Secretion of Trophic Gut Peptides Is Not Different in Bolus- and Continuously Fed Piglets\textsuperscript{1,2}

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Johannes B. van Goudoever\textsuperscript{*}, Barbara Stoll\textsuperscript{*}, Bolette Hartmann\textsuperscript{†}, Jens Juul Holst\textsuperscript{*}, Peter J. Reeds\textsuperscript{*} and Douglas G. Burrin\textsuperscript{3}

\textsuperscript{*}U.S. Department of Agriculture/ARS Children’s Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, Houston, Texas 77030 and \textsuperscript{†}Department of Medical Research Center, Department of Pediatrics, Baylor College of Medicine, Houston, Texas 77025. The pigs were received at 2 wk of age and fed a cow’s milk formula in a randomized, crossover design. During a 6-h period, portal blood flow and arterial and portal concentrations of glucagon-like peptide-2 (GLP-2), peptide YY (PYY) and gastric inhibitory polypeptide (GIP) were measured. All hormone levels were significantly increased within 1 h of the start of the experiment, independent of the feeding modality. There were no differences between bolus and continuous feeding in either the arterial concentrations or secretion rates of GLP-2, PYY and GIP. In both treatment groups, the increases in the plasma concentrations of GLP-2 and GIP after feeding were substantially greater than those for PYY. We conclude that the production or circulating concentrations of GLP-2, PYY and GIP are not significantly different in bolus- and primed continuously fed piglets. J. Nutr. 131: 729–732, 2001.

KEY WORDS: intestinal growth • bolus feeding • continuous feeding • glucagon-like peptide-2 • peptide YY • gastric inhibitory polypeptide

Continuous intragastric feeding is administered to infants with impaired swallowing or gastrointestinal function. However, there is evidence that continuous feeding negatively affects whole body weight gain and feeding tolerance (Schanler et al. 1999). Furthermore, continuous feeding is associated with decreased mucosal and intestinal protein mass in comparison with bolus feeding (Shulman et al. 1994). However, the impact of bolus versus continuous feeding on the physiological signals that affect growth of the gut has not been examined in detail.

Glucagon-like peptide-2 (GLP-2) and peptide YY (PYY) have been implicated as humoral signals that mediate the intestinal trophic effects of enteral nutrition. PYY and, to a lesser extent, GLP-2 have also been reported to suppress gastric motility, gastric and pancreatic secretion and thus are implicated as putative endocrine signals in the ileal brake phenomenon (Holst 1997, Pappas et al. 1986, Wojdemann et al. 1999). Both PYY and GLP-2 are produced locally in the endocrine L cells distributed along the distal ileum and colon (Adrian et al. 1985, Polak et al. 1971). Feeding is a potent stimulus for the secretion of both GLP-2 and PYY into the circulation (Xiao et al. 1999). We recently showed in neonatal pigs that circulating concentrations of GLP-2 and PYY are directly correlated with the levels of enteral nutrient intake as well as with gut growth (Burrin et al. 2000a). Moreover, there is evidence that GLP-2 and, to a lesser extent PYY have a trophic effect on the gut, including in neonates (Burrin et al. 2000b, Gomez et al. 1995, Lovshin and Drucker, 2000). Another gut peptide secreted in response to feeding, gastric inhibitory polypeptide (GIP), is produced in the K cells, which are located mainly in the duodenum (Tseng et al. 1993). GIP not only stimulates the secretion of intestinal glucagon-like peptides (Roberge and Brubaker 1993) but also may have an indirect growth-promoting effect via stimulation of insulin secretion (Fehmann et al. 1995). We hypothesized that bolus feeding compared with continuous feeding is associated with a higher secretion of gut peptides, especially GLP-2 and PYY. Furthermore, we hypothesized that the pattern of secretion of GLP-2 and PYY would parallel that of GIP.

METHODS

Animals. The protocol was approved by the Animal Care and Use Committee of 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 study involved five 30-d-old female crossbred piglets (Large White × Hampshire × Duroc) purchased from the Texas Department of Criminal Justice, Huntsville, TX. The pigs were received at 2 wk of age and fed a cow’s milk replacer formula (Litterlife; Merrick, Union, WI), at a rate of 50 g kg body\textsuperscript{-1} d\textsuperscript{-1}. The composition (per kg dry matter) of Litterlife is

\begin{align*}
\text{Glucose} & : 100 \text{ g kg}^{-1}, \\
\text{Fat} & : 5 \text{ g kg}^{-1}, \\
\text{Protein} & : 20 \text{ g kg}^{-1}, \\
\text{Calcium} & : 0.5 \text{ g kg}^{-1}, \\
\text{Phosphorus} & : 0.35 \text{ g kg}^{-1}, \\
\text{Salt} & : 1 \text{ g kg}^{-1}
\end{align*}

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\textsuperscript{4} Abbreviations used: GIP, gastric inhibitory peptide; GLP-2, glucagon-like peptide-2; PBP, portal blood flow; PDV, portal-drained viscera; PYY, peptide YY.
500 g lactose, 100 g fat and 250 g protein. The calculated energy density is 18 MJ gross energy/kg dry matter. The formula powder was thoroughly mixed with water before feeding to achieve 215 g powder.

**Study design.** The surgical procedures and postoperative care have been described previously (Stoll et al. 1999). In brief, at a postnatal age of 3 wk, piglets underwent surgery after being deprived of food overnight. Catheters were implanted into the stomach, the portal and jugular veins and the common carotid artery. An ultrasonic blood flow probe (Transonic Systems, Ithaca, NY) was placed around the portal vein. After surgery, pigs received intravenous nutrition for 2 d. Weight gain was restored to presurgical rates within 4 d after surgery.

At a postnatal age of 30 d, the piglets were deprived of food from 1800 to 00 h. Baseline (time = 0) arterial and portal blood samples were taken, and the portal blood flow (PBF) was measured for 30 min. In a randomized, crossover design, the piglets received either a bolus feed containing one third of their daily intake (≈80 mL/kg) in their feeder on study day 1 or a priming feed of one twelfth (≈20 mL/kg) of their daily intake, directly followed by a continuous intragastric infusion at a rate of one twenty-fourth of their daily intake per hour (≈10 mL/kg) on study d 2. Importantly, the total formula intake during the 6-h period was the same (80 mL/kg) in both treatment groups. Arterial and portal blood samples were drawn at hourly intervals until 6 h from the start of the feeding. PBF was measured continuously.

**Sample preparation.** Blood samples were drawn into EDTA (4.5 mg) tubes, mixed gently and immediately centrifuged at 2000 × g at 4°C for 10 min to obtain plasma. The chilled plasma samples were quickly frozen in liquid nitrogen and stored at −70°C until analysis. All samples assayed for a given hormone were run in one assay. Plasma GLP-2 concentrations were quantified using a specific N-terminal radioimmunoassay as described previously (Hartmann et al. 2000). In short, plasma samples were extracted with 75% ethanol (final concentration) and centrifuged at 3000 × g for 30 min. The supernatant was decanted, lyophilized, and reconstituted to the original plasma volume in assay buffer of 80 mmol sodium phosphate/L buffer, pH 7.5, containing 1 g valine-pyrrolidide/L (courtesy of Novo Nordisk, Bagsvaerd, Denmark), 0.1% wt/vol human serum albumin (ORHA; 20/21, Behring, Marburg, Germany), 10 mmol EDTA/L and 0.6 mmol thimerosal/L (Sigma-Aldrich Chemical, St. Louis, MO). For standards, we used human GLP-2, and the tracer was bovine GLP-2 with a Thr12→Tyr12 substitution. 125I-labeled according to the chloramine-T method. Approximately 300-μL extracted samples and human GLP-2 standards were incubated with 100 μL rabbit GLP-2 antiserum, code no. 92160 (final dilution 1:25,000), raised against an N-terminal fragment of human GLP-2; this antiserum specifically recognizes the N-terminal region of both the human and porcine GLP-2. Free and bound moieties were separated with plasma-coated charcoal (E. Merck, Darmstadt, Germany). The experimental detection limit of this assay is <5 pmol/L, and the intra-assay coefficient of variation is 5% at a concentration 40 pmol/L. PYY and GIP were measured using a commercially available radioimmunoassay kit specific for the porcine peptides (Peninsula Laboratories, San Carlos, CA). Plasma samples (100 μL) and porcine PYY standards were incubated with rabbit PYY antiserum (final dilution, 1:10,000) raised against the full-length porcine PYY (1–36) peptide. Porcine 125I-PYY was used as a tracer. The sensitivity of this assay is 0.5 pg/tube, and the intra-assay coefficient of variation was 7%.

**Statistical analysis.** All values are shown as the means ± SE. Differences in peptide production rates between the feeding modalities were assessed by one-way ANOVA. Differences in PBF and arterial peptide concentrations during the 6-h sampling period were analyzed by repeated measures ANOVA. A difference of P < 0.05 was considered statistically significant.

**RESULTS**

**PBF.** There was a significant increase in PBF in response to feeding (Fig. 1) that occurred 2 h after the start of the feeding. However, the increase was not related to the modality of feeding. On average, PBF was elevated to ~130–150% of baseline, reaching a maximum at 3 h in the bolus-fed group and at 6 h in the continuously fed piglets. PBF remained above baseline flow rates from 2 h throughout the entire study period, with no significant differences between the two feeding modalities at any time.

**GLP-2.** The baseline arterial and portal concentrations of GLP-2 were 18 ± 8 pmol/L (bolus-fed pigs) and 26 ± 10 pmol/L (continuously fed pigs). The largest increase in concentration occurred within 1 h after the start of the feeding. The concentration at 1 h was significantly different from the baseline concentration in both groups (P < 0.001, Fig. 2). No differences were found in GLP-2 concentrations between the two feeding modalities during the 6-h experiment. Hourly production of GLP-2 by the portal drained viscera was calculated on the basis of the difference in arterial and portal concentration multiplied by the PBF. Almost identical production rates for the two groups were obtained for GLP-2 measured over 6 h (Table 1).

**PYY.** The pattern of PYY secretion was similar to that of GLP-2 (Fig. 2). However, the magnitude of change in arterial concentrations after the start of feeding was much smaller. Although GLP-2 concentrations increased fourfold to eight-fold, PYY concentrations increased by only 50%. Again, we found a significant increase in concentration within 1 h, independent of feeding modality (P < 0.01). Neither arterial nor portal concentrations were different in response to either bolus or continuous feeding. The total PYY production rate by the PDV was not significantly different between groups (Table 1).

**GIP.** Arterial GIP levels increased significantly within 1 h of feeding and remained elevated throughout the experiment in the continuously fed and bolus-fed piglets (P < 0.01, Fig. 2). The magnitude of response to feeding resembled the response of GLP-2, with a fivefold to ninfold increase above baseline concentrations. The total GIP production rate by the PDV was not significantly different between groups (Table 1).
DISCUSSION

Feeding strategies that enhance either intestinal function or the growth rate of preterm infants will improve their clinical outcome and reduce hospitalization costs. Although the nutritional support of preterm infants varies considerably, it has become increasingly evident that providing enteral feedings is beneficial for both gut growth and body weight gain. However, it is less certain whether a bolus or continuous feeding regimen provides the best feeding modality. Bolus feeding increases both weight gain and intestinal growth compared with continuous feeding (Schanler et al. 1999, Shulman et al. 1994). Thus, given the evidence that GLP-2 and PYY stimulate gut growth, we anticipated that the secretion of these trophic peptides would be greater in bolus-fed than in continuously fed piglets. However, we did not find any significant differences in either the arterial concentrations or the PDV production rates of these peptide hormones, despite the fact that circulating GLP-2 concentrations were 30–40% higher in bolus- versus continuously fed pigs. In fact, the portal production rates of both PYY and GIP tended to be higher in continuously fed than bolus-fed pigs but were not statistically significant because of the large variation. These results suggest that a short-term period (i.e., 6 h) of either bolus or primed continuous feeding does not significantly affect the secretion rate or circulating concentration of these gut peptides. It is possible that the small priming feeding in the continuous group was sufficient to stimulate hormone secretion to a concentration that was not different from the bolus group. This was especially true for GIP, for which the circulating concentrations in both groups were increased to similar levels within 1 h of feeding. Thus, given these caveats, we speculate that if differential secretion of these hormones does indeed mediate the intestinal trophic effect of bolus versus continuous feeding, then additional observations are needed to detect such differences or that more prolonged treatment periods (i.e., 5–7 d) are necessary to produce differences in hormone concentrations.

Although the K and L cells producing either GIP or GLP-2 and PYY, respectively, are found at different sites along the gastrointestinal tract (Adrian et al. 1985, Polak et al. 1971, Tseng et al. 1993), all peptide concentrations increased significantly within the 1st h. That GIP concentrations would rise within 1 h was predictable, because K cells are located in the proximal small intestine (Knapper et al. 1995). However, it appears unlikely that within 1 h enteral nutrients would come in direct contact with the L cells located in the distal bowel, which implies that other mechanisms are probably involved in the secretion of GLP-2 and PYY. Indeed, GIP stimulates GLP-1 secretion from the L cell, and thus probably GLP-2 as well (Robege and Brubaker 1993). If so, it seems that GIP has a differential effect on L-cell hormone secretion, because the pattern of secretion of PYY was clearly different from that of GLP-2. Besides the endocrine system, the parasympathetic nerves and the adrenergic system may play a role in the secretion of these gut peptides (Rocca and Brubaker 1999, Sheikh et al. 1989). Thus, it is evident from the present study that direct interaction of nutrients with the L cells per se is not required to obtain a surge in GLP-2 or PYY secretion. Furthermore, based on the differences in the rise in arterial concentrations of PYY and GLP-2, it is likely that PYY secretion, although produced in the same cell as GLP-2, is regulated quite differently than GLP-2 secretion.

| TABLE 1 |
| Portal production rates of GLP-2, PYY and GIP in piglets during a 6-h period after either a bolus feed or a continuous infusion of a cow’s milk formula¹ |

<table>
<thead>
<tr>
<th></th>
<th>Bolus</th>
<th>Continuous</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLP-2</td>
<td>161 ± 57</td>
<td>168 ± 33</td>
</tr>
<tr>
<td>PYY</td>
<td>98 ± 28</td>
<td>122 ± 55</td>
</tr>
<tr>
<td>GIP</td>
<td>247 ± 125</td>
<td>313 ± 89</td>
</tr>
</tbody>
</table>

¹ Means ± se, n = 5.
² GLP-2, glucagon-like peptide-2; PYY, peptide YY; GIP, gastric inhibitory peptide.
Another striking difference between the secretion pattern of GLP-2 and GIP with that of PYY was the magnitude of the increase after feeding. Although maximal PYY concentrations showed a 50% increase, GLP-2 and GIP increased fourfold to eightfold. The magnitude of increase in GLP-2 concentrations after a meal in our study was higher than that found in adult humans (Hartmann et al. 2000, Orskov and Holst 1987, Xiao et al. 1999), whose GLP-2 levels increased 1.3- to 4-fold. This might be a consequence of the stage of development, because circulating concentrations of PYY decrease with age (Adrian et al. 1986). However, the increase in GLP-2 levels in bolus-fed pigs was even greater than the difference that we found previously between neonatal pigs fed either enterally or parenterally (~2-fold) (Burrin et al. 2000a). GIP secretion increases threefold in response to feeding in ~8-wk-old pigs to a maximum of 400 pmol/L, yet the relative increase in the postfeeding plasma concentrations of our younger piglets was much higher.

The circulating concentration of these gut peptides is determined not only by the production rate but also by the clearance rate. Recent evidence shows that the kidneys are the major site of GLP-2 clearance (Tavares et al. 2000). That neonates have lower renal clearance rates is well known and might explain the higher levels we found in our neonatal piglets compared with those of older mammals. We are not aware of any studies that measured the production of gut hormones by the PDV, so we cannot determine whether the relatively high concentrations of hormones we found were due to a lower clearance rate or a higher production rate in the neonatal pig.

In conclusion, we did not find a significant difference in the concentrations of GLP-2, PYY or GIP in response to a primed continuous versus bolus feeding. Moreover, the overall production rate of these trophic peptides by the PDV was not significantly different. Thus, if indeed bolus feeding is more trophic to the gut mucosa than continuous feeding, the response does not appear to be mediated via acute differences in GLP-2, PYY or GIP secretion.

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LITERATURE CITED


