ABSTRACT: The objectives of this study were to use transgenic sows that overexpress IGF-I in milk to investigate the effect of a short-term fast on piglet intestinal morphology and disaccharidase activity and to determine how milk-borne IGF-I influences the response to fasting. After farrowing, litters were normalized to 10 piglets. On d 6, piglets (n = 30) suckling IGF-I transgenic (TG) sows and piglets (n = 30) suckling non-transgenic sows (control) were assigned randomly to three treatments: fed piglets (0 h), which remained with the sow until euthanized on d 7, or fasted piglets, which were removed from the sow at either 6 or 12 h before euthanasia on d 7. Serum IGF-I and IGFBP, intestinal weight and length, jejunal protein and DNA content, disaccharidase activity, and villus morphology were measured. Fasting for 12 h resulted in a negative weight change between d 6 and 7 (quadratic response to fasting; \( P < 0.001 \)). Piglets suckling TG sows tended to have greater intestinal length (\( P = 0.068 \)), but no effect of IGF-I overexpression was noted for intestinal weight. Fasting, however, resulted in linear (\( P < 0.001 \)) and quadratic (\( P = 0.002 \)) decreases in intestinal weight. Serum IGF-I did not differ between control and TG sows, but decreased linearly (\( P = 0.003 \)) with fasting. Serum IGFBP-4 decreased (linear and quadratic; \( P \leq 0.02 \)) with fasting, whereas IGFBP-1 increased quadratically (\( P < 0.001 \)) with fasting. Jejunal villus height, width, and crypt depth were all increased with fasting (linear and quadratic; \( P < 0.04 \)). Disaccharidase activity was not affected by fed state; however, piglets suckling TG sows had greater jejunal lactase-phlorhizin hydrolase (\( P < 0.01 \)) and sucrase-isomaltase (\( P = 0.02 \)) activities than control piglets. In summary, intestinal weight, villus morphology, serum IGF-I, serum IGFBP-1 and -4, and piglet BW change were altered (\( P < 0.02 \)) in response to fasting. Thus, the duration of food deprivation before euthanization should be considered when designing experiments to assess intestinal development or the IGF axis, as the magnitude of differences between the fed and fasted state may exceed those expected as a result of experimental treatment.

Key Words: Enzyme, Fasting, Insulin-Like Growth Factor-I, Intestine, Piglet, Transgenic

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Introduction

The gastrointestinal tract is the first organ system directly affected by changes in nutrient intake, and it displays both acute and long-term adaptation to food restriction (Ferraris and Carey, 2000). As feeding acutely regulates intestinal nutrient transporters (Cui et al., 2003; Pan et al., 2004) and disaccharidase activity (Goda, 2000), it is common to fast piglets before sample collection to decrease the variability in transporter and enzyme activities associated with feeding (Houle et al., 1997; Alexander and Carey, 2001). Intestinal responses to fasting for ≥24 h have been well described in adult rodents (Martins et al., 2001; Hbold et al., 2004). Most pig studies have focused on ion and nutrient transport in weaning-aged animals after a minimum 24-h fast (Carey et al., 1994; Hayden and Carey, 2000). An analysis of the effect of short-term fasting in young piglets had not been conducted.

In previous studies, piglets fed formula containing recombinant human IGF-I exhibited greater intestinal villus height (Burrin et al., 1996) and lactase-phlorizin hydrolase (LPH) activity (Houle et al., 1997, 2000) than piglets fed formula alone. The increased LPH activity was associated with increased LPH mRNA abundance (Houle et al., 2000) and greater efficiency of post-translational processing of the proLPH to the mature LPH (Burrin et al., 2001). In these previous

1This research was funded by a grant from the USDA NRICGP (AG-00-35260-9537) and by the State of Illinois through the Illinois Council for Food and Agricultural Research (C-FAR) Sentinel Program.
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Received July 15, 2004.
Accepted July 5, 2005.

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studies, the magnitude of the response of LPH activity to IGF-I treatment was greater in samples collected from piglets in a postabsorptive state (Park et al., 1999; Houle et al., 2000) than in a fed state (Burrin et al., 2001; Park et al., 2001). Therefore, the effect of duration of fasting on LPH activity in piglets suckling nontransgenic (control) and IGF-I transgenic (TG) sows was studied. We hypothesized that LPH activity would be greater in piglets suckling TG sows than control sows after a short-term fast. Intestinal morphology and circulating IGF-I and IGFBP also were assessed.

Materials and Methods

Animals and Experimental Design

Transgenic swine that exhibit mammary and lactation-specific overexpression of human IGF-I in milk under the direction of the 5′-regulatory elements of the bovine α-lactalbumin gene (Donovan et al., 2001) and control full sib littersmates were used. Eight control Yorkshire Gilts and eight TG Yorkshire Gilts were bred by natural service or artificial insemination to a Yorkshire boar. At d 109 of pregnancy, Gilts were moved into farrowing crates and monitored for signs of parturition. At parturition, three control females and three TG females were selected randomly for use in this project. Within 6 h of farrowing, all litters were normalized to 10 piglets per gilt. If a litter contained <10 piglets, Yorkshire piglets from another gilt from the same treatment (control or TG) that farrowed on the same day were cross-fostered to standardize litter size. Whenever possible, the BW of cross-fostered piglets were approximately the mean birth weight of the piglets in the litter that they were joining. All piglets nursed until 1200 on d 6 postpartum, at which time all piglets were weighed, and piglets within each litter were assigned randomly to one of three treatment groups: fasted piglets were removed from the sow at either 6 or 12 h before (0300 on d 7 or 2100 on d 6, respectively) euthanasia at 0900 on d 7, whereas the 0-h piglets remained with the sow until euthanized at 0900 on d 7, whereas the 0-h piglets remained with the sow until euthanized at 0900 on d 7. All animal protocols and procedures were approved by the Biological Safety Committee and the Laboratory Animal Care Advisory Committee of the University of Illinois and were in compliance with NRC (1996) guidelines for the care and use of laboratory animals.

Sample Collection

Piglets were euthanized via sedation with sodium pentobarbital (0.1 mg/kg of BW; Fatal Plus, Vortech Pharmaceuticals, Dearborn, MI) followed by electrocution and exsanguination. Blood samples were collected, and serum was obtained by centrifugation at 3,500 × g for 15 min at 4°C. The entire small intestine from the pyloric sphincter to the ileocecal valve was immediately removed, and its length was measured. The intestine was divided into segments based on length: duodenum (first 10%), jejunum (middle 75%), and ileum (final 15%). Each segment was flushed with ice-cold saline, and its weight and length were recorded. Duodenal weights were inadvertently not recorded for one replicate; therefore, total intestinal weight could not be calculated, and the number of observations for that outcome is <30 for genotype and 20 for fasting. Beginning at the most proximal region of the jejunum, a jejunal section (2 cm) was fixed in formalin and embedded in paraffin. An additional section of jejunum (3 to 4 cm) was snap frozen in liquid N2. The entire remaining portion of jejunum was opened longitudinally, and the mucosa was scraped from the luminal surface using a glass microscope slide.

Serum IGF. Serum IGF-I content was measured by RIA as previously described (Houle et al., 1997). Serum samples (0.5 mL) were chromatographed in 0.2 M formic acid on a column containing Sephadex G-50 (Pharmacia Biotech Inc., Piscataway, NJ) to dissociate the IGF-I from the IGFBP. The IGF-I fraction was collected, frozen, and lyophilized (Labconco, Kansas City, MO). After lyophilization, samples were resolubilized in RIA buffer (0.03 M sodium phosphate and 0.25% sodium azide; pH 7.5) and diluted to 1:30. The IGF-I was measured using [125I]IGF-I as the radioligand and a polyclonal anti-human IGF-I antibody, which was distributed through the National Hormone and Pituitary Program (http://www.humc.edu/hormones/). After overnight incubation, bound radioactivity was precipitated by centrifugation at 3,000 × g for 30 min after the addition of 1.0% bovine IgG (Sigma Chemical Co., St. Louis, MO) and 20% polyethylene glycol (Sigma Chemical Co.).

Serum IGFBP. Serum IGFBP profiles were characterized by SDS-PAGE and Western ligand blotting as described previously (Houle et al., 1997). Four samples from each treatment group were selected randomly for use in analyses (n = 24). Sera were separated through 4% stacking and 12% running SDS-polyacrylamide gels at 65 V, overnight at 4°C (Hoefer Scientific Instruments, San Francisco, CA). Size-separated proteins were electrotransferred to nitrocellulose (0.45 μm; Micron Separations Inc., Westborough, MA) at 200 mA for 1 h. Membranes were blocked through a series of washes (Houle et al., 1997) and then incubated overnight with 0.45 μCi of [125I]IGF-I. Binding proteins were visualized by autoradiography (Eastman Kodak, Rochester, NY) at −70°C for 4 d. Images were captured using a Kodak Imaging Station 440, and band density was analyzed using Kodak 1D analysis software.

Mucosal Protein and DNA Content. Mucosal protein and DNA content were measured using established methods (Houle et al., 2000). Mucosal samples (0.1 g) were homogenized in 1 mL of buffer containing protease inhibitors (0.45 M NaCl, 0.001 M phenylmethylsulfonyl fluoride, and 0.002 M iodoacetic acid). Protein content was measured by the Lowry method (Peterson,
Fisher Scientific). Calf thymus DNA (Sigma Chemical BioRad, Hercules, CA) using Hoescht H 33258 dye determined fluorometrically (excitation wavelength = 365 and emission wavelength = 460; VersaFluor, BioRad, Hercules, CA) using Hoescht H 33258 dye after dilution in DNA buffer and sonication for 30 s (Fisher Scientific). Calf thymus DNA (Sigma Chemical Co.) was used to generate the DNA standard curve.

**Intestinal Histomorphology.** Ten samples from each treatment group were selected randomly for use in analyses (n = 30). Paraffin-embedded jejunal samples were sliced to approximately 5 μm with a microtome, mounted on slides, and stained with hematoxylin. Villus height from the tip of the villi to the villus/crypt junction, mid-villus width, and crypt depth were measured at 10× magnification using a Nikon microscope and Optiphot-2 software (Nikon, Melville, NY) in 10 to 20 well-oriented villi and crypts. Villus cross-sectional area was calculated by multiplying villus height and mid-villus width (Houle et al., 2000).

**Disaccharidase Activity.** Mucosal disaccharidase activity was measured as described by Dudley et al. (1994). Mucosal samples were homogenized in homogenization buffer containing protease inhibitors. Sucrase-isomaltase (SI) and LPH activities were determined by incubating homogenates with the appropriate disaccharide (sucrose or lactose; Fisher Scientific). Enzyme activities were expressed as μmol of glucose/min·g of mucosal protein.

**Statistical Analyses.** Data were analyzed as a completely randomized design using the GLM procedure of SAS (SAS Inst., Cary, NC). Within litters from control and TG sows, there were 30 piglets, 10 assigned to each fasting treatment (0-, 6-, or 12-h fast). Therefore, data were analyzed as a 2 (sow type) × 3 (fasting time) factorial arrangement of treatments. Separation of means for the effects of fasting time was accomplished using linear and quadratic contrasts. The interaction between sow type and fasting time was not significant for any variable; therefore, only the response to sow type and the linear and quadratic responses to fasting are presented. Comparisons with \( P < 0.05 \) were considered significant, and comparisons with \( P < 0.10 \) are presented as trends.

### Results

**Body Weight and Intestinal Weight and Length.** At sacrifice, BW was similar in all groups. The BW change in response to fasting was calculated by subtracting BW obtained at the same time from all groups on d 6 at 1200 from the BW at sacrifice on d 7 at 0900. Thus, of the 21-h period, 6-h fasted piglets had remained with the sow for 15 h, whereas the 12-h fasted piglets had remained with the sow for 9 h (Table 1). Weight change decreased quadratically (\( P < 0.001 \)) with increasing fasting time. Piglets fasted for either 0 or 6 h had a positive weight gain between the d 6 weighing and sacrifice at d 7, whereas piglets fasted for 12 h lost 69.5 ± 48.39 g, or approximately 2.3% of their d-6 BW. All 0-h piglets had milk in their stomach and/or jejunum at sacrifice, whereas none of the 6- or 12-h fasted piglets had digesta in their stomach or jejunum.

Piglets suckling TG sows tended to have greater intestinal length (\( P = 0.068 \)); however, no effect of fasting was observed on intestinal length. Conversely, intestinal weight was unaffected by IGF-I overexpression, but decreased in a linear (\( P < 0.001 \)) and quadratic (\( P = 0.002 \)) fashion with increasing fasting time (Table 1).

**Serum IGF-I and IGFBP Profiles.** Serum IGF-I concentrations are summarized in Table 2. Serum IGF-I concentrations did not differ, but concentrations tended (\( P = 0.072 \)) to be greater in piglets suckling control sows than TG sows. Fasting linearly decreased serum IGF-I (\( P = 0.003 \)), as piglets fasted for 12 h had the lowest serum IGF-I concentrations. After Western ligand blotting, five IGFBP bands with apparent molecular weights of 48, 46, 32.5, 28, and 24 kDa were visualized. The 48- and 46-kDa bands represent two glycosylated variants of IGFBP-3. The 32.5- and 28-kDa bands have been immunologically identified as IGFBP-2 and IGFBP-1, respectively (McCusker et al., 1991). The 24-kDa band most likely represents IGFBP-4, which has been purified from porcine follicular fluid (Shimasaki et al., 1991). Densitometric analyses of the IGFBP bands are summarized in Table 2. No effect of IGF-I overexpression in milk was noted for piglet serum IGFBP; however, IGFBP-1 and -4 were affected by fasting. The IGFBP-1 tended to increase linearly (\( P = 0.053 \)) and also increased quadratically (\( P < 0.001 \)), whereas serum IGFBP-4 decreased both linearly (\( P = 0.002 \)) and quadratically (\( P = 0.018 \)).

**Histomorphology.** Images depicting representative jejunal histomorphology of piglets suckling control and TG sows at 0, 6, and 12 h are shown in Figure 1, and mean villus height, villus width, crypt depth, and total surface area are summarized in Table 3. No effect of IGF-I overexpression in milk was observed on intestinal villus height (\( P = 0.580 \)), width (\( P = 0.256 \)), surface area (\( P = 0.434 \)), or crypt depth (\( P = 0.826 \)). Villus height, however, increased linearly (\( P = 0.015 \)) and quadratically (\( P = 0.039 \)) in response to fasting. Compared with 0-h piglets, villus height increased approximately 32% after a 6-h fast and 45% after a 12-h fast. Villus width increased (linear, \( P < 0.001 \); quadratic, \( P = 0.019 \)) 67% after a 6-h fast and 85% after a 12-h fast. Concomitant increases in villus height and width resulted in a >100% increase in villus surface area after a 12-h fast (linear, \( P = 0.001 \); quadratic, \( P = 0.001 \).
Table 1. Body weight and intestinal weight in 7-d-old piglets fasted for 0, 6, or 12 h suckling control sows or transgenic (TG) sows that overexpress IGF-I in milk.

<table>
<thead>
<tr>
<th>Item</th>
<th>Control (0 h)</th>
<th>Control (6 h)</th>
<th>Control (12 h)</th>
<th>TG (0 h)</th>
<th>TG (6 h)</th>
<th>TG (12 h)</th>
<th>Genotype</th>
<th>L</th>
<th>Q</th>
</tr>
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<tbody>
<tr>
<td>BW, kg</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.91 ± 0.14</td>
<td>2.82 ± 0.13</td>
<td>2.61 ± 0.14</td>
<td>2.70 ± 0.14</td>
<td>2.75 ± 0.10</td>
<td>2.76 ± 0.17</td>
<td>0.444</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BW change, g</td>
<td>188.89 ± 39.81</td>
<td>143.33 ± 14.14</td>
<td>-68.89 ± 15.50</td>
<td>211.82 ± 32.14</td>
<td>191.11 ± 56.51</td>
<td>-70.00 ± 93.63</td>
<td>0.735</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Intestinal wt, g/kg BW</td>
<td>40.65 ± 1.04</td>
<td>35.38 ± 1.84</td>
<td>32.40 ± 0.90</td>
<td>39.00 ± 4.16</td>
<td>32.77 ± 1.93</td>
<td>35.07 ± 1.59</td>
<td>0.695</td>
<td>&lt;0.001</td>
<td>0.002</td>
</tr>
<tr>
<td>Intestinal length, cm/kg BW</td>
<td>185.93 ± 7.92</td>
<td>198.28 ± 6.66</td>
<td>205.97 ± 6.79</td>
<td>216.39 ± 13.37</td>
<td>200.08 ± 7.55</td>
<td>211.16 ± 8.74</td>
<td>0.068</td>
<td>0.732</td>
<td>0.382</td>
</tr>
</tbody>
</table>

aData are means ± SEM.

bP-values for effect of genotype and the linear (L) and quadratic (Q) effects for the duration of fasting.

cn = 9 to 10 piglets per treatment group.

dBW change was calculated by subtracting BW on d 6 from BW on d 7 after 0, 6, or 12 h of fasting.

Table 2. Serum IGF-I and IGFBP in 7-d-old piglets fasted for 0, 6, or 12 h suckling control sows or transgenic (TG) sows that overexpress IGF-I in milk.

<table>
<thead>
<tr>
<th>Item</th>
<th>Control (0 h)</th>
<th>Control (6 h)</th>
<th>Control (12 h)</th>
<th>TG (0 h)</th>
<th>TG (6 h)</th>
<th>TG (12 h)</th>
<th>Genotype</th>
<th>L</th>
<th>Q</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum IGF-I, ng/mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>92.18 ± 9.88</td>
<td>73.47 ± 10.34</td>
<td>49.45 ± 4.15</td>
<td>66.43 ± 9.21</td>
<td>59.32 ± 5.34</td>
<td>53.49 ± 4.44</td>
<td>0.072</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGFBP-1ds</td>
<td>6.69 ± 1.30</td>
<td>8.21 ± 0.50</td>
<td>25.88 ± 3.61</td>
<td>6.74 ± 1.05</td>
<td>13.55 ± 1.06</td>
<td>18.82 ± 2.86</td>
<td>0.463</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGFBP-2ds</td>
<td>19.69 ± 3.43</td>
<td>25.23 ± 5.96</td>
<td>21.88 ± 0.69</td>
<td>18.49 ± 3.95</td>
<td>23.88 ± 4.87</td>
<td>22.58 ± 3.81</td>
<td>0.596</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IGFBP-3ds</td>
<td>34.46 ± 3.67</td>
<td>37.47 ± 4.28</td>
<td>28.89 ± 5.11</td>
<td>41.63 ± 5.52</td>
<td>36.65 ± 2.99</td>
<td>32.85 ± 4.03</td>
<td>0.963</td>
<td>0.851</td>
<td>0.137</td>
</tr>
<tr>
<td>IGFBP-4ds</td>
<td>39.15 ± 3.96</td>
<td>29.09 ± 1.89</td>
<td>23.38 ± 4.36</td>
<td>33.14 ± 1.63</td>
<td>25.92 ± 2.87</td>
<td>25.72 ± 2.67</td>
<td>0.163</td>
<td>0.002</td>
<td>0.018</td>
</tr>
</tbody>
</table>

aData are means ± SEM.

bP-values for linear (L) and quadratic (Q) effects for the duration of fasting.

cn = 9 piglets per treatment group.

Percent IGFBP data expressed as a percentage of total binding proteins in arbitrary units of pixel intensity.
Figure 1. Jejunal histomorphology of 7-d-old piglets suckling control (CON) sows or transgenic (TG) sows that overexpress IGF-I in milk and fasted for 0, 6, or 12 h before sample collection. Images were captured at 10× magnification, and the bars shown in the 0-h panels are 100 μm in length.

0.017). Crypt depth also increased with fasting (linear, \( P = 0.003 \); quadratic, \( P < 0.001 \)), resulting in a 71% increase in crypt depth after a 6-h fast and an approximately 150% increase in crypt depth after a 12-h fast.

**Jejunal DNA and Protein Content.** No effect of IGF-I overexpression or fasting on jejunal mucosal DNA, protein, or protein-to-DNA ratios was detected (Table 4).

**Disaccharidase Activity.** No effect of fasting was detected on jejunal LPH or SI specific activities (μmol glucose/[min·g of mucosal protein]; Table 5). Lactase-phlorizin hydrolase activity increased approximately 18% (\( P = 0.009 \)) in piglet jejunum in response to IGF-I overexpression in milk. Jejunal SI activity was 45% greater in piglets suckling TG sows than in piglets suckling control sows (\( P = 0.02 \)).

**Discussion**

Piglets used in research studies investigating the effect of diet on gastrointestinal development are often food-restricted before sacrifice (Houle et al., 1997; 2000; Alexander and Carey, 2001) to decrease the variation in digestive and absorptive outcome measures, which are inducible by feeding (Goda, 2000; Cui et al., 2003; Pan et al., 2004). The time interval between suckling episodes during the first week of lactation was reported to be 30 to 70 min (Jensen et al., 1991) and approximately 45 min during d 10 to 14 postpartum (Auldist et al., 2000). Thus, the intestinal response to fasting in sow-reared piglets could be exacerbated by their frequent nursing in a natural setting; however, no previous studies have investigated how piglet intestinal morphology and disaccharidase activity are affected by a short-term fast (6 to 12 h). The current study was designed to address this question in piglets suckling control sows or TG sows with mammary-specific IGF-I overexpression. In addition, we sought to determine how BW and circulating IGF-I and IGFBP responded to a short-term fast.

Body weights of piglets suckling control and TG sows were similar on d 6, which is consistent with earlier data documenting similar growth of piglets fed formula supplemented with or without recombinant IGF-I (Burrin et al., 1996; Houle et al., 1997, 2000). Circulating IGF-I concentrations in piglets suckling control or TG sows did not differ significantly, which is also consistent with previous findings in piglets suckling control or TG sows (Burrin et al., 1996; Houle et al., 1997, 2000). Furthermore, using [125I]IGF-I as a tracer, we have shown that orally administered IGF-I is poorly absorbed in the neonatal
Table 3. Jejunal histomorphology in 7-d-old piglets fasted for 0, 6, or 12 h suckling control sows or transgenic (TG) sows that overexpress IGF-I in milk\textsuperscript{a,b}

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>TG</th>
<th>Fasting</th>
<th>Genotype</th>
<th>L</th>
<th>Q</th>
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</thead>
<tbody>
<tr>
<td>Villus height, (\mu)m</td>
<td>0 h</td>
<td>6 h</td>
<td>12 h</td>
<td>0 h</td>
<td>6 h</td>
<td>12 h</td>
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<tr>
<td></td>
<td>499.02 ± 48.47</td>
<td>694.99 ± 93.46</td>
<td>861.56 ± 27.88</td>
<td>616.56 ± 86.24</td>
<td>782.76 ± 45.25</td>
<td>759.39 ± 51.10</td>
</tr>
<tr>
<td>Villus width, (\mu)m</td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>40.32 ± 2.52</td>
<td>68.78 ± 27.49</td>
<td>83.06 ± 4.33</td>
<td>48.60 ± 5.80</td>
<td>79.80 ± 2.94</td>
<td>81.37 ± 11.25</td>
</tr>
<tr>
<td>Surface area, mm(^2)</td>
<td>200.26 ± 20.36</td>
<td>509.02 ± 126.55</td>
<td>718.61 ± 54.66</td>
<td>318.33 ± 88.78</td>
<td>629.62 ± 56.59</td>
<td>635.30 ± 108.77</td>
</tr>
<tr>
<td>Crypt depth, (\mu)m</td>
<td>54.15 ± 2.97</td>
<td>87.93 ± 15.73</td>
<td>167.27 ± 9.86</td>
<td>65.29 ± 7.90</td>
<td>116.57 ± 4.66</td>
<td>135.70 ± 21.44</td>
</tr>
<tr>
<td>P-value(^c)</td>
<td></td>
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<tr>
<td></td>
<td>0.580</td>
<td>0.015</td>
<td>0.039</td>
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</table>

\begin{itemize}
\item aData are means ± SEM.
\item b\(n = 5\) piglets per treatment group.
\item cP-values for effect of genotype and the linear (L) and quadratic (Q) effects for the duration of fasting.
\end{itemize}

Table 4. Jejunal mucosal DNA, protein, and protein:DNA in 7-d-old piglets fasted for 0, 6, or 12 h suckling control sows or transgenic (TG) sows that overexpress IGF-I in milk\textsuperscript{a,b}

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>TG</th>
<th>Fasting</th>
<th>Genotype</th>
<th>L</th>
<th>Q</th>
</tr>
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<tbody>
<tr>
<td>Item</td>
<td></td>
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</tr>
<tr>
<td>DNA, mg/g of mucosa\textsuperscript{d}</td>
<td>3.18 ± 0.27</td>
<td>4.05 ± 0.82</td>
<td>3.64 ± 0.56</td>
<td>4.12 ± 0.52</td>
<td>3.43 ± 0.39</td>
<td>3.58 ± 0.49</td>
</tr>
<tr>
<td>Protein, mg/g of mucosa\textsuperscript{d}</td>
<td>89.99 ± 10.44</td>
<td>108.88 ± 8.49</td>
<td>96.73 ± 7.57</td>
<td>101.27 ± 5.11</td>
<td>98.96 ± 4.62</td>
<td>91.66 ± 6.10</td>
</tr>
<tr>
<td>Protein:DNA</td>
<td>30.44 ± 4.43</td>
<td>32.00 ± 4.66</td>
<td>30.78 ± 3.98</td>
<td>28.80 ± 4.18</td>
<td>32.00 ± 4.66</td>
<td>29.40 ± 3.72</td>
</tr>
<tr>
<td>P-value(^c)</td>
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<tr>
<td></td>
<td>0.772</td>
<td>0.371</td>
<td>0.405</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\begin{itemize}
\item aData are means ± SEM.
\item b\(n = 9\) to 10 piglets per treatment group.
\item cP-values for effect of genotype and the linear (L) and quadratic (Q) effects for the duration of fasting.
\item dWet tissue basis.
\end{itemize}
Table 5. Jejunal disaccharidase activity (LPH = lactase phlorhizin hydrolase; SI = sucrase-isomaltase; μmol of glucose/[min · g of mucosal protein]) in 7-d-old piglets fasted for 0, 6, or 12 h suckling control sows or transgenic (TG) sows that overexpress IGF-I in milk.a,b

<table>
<thead>
<tr>
<th>Response</th>
<th>Genotype</th>
<th>Fasting</th>
<th>P-value</th>
<th>L</th>
<th>Q</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6 h</td>
<td>12 h</td>
<td>b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LPH</td>
<td>336.89 ± 30.42</td>
<td>346.95 ± 36.41</td>
<td>328.73 ± 35.25</td>
<td>0.009</td>
<td>0.301</td>
</tr>
<tr>
<td>SI</td>
<td>5.74 ± 1.29</td>
<td>8.79 ± 2.02</td>
<td>10.08 ± 1.17</td>
<td>0.020</td>
<td>0.191</td>
</tr>
</tbody>
</table>

aData are means ± SEM. 
b = 9 to 10 piglets per treatment group. 
P-values for effect of genotype and the linear (L) and quadratic (Q) effects for the duration of fasting.

piglet (Donovan et al., 1997). The length of time piglets were fasted influenced BW change and circulating IGF-1 concentrations. Piglet BW change decreased quadratically with fasting. Piglets fasted for 12 h lost an average of 70 g, or 2% of their d 6 BW, whereas piglets fasted for only 6 h before sacrifice maintained a positive weight gain between d 6 and 7. Serum IGF-I decreased linearly with increased fasting time. A 6-h fast resulted in a 16% decrease in serum IGF-I, whereas a 12-h fast resulted in a 34% decrease in serum IGF-I concentrations.

In addition, changes in circulating IGFBP profiles in response to fasting were observed. The IGFBP-4 decreased 31% with a 6-h fast. Although the greatest percentage decrease in IGFBP-4 was observed within 6 h of fasting, IGFBP-4 continued to decrease with additional fasting time. In contrast to IGFBP-4, IGFBP-1 increased with fasting time. Serum IGFBP-1 increased 30% after 6 h and showed a further increase after 12 h of fasting to levels threefold greater than fed (0-h) piglets.

Food restriction over several days has been shown to alter piglet serum IGFBP profiles (McCusker et al., 1991; Zjilstra et al., 1997), but documentation of the effect of short-term food deprivation in livestock is limited, and most fasting studies have been conducted in human subjects (Suikkari et al., 1988). Katz et al. (2002) demonstrated that serum IGFBP-1 increases steadily during fasting, following an inverse relationship with insulin. The increase in IGFBP-1 is thought to serve as a glucose counter-regulatory hormone during fasting and hypoglycemia by binding free IGF-I and inhibiting its ability to bind to IGF receptors. Thus, the response of circulating IGF-I (−34%) and IGFBP-1 (+300%) to fasting illustrates the need to control for postabsorptive state when aspects of the IGF axis are included in the outcome variables.

The gastrointestinal tract often displays striking structural and functional changes to food restriction (Ferraris and Carey, 2000). We observed decreased intestinal weight (g/kg of BW) within 6 h of fasting, whereas a change in overall BW was not detected until after 12 h of food restriction. Although we did not directly measure tissue hydration, we speculate that the change in intestinal weight may reflect short-term changes in tissue wet weight that arise from decreased luminal nutrient transport (Meinild et al., 1998) and blood flow to the tissue (Nowicki et al., 1983; Niinikoski et al., 2004). Glucose and galactose, produced from dietary lactose by the action of LPH, are transported across the brushborder membrane via the sodium-glucose co-transporter. It has been reported that the sodium-glucose co-transporter functions as a molecular water pump, wherein the transmembrane transport of one sugar molecule is coupled with two sodium ions and the influx of 210 water molecules (Meinild et al., 1998). From the serosal side, it was demonstrated in nonanesthetized, awake 2-d-old piglets that within 30 min of feeding, oxygen delivery and
the average volume of nonabsorbing enterocytes was
shrink and lose up to 30% of their volume. In hamsters,
in the volume of individual enterocytes lining the villi
from hamsters, rats, and humans document changes
Mooseker, 1992). In support of our observations, data
dynamic under physiological conditions including feed-
ations in villus cell number, although fasting periods
were observed in fasted vs. fed piglets. This result was
unexpected, as most studies have shown a decrease in
villus height and crypt depth observed at 24 h, and the decreased cell pro-
and cell survival observed after 48 h on TPN (Niinikoski et al., 2004). The time points studied
herein, 6 and 12 h, coincide with the period of time of
decreased blood flow, but before the onset of villus
atrophy.

Despite the decrease in intestinal weight, piglets
fasted for 6 or 12 h had 32 and 45% greater villus
height than fed piglets, respectively. Increases in mid-
villus width, total surface area, and crypt depth also
were observed in fasted vs. fed piglets. This result was
unexpected, as most studies have shown a decrease in
villus height and crypt depth in response to food
privation (Mayhew, 1987; Carey et al., 1994; Kong
et al., 2000); however, the duration of food restriction
in these studies was 48 to 72 h. The lack of change in
mucosal protein and DNA content with fasting sug-
gests that changes in morphology were not due to alter-
ations in villus cell number, although fasting periods
>24 h were associated with decreased cell number
along the crypt-villus axis (Raul and von der Decken,
1985). The intestinal brush border architecture is dy-
namic under physiological conditions including feeding,
fasting, or exposure to lectins (Heintzelman and
Mooseker, 1992). In support of our observations, data
from hamsters, rats, and humans document changes
in the volume of individual enterocytes lining the villi
in response to feeding (Pappenheimer and Michel,
2003). During sodium-coupled absorption, enterocytes
shrink and lose up to 30% of their volume. In hamsters,
the average volume of nonabsorbing enterocytes was
1,503 μm³ vs. 1,040 μm³ during the absorption of glu-
cose (Pappenheimer and Michel, 2003). Thus, it is rea-
sonable to speculate that our observed increase in
overall villus height and width after 6 to 12 h fasting
may reflect short-term changes in enterocyte volume
during the postabsorptive state.

In contrast to the effect of fasting, no effect of IGF-
I on villus morphology was detected. Others have docu-
mented improvements in villus architecture in re-
response to oral IGF-I supplementation of artificial for-
mula (Burrin et al., 1996; Houle et al., 1997, 2000); however, this effect has not been observed by all (Alex-
ander and Carey, 1999; Burrin et al., 2001). The differ-
ent model used in this project (sow-reared) vs. artificial
rearing in the previous studies may contribute to this
discrepancy. Additionally, other bioactive components
in sow milk (Donovan and Odle, 1994) may modulate
the actions of IGF-I on villus morphology.

Several previous studies have investigated the effect
of enterally administered IGF-I on nutrient transport
(Alexander and Carey, 1999, 2001, 2002) and intesti-
ral development (Burrin et al., 1996, 2001) in piglets.
A particular focus of our laboratory has been on the
effect of IGF-I on intestinal LPH activity (Houle et al.,
2000; Park et al., 1999, 2001), and mRNA abundance
(Houle et al., 2000), synthesis, and processing (Burrin
et al., 2001; Park et al., 2001), all of which were shown
to be greater in piglets fed sow milk replacer + IGF-I
compared with piglets receiving sow milk replacer
alone. The magnitude of increase in LPH activity in
response to IGF-I was greater when piglets were killed
in a postabsorptive state (Houle et al., 1997, 2000;
Park et al., 1999) than when piglets were killed in a
fed state (Burrin et al., 2001; Park et al., 2001). The
latter two studies involved stable isotope protocols
that required piglets receive a continuous infusion of
luminal nutrients until immediately before intestinal
collection (Burrin et al., 2001; Park et al., 2001). The
stable isotope enrichment findings sug-
gested that enterally administered IGF-I suppressed
intestinal proteolysis and substantially increased the
portion of newly synthesized pro-LPH that was pro-
cessed and inserted in the brush border membrane
(e.g., increased processing efficiency; Burrin et al.,
2001). Greater efficiency of insertion of LPH into the
brush border may, in turn, affect the turnover rate of
LPH, allowing for LPH activity to be maintained at
greater levels in the postabsorptive state. Therefore,
we wished to assess the effect of duration of food re-
striction on LPH activity. Piglets suckling TG sows
had greater jejunal LPH and SI activities, but no effect
of fasting on disaccharidase activity was apparent, de-
spite the observed morphological changes. Nonethe-
less, simply measuring disaccharidase activity after a
fast of only 6 or 12 h might not have been sufficiently
sensitive to detect differences in LPH turnover.

In conclusion, using a transgenic model of mam-
mary-specific IGF-I production, we have confirmed
that oral IGF-I upregulates jejunal LPH and SI activi-
ties, whereas disaccharidase activities were una-
fected by a short-term fast. Food deprivation of piglets
for as little as 6 to 12 h before euthanasia decreased
serum IGF-I concentrations, altered serum IGFBP
profiles, and decreased BW and intestinal weight. A
unique observation was the marked villus morphologi-
ical changes in response to short-term fasting, which
are counter to what has been reported with longer-
term food restriction.
Implications

Piglets used to investigate gastrointestinal development are frequently food-deprived overnight before euthanasia; however, differences in the duration of food restriction could be a confounding factor when comparing results among experiments. We showed that a short-term fast before sample collection altered intestinal weight, villus morphology, and serum insulin-like growth factor-I and insulin-like growth factor-I binding protein levels compared with the fed state. Therefore, duration of food deprivation before euthanasia should be considered when designing experiments, as the magnitude of differences between the fed and fasted state may exceed those expected as a result of the experimental treatment. In addition, the duration of food restriction before sample collection should be noted in publications to better enable investigators to compare results among experiments.

Literature Cited


Park, Y. K., M. H. Monaco, and S. M. Donovan. 1999. Enteral insulin-like growth factor-I augments intestinal disaccharidase activ-
Fasting and IGF-I on piglet intestine


