Glucagon-like peptide 2 treatment may improve intestinal adaptation during weaning

T. Thymann,* I. Le Huërou-Luron,† Y. M. Petersen,* M. S. Hedemann,‡ J. Elinf,§ B. B. Jensen,‡ J. J. Holst,# B. Hartmann,# and P. T. Sangild*

*University of Copenhagen, Dep. of Human Nutrition, Exercise and Sports, DK-1958 Frederiksberg, Denmark; †INRA UR1341 ADNC, F-35590 Saint-Gilles, France; ‡Aarhus University, Faculty of Agricultural Sciences, Dep. of Animal Nutrition and Physiology, DK-8830 Tjele, Denmark; §University of Copenhagen, Department of Basic Animal and Veterinary Sciences, DK-1870 Frederiksberg, Denmark; and #University of Copenhagen, The NNF center for basic metabolic research, Dep. of Biomedical Sciences, DK-2200 Copenhagen, Denmark

ABSTRACT: Transition from sow’s milk to solid feed is associated with intestinal atrophy and diarrhea. We hypothesized that the intestinotrophic hormone glucagon-like peptide 2 (GLP-2) would induce a dose- and health status-dependent effect on gut adaptation. In Exp. 1, weaned pigs (average BW at weaning 4.98 ± 0.18 kg) were kept in a high-sanitary environment and injected with saline or short-acting GLP-2 (80 μg/(kg BW·12 h); n = 8). Under these conditions, there was no diarrhea and GLP-2 did not improve gastrointestinal structure or function. In Exp. 2, weaned pigs (average BW at weaning 6.68 ± 0.27 kg) were kept in a low-sanitary environment, leading to weaning diarrhea, and injected with saline or short-acting GLP-2 (80 μg/(kg BW·12 h); n = 11). Treatment with GLP-2 increased goblet cell density (P < 0.05) and reduced short chain fatty acid concentration in the colon (P < 0.01) but had limited effects on diarrhea. In Exp. 3, weaned pigs (average BW at weaning 6.90 ± 0.32 kg) were kept in a low-sanitary environment and injected with saline or a long-acting acylated GLP-2 analogue (25 μg/(kg BW·12 h); n = 8). In this experiment, GLP-2 increased intestinal weight (+22%; P < 0.01) and activity of brush border enzymes (+50–100%; P < 0.05). Circulating GLP-2 levels were in the pharmacological range in Exp. 3 (constant levels >20,000 pmol/L) and Exp. 2 (increases to 20,000 pmol/L for a few hours each day) while they were in the supraphysiological range in Exp. 1 (50–200 pmol/L). In conclusion, GLP-2 may improve gut structure and function in weaning pigs. However, the effects may be significant only under conditions of diarrhea and if GLP-2 exposure time is extended using long-acting analogues.

Key words: adaptation, dietary transition, glucagon-like peptide 2, intestine, pigs, weaning

INTRODUCTION

Glucagon-like peptide 2 (GLP-2) stimulates intestinal growth and adaptation and is secreted from entero-endocrine L-cells in response to ingestion of nutrients such as carbohydrates and fat (Drucker, 1998). While GLP-2 from human, bovine, rat, and mouse are all 33 amino acid peptides, porcine GLP-2 is a 35 amino acid peptide with Ser and Leu at the C-terminal end (Pedersen et al., 2008). After secretion, the intact peptide is rapidly degraded by the enzyme dipeptidyl peptidase IV (DPP IV) to form a truncated, inactive peptide metabolite (Hansen et al., 2007). The native peptide has a short half-life of 7 to 8 min in both humans and pigs (Pedersen et al., 2008), so long acting analogues may show superior effects by maintaining circulating GLP-2 at higher levels for an extended period of time.

The therapeutic potential of GLP-2 has mostly been investigated in conditions where the total mucosal surface is compromised, such as in short bowl syndrome patients or subjects receiving total parenteral nutrition. Another, yet unexplored therapeutic potential of GLP-2 is the weaning-induced intestinal atrophy and dysfunction that occurs in pigs. Abrupt weaning of pigs in early life associates with intestinal atrophy and development.
Intestinal adaptation during weaning

This study aimed to minimize the role of the gut microflora on diarrhea development. We aimed at minimizing pig-to-pig fecal contamination by keeping weaned pigs in individual cages that were cleaned daily. These procedures previously led to a low prevalence of weaning diarrhea in the same unit. All the weaned pigs had ad libitum access to water and feed, whereas no solid feed was offered during the suckling period to any group. To maximize the dietary challenge induced by the weaning transition, the feed was formulated to contain plant products only. Main ingredients were wheat, maize, barley, and soybean meal. The individual feed consumption was recorded daily.

**Sample Collections.** Blood samples for measurement of plasma GLP-2 were collected on d 18, 21, 23, 25, and 26 before daily GLP-2 injection. As GLP-2 is influenced by ingestion of feed, the pigs were feed deprived for 3 h before each blood collection. Blood samples were collected in ice-chilled tubes containing EDTA and an inhibitor of DPP IV (Valine-Pyrrolidide; Novo Nordic). Plasma was isolated on centrifugation and stored at -20°C for later analysis of GLP-2.

Two hours before killing on d 5 to 6 postweaning, the pigs received an intravenous dose of bromodeoxyuridine (BrdU; 50 mg/kg BW; Sigma Aldrich, St. Louis, MO) for later histological evaluation of proliferating cells in the small intestinal tissue. The pigs were subsequently killed with an intravenous injection of sodium pentobarbitone. The whole gastrointestinal tract was quickly removed and weights of the stomach, pancreas, and small and large intestine were recorded. Samples at 16 (proximal) and 84% (distal) of the small intestinal length were excised and snap frozen in liquid nitrogen for analysis of digestive enzyme activity. For histology, additional samples from the same locations were fixed for 24 h in formaldehyde or Carnoy buffer and subsequently transferred to 70% ethanol for storage. A 10-cm section from the proximal, middle, and distal small intestine and the colon were excised and slit along their lengths so the mucosal side was exposed. The mucosa was gently scraped off with a blade and its weight determined on both wet and dry basis.

**Glucagon-Like Peptide 2 Radioimmunoassay.** Plasma concentrations of active GLP-2 were determined by RIA as described previously (Hartmann et al., 2000) using a rabbit anti-human antibody targeted at the NH2-terminal region of GLP-2 and cross-reacting 100% with porcine GLP-2.

**Intestinal Morphology.** Villous height and crypt depth were measured on formalin-fixed tissue samples from the proximal and distal small intestine using a microdissection technique. Carnoy-fixed samples from the proximal and distal small intestine were embedded in paraffin, sliced, and incubated with a mouse anti-BrdU/nuclease antibody (Amersham; Pharmacia Biotech, Piscataway, NJ). Subsequently, a secondary biotinylated anti-mouse antibody was applied and visualized with peroxidase (Vector Laboratories, Burlingame, CA). The slices were counterstained with hematoxylin and eosin and the number of stained nuclei were counted and expressed relative to the total number of nuclei per crypt.

**Enzyme Activity.** Activities of lactase (Enzyme Commission number [EC] 3.2.1.23-62), sucrase (EC 3.2.1.48-10), maltase (EC 3.2.1.20), DPP IV (EC 3.4.14.5), aminopeptidase N (ApN; EC 3.4.11.2), and...
aminopeptidase A (ApA; EC 3.4.11.7) in homogenates of proximal and distal small intestinal tissue were determined spectrophotometrically using lactose, sucrose, maltose, glycy1-L-prolin-4-nitroanilide, L-alanine-4-nitroanilide, and d-L-glutamic acid 4-nitroanilide, respectively, as substrates, according to a preestablished protocol (Sangild et al., 2002). Enzyme activities were expressed per gram of wet intestine and a hydrolytic rate of 1 μmol substrate released/min at 37°C represented 1 U of enzyme activity.

Experiment 2

Experimental Design. On d 24 postpartum, 22 suckling pigs from 4 different litters were separated from their sows (Landrace × Yorkshire). The experimental setting was a commercial pig farm (Ugerløse, Denmark), selected based on a long history of severe weaning-associated diarrhea. One group of suckling pigs was killed for tissue collection immediately after separation from the sow (preweaning; n = 8), whereas the rest were weaned. The newly weaned pigs were allocated to 2 groups with equal representation from the 4 litters. Pigs in one treatment group were injected intra muscular (i.m.) with GLP-2 (200 μg/(kg BW·12 h); GLP-2 group; n = 11), whereas the other was injected with a similar volume of saline (control; n = 11). Both groups were injected for 7 d starting from the day of weaning.

Housing and Feeding. No solid feed was offered during the suckling period. The GLP-2 and control groups were group housed in 2 cages with poor sanitary conditions to maximize spontaneous development of diarrhea. The cages were not cleaned before use, and there was no daily cleaning during the experiment. There was ad libitum access to water and dry feed and the feed was formulated to contain ingredients of plant and animal origin suitable for young weanling pigs. Main feed ingredients were wheat, fish meat, swine fat, potato, and dairy products. Feed consumption on a group basis was measured daily, and pigs were weighed at weaning and at the time of tissue collection.

Sample Collection. To document increased plasma levels of GLP-2 after injection, blood samples were collected at 0, 45, 90, 180, and 300 min postinjection on d 7 postweaning. Next, the pigs were anesthetized with zolazepam/tiletamin (Zoletil; Boehringer Ingelheim, Copenhagen, Denmark) and killed with sodium pento-barbitone. The intestine was immediately removed and tissue was sampled at 80 cm distal to the pyloric sphincter (proximal), 80 cm proximal to the ileocecal valve (distal), and from the middle colon, respectively, and transferred to an oxygenated bath of Ringers solution for ex vivo nutrient absorptive function was done using the everted-sleeve technique according to an established protocol (Karason and Diamond, 1983). Briefly, tissue samples from the proximal and distal small intestine and middle colon were everted, mounted on steel rods, and kept in oxygenated saline baths containing 50 mM of either glucose, fructose, butyrate (containing 0.01 mM 14C-labeled tracer), Leu, or Pro (containing 0.01 mM 3H-labeled tracer) or tracer alone. The samples were weighed and radioactivity levels were subsequently measured (Wallac, PerkinElmer, Boston, MA). Calculations of nutrient accumulation rates of each individual nutrient were done according to Karason and Diamond (1983). Furthermore, as an indication of carrier dependency, ratios of nutrient accumulation in the presence of tracer alone to presence of tracer in 50 mM of nonlabeled nutrients were calculated according to previously established protocols (Buddington et al., 2000).

Morphology. After 24 h in formaldehyde, the tissue samples from proximal and distal small intestine and the colon were transferred to a fresh solution of 10% neutral buffered formaldehyde and subsequently dehydrated with alcohol, infiltrated with paraffin wax, and sliced. Three slides were prepared from each sample, and each slide contained a minimum of 4 sections cut at 4 μm, at least 50 μm apart. The slides were processed for carbohydrate histochemistry using either periodic acid-Schiff (PAS; to stain neutral mucins) or Alcian blue at pH 2.5 (to stain carboxylated or sulfated types of acidic mucins) or pH 1.0 (to stain sulfomucins). Carbohydrate histochemistry on the PAS and Alcian blue stained samples were evaluated as described previously (Brunsgaard, 1997). Briefly, 15 well-oriented villi and crypts were selected on each slide and for each villus and crypt the area of mucin granules with a clear positive reaction for either neutral, acidic, or sulfomucins were determined using a computer-integrated microscope and an image analysis system (Quantimet 500MC; Leica, Cambridge, UK). The PAS-stained slides were further used to determine villus height and crypt depth using the image analysis system.

Enzyme Activity. Activities of brush border enzymes lactase, sucrase, maltase, DPP IV, ApA, and ApN in proximal and distal small intestinal tissue were determined as in Exp. 1. The activity of amylase, trypsin, and chy-
motrypsin were analyzed in pancreatic homogenates according to an established protocol (Sangild et al., 2002). Ethyldened-p-nitrophenyl, D-maltoheptasidase was used as a substrate for amylyase and the liberated nitrophenol determined spectrophotometrically at 405 nm (577-50P; Sigma). Benzoyl-L-arginine-p-nitroanilide was used as the substrate for trypsin after activation of trypsinogen with enterokinase (B 4875 and E 0632; Sigma), and chymotrypsin was measured using Succinyl-Ala-Ala-Pro-Phe-p-nitroanilide (S 7388; Sigma). For all enzymes, a hydrolytic rate of 1 µmol substrate released/min at 37°C represented 1 U of enzyme activity.

Short Chain Fatty Acids. The concentrations of short chain fatty acids (SCFA) were measured as described previously (Jensen et al., 1995). Briefly, 10 g of contents from the colon were diluted 10-fold in a sodium hydroxide solution containing 2-ethylbutyric acid as an internal standard and homogenized for 2 min. One milliliter of the diluted sample was extracted and quantification of SCFA was performed on a gas chromatograph and the chromatograms were analyzed using HP GC ChemStation software (Agilent Technologies, Santa Clara, California).

Experiment 3

Experimental Design. Like Exp. 2, this experiment was conducted in an experimental setting known to have a high prevalence of weaning-associated diarrhea (Bårup, Denmark). On d 25 postpartum, 16 suckling pigs from 3 litters (Landrace × Yorkshire) were selected and injected i.m. with either a stabilized acylated GLP-2 receptor agonist (GLP-2 group; n = 8; 25 µg/kg BW·12h; Novo Nordic) or a similar dose of saline (control; n = 8). The pigs were subsequently weaned on d 28 and transferred to a housing facility characterized by low-sanitary conditions.

Housing and Feeding. The GLP-2 and control groups were kept in 2 separate cages, and the cages were not cleaned before or during the experiment. Daily recordings included feed consumption (on a group basis) and individual BW. The diet was formulated to contain ingredients of plant and animal origin suitable for young weanling pigs. Main feed ingredients were wheat, fish meal, potato protein, and dairy products.

Sample Collection. To document circulating levels of GLP-2, jugular blood samples were retrieved from the GLP-2 group at 0, 1, and 19 h after injection on d 5 after weaning. To document endogenous levels, a blood sample was collected from each control pig on d 5 after weaning. After the blood sampling, pigs were anesthetized and killed as previously described. Recordings included weight of all visceral organs and length of the small intestine and a score was given to colon contents (1 = firm, 2 = well shaped, 3 = soft, and 4 = watery). Sample collection included tissue samples from the proximal (80 cm distal to the pylorus) and distal (80 cm proximal to the cecum) small intestine. The tissue samples were either snap frozen in liquid nitrogen and subsequently stored at −80°C or fixed in 4% buffered formaldehyde. After 24 h fixation, tissues were dehydrated in 70% ethanol, embedded in paraffin, and sliced and stained with hematoxylin and eosin (H&E). In each sample, villus height and crypt depth were measured on 10 to 15 well-oriented villi and crypts using Softworx Explorer 1.0 (Applied Precision, Issaquah, Washington). Plasma GLP-2 concentrations, percentage of mucosa in the small intestine, and activities of lactase, sucrase, maltase, DPP IV, ApN, and ApA were determined as described in Exp. 1 and 2.

Statistics. Experiments 1, 2, and 3 were analyzed separately and no comparisons were made among them. All data were analyzed using pig and litter as random variables and treatment and intestinal region as fixed variables in the Mixed procedure of the SAS statistical software program (SAS version 9.1; SAS Inst. Inc., Cary, NC). Probability levels below 0.05 were considered significant.

RESULTS

Experiment 1. Weaning pigs (GLP-2 and control) kept in high-sanitary conditions did not develop diarrhea and there was a positive weight gain for both groups (Table 1). In controls relative to preweaning levels there was reduced villus height in the distal small intestine whereas brush border enzyme activities (expressed per gram of tissue) were markedly increased (P < 0.01) for maltase, sucrase, ApN, DPP IV, and ApA (Table 2). These weaning adaptations in the small intestine were also associated with increased stomach and pancreas weights (relative to BW) in weaned control pigs versus preweaning pigs (Table 1).

Average basal plasma GLP-2 levels (before each daily injection) were significantly higher in GLP-2 treated pigs versus control (210 ± 127 vs. 45 ± 21 pmol/L; P < 0.001). Treatment with GLP-2 caused significantly reduced feed intake compared with controls whereas daily weight gain was not different (Table 1). Although this may indicate improved utilization of feed in the GLP-2 group relative to controls, GLP-2 treatment did not affect gut indices including brush border enzyme activity (lactase, maltase, sucrase, ApA, ApN, and DPP IV), small intestinal weight, villus height, or crypt depth (Fig. 1). There was no effect of intestinal region. Cell proliferation measured by the BrdU technique showed major differences between the proximal and distal small intestine but no difference between GLP-2 and controls. Hence, across treatment groups the number of positively stained cells relative to total number of crypt cells was approxi-
Relative to control, GLP-2 treatment did not increase weights (relative to BW) of the stomach, colon, and kidney while weights for the heart and lungs were reduced (Table 1). These adaptations in relative weights of organs were not due to changes in BW as there were no significant differences in BW before or after weaning among groups (Table 1).

Plasma GLP-2 levels after a single intramuscular injection reached a peak of approximately 30,000 pmol/L at 45 min, decreasing to approximately 6,000 pmol/L at 300 min (Fig. 2). Endogenous levels in control pigs were 130 ± 20 (preweaning) and 140 ± 20 pmol/L (postweaning). Relative to control, GLP-2 treatment did not change daily weight gain or weight of the small intestine, mately 100-fold higher in the proximal relative to the distal small intestine.

**Experiment 2.** Weaning pigs (both GLP-2 and control) all developed diarrhea and there was no weight gain for either group relative to the preweaning level (Table 1). This was associated with marked villus atrophy, crypt elongation, and reduction in activity of digestive enzymes (lactase, ApN, ApA, DPP IV, and amylase) in control relative to preweaning levels (Tables 1 and 2). Furthermore, compared with preweaning levels, weaning pigs from both GLP-2 and control groups showed increased weights (relative to BW) of the stomach, colon, and kidney while weights for the heart and lungs were reduced (Table 1). These adaptations in relative weights of organs were not due to changes in BW as there were no significant differences in BW before or after weaning among groups (Table 1).

**Table 1.** Organ dimensions in preweaning pigs or weaned pigs housed in either high- or low-sanitary conditions and treated with either native or acylated glucagon-like peptide 2 (GLP-2). Preweaning pigs were 18 d old (Exp. 1) or 24 d old (Exp. 2 and 3) and weaning values represent tissue collected 5 to 7 d after weaning. Data represent means ± SEM.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Exp. 1: high sanitation native GLP-2</th>
<th>Exp. 2: low sanitation native GLP-2</th>
<th>Exp. 3: low sanitation, acylated GLP-2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GLP-2</td>
<td>Control</td>
<td>GLP-2</td>
</tr>
<tr>
<td>Intestinal length, cm/kg</td>
<td>141 ± 7</td>
<td>130 ± 7</td>
<td>121 ± 6</td>
</tr>
<tr>
<td>Percent dry mucosa</td>
<td>66 ± 5 a</td>
<td>73 ± 1 b</td>
<td>69 ± 9 ab</td>
</tr>
<tr>
<td>Stomach, g/kg</td>
<td>4.1 ± 0.2 a</td>
<td>5.1 ± 0.1 b</td>
<td>5.4 ± 0.2 b</td>
</tr>
<tr>
<td>Colon, g/kg</td>
<td>1.1 ± 0.04 a</td>
<td>1.3 ± 0.1 b</td>
<td>1.5 ± 0.1 b</td>
</tr>
<tr>
<td>Heart, g/kg</td>
<td>9.8 ± 1.0 a</td>
<td>15.9 ± 0.8 b</td>
<td>15.7 ± 1.0</td>
</tr>
<tr>
<td>Pancreas, g/kg</td>
<td>3.6 ± 0.3 a</td>
<td>2.7 ± 0.2 b</td>
<td>2.7 ± 0.3 b</td>
</tr>
<tr>
<td>Amylase, U/g</td>
<td>17.2 ± 4.1 a</td>
<td>9.3 ± 1.4 b</td>
<td>8.9 ± 1.1 b</td>
</tr>
<tr>
<td>Chymotrypsin, U/g</td>
<td>2.6 ± 0.4</td>
<td>2.8 ± 0.6</td>
<td>2.4 ± 0.6</td>
</tr>
<tr>
<td>Trypsin, U/g</td>
<td>5.1 ± 1.6</td>
<td>5.3 ± 1.0</td>
<td>3.9 ± 0.7</td>
</tr>
</tbody>
</table>

**Table 2.** Changes in gut indices (distal small intestine) in weaned control pigs housed in high- or low-sanitary conditions compared with preweaning values. Preweaning pigs were 18 d old (Exp. 1) or 24 d old (Exp. 2) and weaning values represent tissue collected 7 d after weaning. Data represent means ± SEM.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1 Exp. 1: high sanitation</th>
<th>2 Exp. 2: low sanitation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prewean</td>
<td>Control</td>
</tr>
<tr>
<td>LacN, U/g</td>
<td>0.21 ± 0.02</td>
<td>0.27 ± 0.07</td>
</tr>
<tr>
<td>Maltase, U/g</td>
<td>1.69 ± 0.44</td>
<td>7.53 ± 1.08**</td>
</tr>
<tr>
<td>Sucrase, U/g</td>
<td>0.21 ± 0.07</td>
<td>1.37 ± 0.20**</td>
</tr>
<tr>
<td>ApN, U/g</td>
<td>1.48 ± 0.37</td>
<td>4.49 ± 0.56**</td>
</tr>
<tr>
<td>DPP IV, U/g</td>
<td>0.52 ± 0.13</td>
<td>1.56 ± 0.23**</td>
</tr>
<tr>
<td>ApA, U/g</td>
<td>1.76 ± 0.55</td>
<td>6.32 ± 0.86**</td>
</tr>
<tr>
<td>villus height, µm</td>
<td>733 ± 33</td>
<td>426 ± 7***</td>
</tr>
<tr>
<td>Crypt depth, µm</td>
<td>91 ± 6</td>
<td>126 ± 18</td>
</tr>
</tbody>
</table>

1Significant differences are indicated within each row (***P < 0.001, **P < 0.01, and *P < 0.05). Comparisons are made between control and preweaning values within each experiment while no comparisons between Exp. 1 and 2 are made.

2For all enzymes, a hydrolytic rate of 1 µmol substrate released/min at 37°C represented 1 U of enzyme activity per gram of pancreatic tissue.
Intestinal adaptation during weaning

stomach, colon, pancreas, heart, lungs, liver, kidney, or adrenals (all relative to BW; Table 1). Likewise, there were no differences between GLP-2 and control in activity of digestive enzymes in the intestine or pancreas or histological dimensions of the small intestine (Table 1; Fig. 1). In the colon, however, the total concentration of SCFA in contents was lower in GLP-2 pigs compared with controls (93 ± 5 vs. 68 ± 7 mmol/kg; \( P < 0.01 \)). The most dominating SCFA were acetic acid, propionic acid, and butyric acid, which either tended to be lower (acetic acid, \( P = 0.12 \), and buturic acid; \( P = 0.09 \)) or were significantly lower (propionic acid, \( P < 0.01 \)) in GLP-2 versus control (data not shown). This was associated with a significantly higher density of mucin-containing goblet cells in the colon in GLP-2 relative to controls (+50%; \( P < 0.05 \)) while preweaning pigs had an even higher level (Fig. 3). Histological stainings with Alcian blue at both pH 1.0 and 2.5 yielded results similar to PAS staining (data not shown).

Between GLP-2 and controls, measurement of absorptive function ex vivo showed similar results for glucose, fructose, Leu, and Pro (Fig. 4). Relative to preweaning pigs, there was a marked reduction in absorption of glucose and Pro (in the proximal intestine) and Leu and Pro (in the distal intestine) in both the control and the GLP-2 group. Finally, fructose absorption in the proximal small intestine was significantly higher in preweaning pigs relative to GLP-2 pigs, with intermediate values in the control group. There was no specific GLP-2-induced regulation of the carrier-dependent nutrients (glucose and Leu) versus the less carrier-dependent nutrients (fructose and Pro).
Experiment 3. Body weight at weaning was similar between GLP-2 and control and did not change significantly for either group after weaning (Table 1). Blood samples collected at time \( t = 0, 1 \), and 19 h after a single injection of acylated GLP-2 on d 5 showed very high and stable GLP-2 plasma levels (Fig. 2), whereas endogenous level in control pigs was approximately 1% of this level (237 ± 43 pmol/L; \( P < 0.001 \)). Treatment with acylated GLP-2 improved gut health as indicated by a lower score for colon content in GLP-2 pigs vs. controls (2.25 ± 0.31 vs. 3.50 ± 0.26; \( P < 0.01 \)). This was further associated with markedly increased small intestinal brush border enzyme activity for sucrase, maltase, ApN, ApA, and DPP IV in the GLP-2 group versus control (Fig. 1). Moreover, the GLP-2 group showed higher intestinal weight, larger villi, and deeper crypts (Fig. 1) and larger small intestinal circumference (+14%; \( P < 0.05 \); data not shown). Finally, GLP-2 showed a trophic effect on liver weight whereas other organs were not different among the groups (Table 1).

DISCUSSION

In low-sanitary conditions the acylated, long-acting GLP-2 improved intestinal function markedly and improved the score of colon luminal content in weanling pigs. Injections with the native GLP-2 under similar conditions had more marginal effects on diarrhea scores, although it did increase the density of goblet cells and reduced the concentration of SCFA in the colon, indicating a beneficial effect of GLP-2 on mucosal protection and nutrient fermentation. When weaning diarrhea was completely absent in the high-sanitary conditions, native GLP-2 failed
to affect intestinal structure and function. Collectively, these data indicate that administration of GLP-2 may indeed affect gut function and diarrhea sensitivity around weaning but that the effects are most pronounced under low-sanitary conditions with a high prevalence of weaning-associated diarrhea and that prolonged pharmacological levels of GLP-2 are required to exert this effect.

Based on the fact that the presence of nutrients in the gut lumen exerts trophic effects on the intestine via stimulation of growth factors such as GLP-2 (Burrin et al., 2003; Drucker, 2002), we hypothesized that treatment with exogenous GLP-2 would reduce diarrhea symptoms in the weaning-compromised gut. Whereas the role of GLP-2 in gut maturation in neonatal animals has been studied (Burrin et al., 2000; Petersen et al., 2003), its role around weaning remains largely unexplored. Using the same animals as in Exp. 1, Petersen et al. (2003) showed that endogenous plasma GLP-2 levels during the first 4 d postweaning followed the transient decrease and subsequent resumption in voluntary feed intake, while GLP-2 receptor mRNA levels became downregulated relative to preweaning levels. Moreover, it has been shown in weanling rats that anti-GLP-2 treatment significantly inhibited enterocyte proliferation in the distal small intestine indicating sensitivity around the time of weaning (Ishizuka et al., 2009). Although the sensitivity toward GLP-2 may be higher in the neonatal period, these data and the data from the present studies indicate that some degree of GLP-2 sensitivity remains during the weaning transition.

In Exp. 1, daily feed intake following weaning was transiently reduced to approximately 10 g on d 1 (for both GLP-2 and controls), increasing to 182 (GLP-2) and 239 g (control) on d 4. In comparison, the level of nutrient intake in suckling pigs of similar age is approximately 150 g DM/d (Auldist et al., 2000). Combined with the clinical observations, these data indicate that low feed intake is associated with weaning but does not per se result in clinical diarrhea when pigs are kept in high-sanitary conditions. In contrast, low feed intake and low-sanitary conditions combined triggered development of diarrhea as observed in Exp. 2. Whether continued feed intake at a level similar to preweaning levels would prevent diarrhea development in low-sanitary conditions remains speculative. Regardless, epidemiological data indicate that low feed intake correlates strongly with presence of diarrhea (Madec et al., 1998), but direct causality remains questionable.

In Exp. 1 and 2, villus atrophy and crypt elongation took place after weaning in both GLP-2 and control pigs regardless of presence or absence of diarrhea. This indicates that normal physiological changes in villus–crypt dimensions take place as a result of weaning in both pathological and healthy conditions. Although we did not observe an effect of native GLP-2 on villus–crypt dimensions in these first 2 experiments, the data from Exp. 3 does indicate that stabilized acylated GLP-2 has a positive effect on villus–crypt dimensions and that it reduces the severity of weaning diarrhea. Under the high-sanitary conditions (Exp. 1), weaning improved gut function, as indicated by markedly increased digestive enzyme activities (ApA, ApN, DPP IV, sucrase, and maltase). This weaning-associated stimulation of brush border enzymes was absent in the low-sanitary conditions in Exp. 2 where activity of all aminopeptidases (ApA, ApN, and DPP IV) were markedly reduced and sucrase and maltase activity remained unchanged compared with preweaning pigs. Under these conditions, we showed in Exp. 3 that pigs may benefit from being exposed to high and prolonged levels of circulating GLP-2, as indicated by improved digestive enzyme activities after treatment with acylated GLP-2. The enzyme maturational effects of acylated GLP-2 were most pronounced in the distal small intestine, whereas villus and crypt elongation were more pronounced in the proximal small intestine.

A compromised digestive system induced by low-sanitary conditions may lead to increased availability of fermentable substrate in the colon. From this, an increased production of SCFA by the resident microbiota may exceed the absorptive capacity and cause water accumulation rather than absorption in the colon leading to osmotic diarrhea. In Exp. 2, native GLP-2 significantly reduced the total concentration of SCFA and increased the density of goblet cells in the colon. This indicates a positive effect on colon function, although this effect appeared inadequate to prevent weaning diarrhea. Whether the GLP-2 induced reduction in concentration of SCFA in the colon resulted from increased absorption of SCFA, reduced water absorption, reduced bacterial fermentation, or reduced availability of substrate is not clear. The data from Exp. 2 on nutrient absorptive rate suggest that there was little effect of GLP-2 on the absorptive mechanism per se. Other studies in rats and parenterally fed newborn pigs have shown that GLP-2 increases Sodium-Glucose Linked Transporter-1 (SGLT-1) mRNA levels and protein abundance and stimulates glucose uptake (Cheeseman, 1997; Iordache et al., 2005). The lack of response to GLP-2 on glucose uptake in Exp. 2 may be related to a general dysfunction of the gut but could also be due to reduced expression of the GLP-2 receptor. Petersen et al. (2003) showed that mRNA levels for the receptor in the small intestine are reduced following weaning and other studies have not been able to document that GLP-2 increases nutrient absorption during the weaning process (Iordache et al., 2005). Hence, GLP-2 may be less efficient to increase glucose absorptive function in weanling pigs relative to parenterally fed neonatal pigs.

The effects of GLP-2 may vary widely according to the dose, administration method, and physiologic state of
the intestine (Kaji et al., 2008, 2009). Using an adult rat model of intestinal resection, Kaji et al. (2009) showed that GLP-2 treatment stimulated growth of the intestine (length, weight, and width) during the early postresection period while later, GLP-2 treatment mainly stimulated villus height and protein abundance of SGLT-1 and Glucose transporter-5 (GLUT-5) transporters. Our incomplete understanding of GLP-2 effects is also illustrated by recent work on a short bowel pig model, where GLP-2 treatment had adverse effects (Pereira-Fantini et al., 2008). Although this is a surprising result, it suggests that GLP-2 treatment must be targeted to specific clinical situations where the organism is receptive.

Whether the lack of response to GLP-2 treatment in Exp. 1 was due to lack of sensitivity to the hormone is speculative. The absence of diarrhea is likely explained by the high-sanitary conditions but it remains difficult to explain that enterocyte proliferation rate remained unaffected while other studies on healthy animals have shown dose-dependent effects of GLP-2 on enterocyte proliferation (Burrin et al., 2005). It is possible that the route of nutrition, enteral versus parenteral, also plays an important role. Most of the studies that document trophic effects of GLP-2 have used parenterally fed animals or humans (Burrin et al., 2000; Chance et al., 2006; Cottrell et al., 2005, 2006; Howard et al., 2004; Jeppesen et al., 2005; Martin et al., 2004) and there is less evidence in favor of effects during enteral feeding (Liu et al., 2006). Whether the marginal effect of native GLP-2 seen in our studies (Exp. 1 and 2) is due to altered feed intake or unresponsiveness in weanling pigs should be studied using more controlled nutritional regimens such as tube feeding or total parenteral nutrition. However, the fact that stabilized acylated GLP-2 (Exp. 3) did induce significant effects indicates that exogenous GLP-2 can provide an effect in weanling pigs also during enteral nutrition provided the dose is sufficiently high.

The discrepancy in effect between the native and acylated form of GLP-2 in low-sanitary conditions is intriguing. The reduced responsiveness to native human GLP-2 may be related to dosage and fast degradation of the peptide, which may affect local GLP-2 concentration around the receptor. Thulesen and coworkers (Thulesen et al., 2002) have shown that the truncated metabolite of GLP-2 (GLP-2a3-33) is a partial GLP-2 receptor agonist. As high doses of native GLP-21-33 are quickly truncated by DPP IV to form the GLP-2a3-33 metabolite, there may be competition for the receptor leading to reduced effect of the intact GLP-21-33 peptide. This may partly explain the marginal effects of native GLP-2 compared with acylated GLP-2, which is probably not degraded, and is further supported by the finding that plasma levels following injection of acylated GLP-2 remained high for at least 19 h whereas approximately 80% of the native GLP-2 was cleared within 5 h (Fig. 2).

We conclude that acylated GLP-2 significantly improves gut adaptation during dietary transition phases such as weaning. However, the potential of GLP-2 treatment to improve the weaning-associated gut adaptation is most pronounced during conditions that lead to a high prevalence of weaning-associated diarrhea (e.g., high environmental stress, low sanitation).

**LITERATURE CITED**


