the celiac blood flow is distributed to the stomach and spleen, which was suppressed by GLP-2, whereas only 10% of the flow goes to the duodenum and pancreas, where flow was stimulated by GLP-2. The finding that GLP-2 increased pancreatic blood flow warrants further study as to whether the GLP-2R mRNA is expressed in this tissue, which might not be surprising given that this is a key tissue that expresses the GLP-1 receptor. We observed a stimulation of relative proximal small intestinal blood flow at the low and high GLP-2 infusion rates compared with baseline. However, it is notable that we did not detect significant changes in either the absolute flow rates (Fig. 1) or the calculated SMA flow rate (Fig. 4) at the low GLP-2 infusion rate. We should note that the low GLP-2 infusion rate...
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was identical to that found previously to increase portal blood flow in TPN-fed piglets. However, the GLP-2 infusion rates used in this study were pharmacological, producing circulating concentrations of the biologically active GLP-2 (1–33) peptide ranging from 5- to 25-fold higher than normal physiological levels observed in enterally fed piglets (5). The low GLP-2 infusion dose is comparable to that being used in recent clinical trials for treatment of short-bowel syndrome in adult humans (17). We suggest that two factors may explain the lack of response to the low GLP-2 infusion rate. First, and most likely, is the fact that the piglets were studied under general isoflurane anesthesia, whereas the piglets were conscious in our previous report on portal blood flow (14). Isoflurane has been shown to decrease GI blood flow in the stomach, small intestine, colon, and pancreas in studies using a similar microsphere deposition technique (15, 18, 19). Interestingly, although isoflurane has been shown to decrease mean arterial pressure and increase heart rate, especially at higher exposure rates, we observed no significant change in either MAP or heart rate across the 120-min period in either GLP-2 group. A second factor contributing to the lack of response to the low GLP-2 infusion rate is the inherently higher variance associated with single time-point blood flow estimates using the microsphere deposition method compared with continuous blood flow measurements with implanted ultrasonic flow probes. Given this fact, it is notable that in the current study, the relative increases in proximal intestinal blood flow at the low GLP-2 infusion rate were ~30–50% higher than baseline, a response that is consistent with the relative changes in portal blood flow observed previously in conscious piglets (14).

The decrease in the blood flow to the stomach, spleen, and kidneys was also of interest. Despite the fact that the GLP-2R is expressed in the stomach, the finding of GLP-2-induced suppression of stomach blood flow is consistent with our previous report that GLP-2 suppresses the stomach tissue protein synthesis rate (5). Taken together, these observations coupled with previous evidence of GLP-2-mediated reduced secretory and motor function (33, 34) are congruent with the idea that GLP-2 generally suppresses metabolism and function of the stomach. The GLP-2-associated reductions in splenic and kidney blood flow were unexpected, as expression of the GLP-2R was not detected previously in the kidney (24) and has not been reported in the spleen. The close proximities of the arteries that supply these organs, namely, the celiac and renal, are cranial or caudal to the SMA, and this raises the possibility that increased flow to the SMA indirectly decreased flow to the celiac artery and kidneys. Whether these changes in blood flow alter function and metabolism of the spleen or kidney is unknown, although we found no changes in spleen protein synthesis or growth after GLP-2 treatment in a previous study (5). Furthermore, we did not observe any change in brain blood flow, even though the GLP-2R has been reported to be localized in the hypothalamus. The finding of no effect on brain-blood flow is also an important finding, particularly, if GLP-2 is ever used clinically in human neonates or infants.

In summary, the current study extends our previous finding that GLP-2 stimulates gastrointestinal or portal blood flow and demonstrates a specific effect on the SMA-derived tissues, namely, the jejunum, but also the duodenum and pancreas. In other gastrointestinal tissues, we found that GLP-2 either had no effect or a modest suppression of blood flow in the stomach and colon but also slightly decreased spleen and kidney blood flow at the highest infusion rate. These findings may be clinically relevant for the prevention and treatment of intestinal dysfunction in infants, especially those supported by TPN, to maintain mucosal growth and function. These results also may be clinically relevant by serving to augment blood flow and minimize tissue ischemia in chronic rejection in small bowel-transplanted patients and during pancreatitis (21, 23).

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