Oral IGF-I enhances nutrient and electrolyte absorption in neonatal piglet intestine

ANDREW N. ALEXANDER AND HANNAH V. CAREY
Department of Comparative Biosciences, School of Veterinary Medicine, University of Wisconsin, Madison, Wisconsin 53706

Alexander, Andrew N., and Hannah V. Carey. Oral IGF-I enhances nutrient and electrolyte absorption in neonatal piglet intestine. Am. J. Physiol. 277 (Gastrointest. Liver Physiol. 40): G619–G625, 1999.—The effect of orally administered insulin-like growth factor-I (IGF-I) on small intestinal structure and function was studied in 5-day-old colostrum-deprived piglets. Human recombinant IGF-I (3.5 mg·kg−1·day−1) or control vehicle was given orogastrically for 4 days. Body weights, jejunal and ileal mucosa wet and dry weights, and serum IGF-I levels were similar in the two groups. Small intestinal villus height and crypt depth and jejunal enterocyte microvillar dimensions were also similar between groups. Oral IGF-I produced higher rates of jejunal sodium transport because of increased basal Na+ absorption. Short-circuit current responses to mucosal addition of α-glucose and l-alanine and net transepithelial absorption of 3-O-methylglucose were increased by IGF-I. Carrier-mediated uptake of α-glucose per milligram in everted jejunal sleeves was greater in IGF-I-treated piglets because of a significantly greater maximal rate of uptake. We conclude that rates of net Na+ and Na+-dependent nutrient absorption are enhanced in piglets treated with oral IGF-I, and this effect is independent of changes in mucosal mass or surface area.

IGF-I is a 70-amino-acid-long polypeptide whose structure is highly conserved among species and shares 100% homology among human, porcine, and bovine structures. Human recombinant IGF-I (3.5 mg·kg−1·day−1) or control vehicle was given orogastrically for 4 days. Body weights, jejunal and ileal mucosa wet and dry weights, and serum IGF-I levels were similar in the two groups. Small intestinal villus height and crypt depth and jejunal enterocyte microvillar dimensions were also similar between groups. Oral IGF-I produced higher rates of jejunal sodium transport because of increased basal Na+ absorption. Short-circuit current responses to mucosal addition of α-glucose and l-alanine and net transepithelial absorption of 3-O-methylglucose were increased by IGF-I. Carrier-mediated uptake of α-glucose per milligram in everted jejunal sleeves was greater in IGF-I-treated piglets because of a significantly greater maximal rate of uptake. We conclude that rates of net Na+ and Na+-dependent nutrient absorption are enhanced in piglets treated with oral IGF-I, and this effect is independent of changes in mucosal mass or surface area.

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understood. Thus the aim of our study was to determine whether orally administered IGF-I influences intestinal nutrient and electrolyte transport in neonatal piglets. We focused our functional studies on the jejunum, which is the major site of nutrient absorption in the small intestine. To complement the functional studies, we determined the effect of oral IGF-I on jejunal mucosal morphology at light and electron microscope levels. We also carried out limited measurements on ileal structure because others have reported that orally administered IGF-I may selectively enhance ileal mucosal growth (4, 18).

MATERIALS AND METHODS

Experimental animals and diets. The University of Wisconsin Institutional Animal Care and Use Committee approved the protocol used in this study. Colostrum-deprived neonatal crossbred pigs (1–2 kg body wt) were obtained through the University of Wisconsin Swine Teaching and Research herd. To ensure that piglets did not consume mammary gland-derived IGF-I, they were removed from the sow immediately postpartum. All piglets were provided free access to porcine breast milk replacer (Milik Specialties, Dundee, IL) throughout the study. At birth, piglets were randomly assigned to one of two groups. Group 1 received rhIGF-I (Genentech, South San Francisco, CA) dissolved in 2 ml of milk replacer delivered by orogastric gavage, and group 2 received the same volume of milk replacer given orogastrically with no IGF-I added. The dose of IGF-I administered was 3.5 mg·kg⁻¹·day⁻¹ for 4 days. We chose this dose of oral IGF-I because Burris et al. (4) showed previously that it stimulated intestinal growth in ileal mucosal structure because others have reported that orally administered IGF-I may selectively enhance ileal mucosal growth (4, 18).

Basal electrical parameters. Flat sheets of proximal jejunum were stripped of outer muscle layers and mounted in Ussing chambers with an aperture of 1.13 cm². The Krebs solution that bathed mucosal and serosal sides of tissues contained (in mM) 148.5 Na⁺, 6.3 K⁺, 139.7 Cl⁻, 0.3 H₂PO₄⁻, 1.3 HPO₄²⁻, 19.6 HCO₃⁻, 3.0 Ca²⁺, and 0.7 Mg²⁺. D-Glucose (11.5 mmol/l) or mannitol (11.5 mmol/l) was present in serosal and mucosal solutions, respectively. Tissues were bathed with 10 ml of solution by recirculation from a reservoir maintained at 39°C (porcine core temperature). Solutions were bubbled with a 95% O₂–5% CO₂ mixture, and solution pH was ~7.4. A short-circuit current (∂Isc) was delivered by a voltage-damp apparatus; the magnitude of the ∂Isc reflected active ion transport. Transepithelial potential difference (PD) was recorded under open circuit conditions every 10 min. Tissue conductance (Gt) was calculated from ∂Isc and PD values using Ohm’s law. For transepithelial flux studies, tissues from the same animal having Gt values within 25% of each other were paired to determine net fluxes.

**Transbiophasal fluxes of Na⁺ and Cl⁻.** Methods for measuring unidirectional and net Na⁺ and Cl⁻ fluxes in piglet jejunum were similar to those described previously (8). Briefly, unidirectional Na⁺ and Cl⁻ fluxes were measured by adding 3 µCi of 2⁰Na⁺ and 7 µCi of 3⁰Cl⁻ to mucosal or serosal bathing solutions. After 20 min to achieve isotopic steady state, five fluid samples (0.5 ml) separated by 10 min were withdrawn and replaced with nonradioactive aliquots of similar composition and volume. At the completion of the experiment, 0.5 ml of fluid was withdrawn from the “hot” side, diluted into 50 ml of H₂O, and used as a baseline sample. The samples were assayed with a gamma counter (for Na⁺ flux), and then 4 ml of scillitation fluid were added to each vial and mixed. The samples were then counted on a liquid scillation counter (for Cl⁻ flux). The Na⁺ activity was subtracted from the combined activity of the two isotopes to yield Cl⁻ activity. The four flux values obtained for each tissue were averaged to yield one unidirectional flux value for each ion. Unidirectional Na⁺ and Cl⁻ flux values were paired based on Gt to obtain net fluxes. Residual ion flux (∆J ∂Isc), i.e., that portion of the ∂Isc not accounted for by Na⁺ and Cl⁻ fluxes, was calculated as J β = J ∂Isc (J net = J ∂Isc – J β). Mucosal addition of D-glucose and L-alanine. Methods and solution compositions were similar to those described for measurement of basal electrical parameters. Twenty minutes after they were mounted, jejunal tissues were exposed to D-glucose (10 mM) or L-alanine (10 mM) added to the mucosal solution, and the maximal change in ∂Isc was recorded.

Transbiophasal nutrient absorption. Methods were similar to those reported previously (5, 7). Briefly, transepithelial glucose fluxes were determined with the nonmetabolizable glucose analog 3-O-methylglucose (3-OMG) to avoid metabolism of D-glucose in enterocytes during the flux experiments. For these experiments the Krebs buffer contained nonradioactive 3-OMG (10 mM) in the mucosal and serosal solutions. After the tissues were mounted, 20 µCi of 3-O-methyl-D-[1⁻³H]glucose (Amersham) was added to either mucosal or serosal solutions. After isotopic equilibration (25–30 min), five flux samples separated by 10 min were taken from the unlabeled side to obtain four fluxes that were averaged for each tissue. For each piglet, 3–6 tissues were used to determine unidirectional and net fluxes.
Kinetics of active D-glucose transport in intact jejunal tissues. Methods were similar to those previously described (9, 33, 39, 48). After harvest from the animal, jejunal tissues were bathed continually in ice-cold Krebs solution and bubbled with 95% O₂-5% CO₂ during tissue preparation. Tissues were everted, and 1-cm sleeves were mounted on grooved metal rods (7 mm diameter) suspended in a warmed (39°C) oxygenated Krebs solution over a stir bar rotating at 1,200 rotations/min. Sleeves were preincubated in isotope-free solution for 5 min and then transferred to incubation solutions containing varying concentrations of unlabeled D-glucose and radiolabeled probes. Incubation solutions containing unlabeled D-glucose were prepared by isosmotic replacement of mannitol to obtain a solution osmolality of ~290 mosmol/kgh₂O. Uptake studies used 4 μCi of D-[³H]glucose (American Radiolabeled Chemical, St. Louis, MO) added to each incubation solution of cold D-glucose. After incubation, sleeves were rinsed in Krebs solution for 20 s, blotted on filter paper, placed into tared vials, and weighed. Tissue solubilizer (500 μl, Solvable, Packard) was added, and 24 h later 4 ml of aqueous counting scintillant (Ultima Gold, Packard) were added to each vial. Radiotracer counting procedures and data analyses were performed as previously described (21). Uptake rates were corrected for solute present in adherent fluid by addition of tracer amounts of [¹⁴C]polyethylene glycol. Passive permeability coefficients (Pₚ) were calculated from uptake rates of tracer quantities of L-glucose, which is not transported by the Na⁺-glucose transporter (SGLT1), with the use of methods described by Karasov and Diamond (21). Carrier-mediated D-glucose uptake was computed by subtracting passive uptake (Pₛ) from the total glucose uptake rate at each D-glucose concentration. Kinetic data were analyzed with regression models outlined by Carey and Sills (9). The method of Motulsy and Ransnas (26) was used to fit data to linear or nonlinear regression models. Significance of differences between control and IGF-I-treated pigs were determined by methods previously described (24).

Statistics. Values are reported as means ± SE. Student's t-tests were used to determine significance of differences between means. A probability level of P < 0.05 was considered statistically significant.

RESULTS

There were no differences in overall animal demeanor or fecal consistency between IGF-I-treated and control piglets. However, a small percentage (~10%) of the piglets that included animals from both treatment groups failed to thrive and were removed from the study.

Piglet body weights and serum IGF-I levels. Control and IGF-I-treated piglets had similar rates of weight gain (177.0 ± 35.2 g/day and 172.5 ± 38.2 g/day, respectively). Mean body weights on day 5 in IGF-I-treated and control piglets were similar (2.1 ± 0.1 kg vs. 1.9 ± 0.1 kg, respectively; P = 0.20). Serum IGF-I levels determined by radioimmunoassay were similar in IGF-I-treated and control piglets (66.3 ± 11.0 µg/l, n = 6, vs. 58.5 ± 6.3 µg/l, n = 10, respectively; P = 0.55).

Intestinal mucosa and brush-border morphology. Oral administration of IGF-I had no significant effect on mucosal wet or dry weight per centimeter, villus height, or crypt depth in the jejunal and ileum compared with control piglets (Table 1). Ultrastructural analysis was carried out on three jejunal enterocytes from each of five piglets per treatment group. There were no significant differences in jejunal microvillus length (6.9 ± 0.3 µm in IGF-I-treated and 5.9 ± 0.3 µm in control piglets; P = 0.45) or density (5.2 ± 0.04 microvilli/µm for 5 IGF-I-treated pigs vs. 5.0 ± 0.14 microvilli/µm for controls; P = 0.10) between the two groups.

Table 1. Intestinal mucosa morphology in piglets

<table>
<thead>
<tr>
<th>Segment</th>
<th>Diet</th>
<th>No. of Pigs</th>
<th>No. of Tissues</th>
<th>Mucosal Wet Wt, mg/cm</th>
<th>Mucosal Dry Wt, mg/cm</th>
<th>Villus Height, µm</th>
<th>Crypt Depth, µm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jejunum</td>
<td>Control</td>
<td>15</td>
<td></td>
<td>128 ± 10</td>
<td>21 ± 5</td>
<td>563 ± 58</td>
<td>139 ± 5</td>
</tr>
<tr>
<td></td>
<td>IGF-I</td>
<td>18</td>
<td></td>
<td>133 ± 11</td>
<td>17 ± 1</td>
<td>492 ± 49</td>
<td>142 ± 5</td>
</tr>
<tr>
<td>Ileum</td>
<td>Control</td>
<td>15</td>
<td></td>
<td>143 ± 10</td>
<td>26 ± 5</td>
<td>391 ± 28</td>
<td>123 ± 4</td>
</tr>
<tr>
<td></td>
<td>IGF-I</td>
<td>18</td>
<td></td>
<td>148 ± 14</td>
<td>28 ± 7</td>
<td>423 ± 43</td>
<td>136 ± 6</td>
</tr>
</tbody>
</table>

Values are means ± SE. IGF-I, insulin-like growth factor-I.

Table 2. Basal electrical parameters in piglet jejunum

<table>
<thead>
<tr>
<th>Diet</th>
<th>No. of Pigs</th>
<th>No. of Tissues</th>
<th>PD, mV</th>
<th>Iₛ, µA/cm²</th>
<th>Gₛ, mS/cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14</td>
<td>112</td>
<td>4.2 ± 0.8</td>
<td>114.5 ± 6.7</td>
<td>31.4 ± 1.0</td>
</tr>
<tr>
<td>Oral IGF-I</td>
<td>16</td>
<td>112</td>
<td>4.9 ± 0.2</td>
<td>141.6 ± 5.6</td>
<td>30.7 ± 0.8</td>
</tr>
</tbody>
</table>

Values are means ± SE. Iₛ, short-circuit current; PD, transepithelial potential difference; Gₛ, tissue conductance. *P < 0.01 vs. control piglets.
Consistent with previous experiments, basal $I_{sc}$ was significantly higher in IGF-I piglets compared with controls (76.3 ± 4.6 μA/cm² vs. 45.0 ± 5.5 μA/cm², respectively; P < 0.01).

Wet weights of everted jejunal sleeves were not significantly different between four IGF-I-treated and four control piglets (154.6 ± 2.6 mg/cm vs. 161.7 ± 3.6 mg/cm, respectively; P = 0.20). The curves representing nonlinear least-squares fits of total D-glucose uptake data to the Michaelis-Menton ($K_{m}$) equation for the two treatment groups were significantly different ($F = 9.98$, degrees of freedom (df) = 2,114; P < 0.001). P* calculated from L-glucose uptakes were similar (Table 4). Carrier-mediated uptake rates of D-glucose normalized to tissue mass for control and IGF-I-treated piglets are shown in Fig. 2. The data for both groups were best fit to a nonlinear model, and the curves were significantly different from one another ($F = 10.32$, df = 2,130; P < 0.001). Kinetic analysis revealed a significantly greater maximal rate of D-glucose transport ($J_{max}$) per milligram in IGF-I-treated piglets compared with controls (Table 4). There were no differences in apparent $K_{m}$ between groups.

**DISCUSSION**

The goal of this study was to investigate the effect of orally administered IGF-I on intestinal ion and nutrient transport in neonatal piglets. The concentration of IGF-I we used (3.5 mg·kg⁻¹·day⁻¹) was shown by others (4) to stimulate intestinal mucosal growth in newborn piglets. We used the same dosing regime to determine whether this pharmacological concentration also influenced intestinal function in a similar animal model. We administered IGF-I orally to mimic the natural presence of the peptide in the neonatal gut lumen. There is evidence for apically located IGF-I receptors on jejunal enterocytes during the suckling period (25) that could potentially mediate effects of luminal IGF-I on epithelial function. Furthermore, IGF-I is thought to retain bioactivity in the neonatal intestinal lumen, possibly because of the lower pH of gastric contents in neonates and protection of the peptide from luminal proteolysis by high concentrations of casein in milk and milk replacers (13, 43).

In our study, oral administration of IGF-I had no effect on serum IGF-I levels, which is consistent with the findings of Burrin et al. (4). However, other studies suggest that luminal IGF-I may cross the intestinal mucosa in neonates and accumulate within the gastrointestinal wall and/or in the systemic circulation (31, 45). The contrasting findings in these studies may reflect differences in the detection methods used to measure IGF-I in serum. Our study and that of Burrin et al. (4) used radioimmunoassay, whereas those studies that documented an elevation in serum IGF-I administered radiolabeled IGF-I and subsequently detected the radiolabel in serum and tissues (31). The half-life of IGF-I in serum ranges from minutes for free IGF-I to hours for IGF-I bound to IGF-I-binding proteins (40). Serum levels of IGF-I-binding proteins were not measured in our study, and thus their influence on serum IGF-I levels is unknown.

Oral IGF-I had no effect on piglet body weight nor did it affect the mass or mucosal architecture of either the jejunum or ileum. The study of Burrin et al. (4), which used the same oral IGF-I concentration, reported no effect of the peptide on piglet body weight; however, oral IGF-I significantly increased intestinal weight as well as jejunal and ileal villus heights. Recently, the same investigators reported minimal effects of IGF-I on intestinal growth of mouse pups suckling from dams that overexpressed IGF-I in mammary secretions (32). Houle et al. (18) administered oral IGF-I to 14-day-old piglets at a concentration similar to that found in sow colostrum (200 μg·kg⁻¹·day⁻¹) for 14 days and reported no effect of the peptide on body weight, but it significantly increased ileal (but not jejunal) villus height. In our study, oral IGF-I also had no effect on...
Table 4. Kinetic parameters for active uptake of D-glucose in control and IGF-I-treated piglets

<table>
<thead>
<tr>
<th>Diet</th>
<th>No. of Pigs</th>
<th>J_{max} (nmol/min·mg)</th>
<th>K_{m} (mM)</th>
<th>P* (µl/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4</td>
<td>0.86 ± 0.07</td>
<td>0.75 ± 0.33</td>
<td>0.020 ± 0.007</td>
</tr>
<tr>
<td>IGF-I treated</td>
<td>4</td>
<td>1.40 ± 0.05*</td>
<td>1.04 ± 0.23</td>
<td>0.020 ± 0.003</td>
</tr>
</tbody>
</table>

Values are means ± SE from 13–15 tissues per animal. Maximum uptake rate (J_{max}) and affinity constant (K_{m}) were calculated from nonlinear fits to the Michaelis-Menten equation using total uptakes of D-[3H]glucose that were corrected for diffusional uptake. Diffusional uptake at each concentration was estimated using passive (diffusional) uptake from uptake of L-[3H]glucose in separate experiments using 4 control and 4 IGF-I-treated piglets. *P < 0.001 vs. control piglets.

eнтероцит микровилл размеров, включая микровилл высоту и плотность. В единственной из предыдущих исследований, которые не согласуются с нашими результатами, указывается, что небольшое количество илеуса является главным причиной нарушений барьерной функции кишечника.

IGF-I significantly increased the absorptive capacity for two different Na+-coupled nutrients, D-glucose and L-alanine. This was supported by three separate experiments. First, the increase in I_{sc} induced by mucosal administration of either nutrient was greater in IGF-I-treated piglets. Because both nutrients are cotransported with Na^+, the D-glucose- and L-alanine-stimulated changes in I_{sc} are indirect measures of each nutrient's absorption rate. These electrical findings were confirmed by the enhanced rates of net transepithelial absorption of 3-OMG observed in IGF-I-treated piglets. An effect of IGF-I on stimulating Na^+-coupled solute transport has been demonstrated in other cells, including kidney epithelial cells (11) and osteoblasts (35), in which it stimulates Na^+-dependent phosphate absorption.

Studies with everted jejunal sleeves provided a third measure of the peptide's effects on nutrient absorption by focusing on changes in sugar uptake across the brush-border membrane into enterocytes. Piglets receiving oral IGF-I had significantly greater total and carrier-mediated D-glucose uptake rates when normalized to tissue mass. Total uptake represents the rate of solute transport through both the nonselective paracellular pathway and the brush-border Na^+-glucose transporter (SGLT1). We calculated P* using L-glucose fluxes, and used these to derive carrier-mediated uptake rates in the two groups of piglets. The observation that P* values were similar in control and IGF-I-treated piglets lends further support to the idea that oral IGF-I does not alter paracellular permeability of the jejunal epithelium.

Kinetic analysis of carrier-mediated D-glucose uptake indicated that jejunal tissues from animals given IGF-I had significantly greater J_{max} but the affinity of the sugar for SGLT1 (K_{m}) was unchanged. Several mechanisms could account for a greater J_{max} in IGF-I-treated piglets. One is an increase in SGLT1 expression secondary to increased proliferation of enterocytes in IGF-I-treated piglets. This mechanism is unlikely to account for our results because we found no differences in jejunal tissue weight, villus height, crypt depth, or changes in paracellular permeability, and the absence of an effect of IGF-I on this transport route suggests the peptide did not compromise the barrier function of the intestinal mucosa.

The effects of oral IGF-I on jejunal permeability were mirrored by its effects on transepithelial ion fluxes. The higher basal I_{sc} of IGF-I-treated piglets was due primarily to increased net absorption of Na^+, due to an increase in mucosal-to-serosal fluxes of the cation. Although mucosal-to-serosal Cl^- flux was increased by the peptide, the effect on net Cl^- movement was not significant. Similarly, J_K^w, which most likely reflects bicarbonate secretion, was reduced by IGF-I, but the effect was not significant. These trends for reduced secretion of both anions, which tend to reduce I_{sc} values, likely accounted for the disparity between the increase in I_{sc} induced by the peptide (about twofold) and the larger increase in net Na^+ flux (about threefold).

In addition to its effect on net Na^+ absorption, oral IGF-I also increased the absorptive capacity for two different Na^+-coupled nutrients, D-glucose and L-alanine. This was supported by three separate experiments. First, the increase in I_{sc} induced by mucosal administration of either nutrient was greater in IGF-I-treated piglets. Because both nutrients are cotransported with Na^+, the D-glucose- and L-alanine-stimulated changes in I_{sc} are indirect measures of each nutrient's absorption rate. These electrical findings were confirmed by the enhanced rates of net transepithelial absorption of 3-OMG observed in IGF-I-treated piglets. An effect of IGF-I on stimulating Na^+-coupled solute transport has been demonstrated in other cells, including kidney epithelial cells (11) and osteoblasts (35), in which it stimulates Na^+-dependent phosphate absorption.

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microvillar surface morphology as the result of IGF-I treatment. Second, there could be an increase in density of SGLT1 molecules in brush-border membranes of individual enterocytes after IGF-I treatment. Recently, Cheeseman et al. (12) demonstrated an increase in SGLT1 abundance in brush-border membranes in rats perfused in vivo with glucagon-like peptide 2; thus there is precedent for hormonal regulation of this transporter. Furthermore, EGF, another peptide growth factor present in high concentrations in porcine colostrum, has been reported to increase SGLT1 abundance in intestinal tissues after massive small bowel resection in adult rats (14).

Another mechanism to explain the ability of IGF-I to increase the J\textsubscript{\text{max}} for d-glucose in piglet jejunum is an increase in the electrochemical driving force for Na\textsuperscript{+}-coupled nutrient transport. This possibility is supported by the enhanced rates of net Na\textsuperscript{+} absorption as well as the greater change in \Delta\varepsilon\textsubscript{Na} induced by L-alanine in IGF-I-treated piglets. Both of these processes would also be favored by a change in the Na\textsuperscript{+} electrochemical gradient across the brush-border membrane. Because the Na\textsuperscript{+} electrochemical gradient is dependent on basolateral Na\textsuperscript{+}-K\textsuperscript{+} -ATPase activity, an increase in the number and/or activity of Na\textsuperscript{+}-K\textsuperscript{+} -ATPase pumps could be involved in the upregulation of nutrient absorption in IGF-I-treated piglets. Treatment of rat arterial smooth muscle cells in vitro with IGF-I was reported to stimulate Na\textsuperscript{+}-K\textsuperscript{+} -ATPase activity (37), and in preliminary studies we observed greater Na\textsuperscript{+}-K\textsuperscript{+} -ATPase activity in enterocytes from IGF-I-treated piglets compared with control animals (unpublished observations).

In summary, our results provide evidence that oral administration of IGF-I to neonatal piglets can enhance intestinal epithelial Na\textsuperscript{+} and Na\textsuperscript{+}-coupled nutrient absorption in the absence of changes in enterocyte mass or architecture. Because orogastric administration of IGF-I did not alter serum IGF-I levels, we speculate that luminal IGF-I exerted its effects via stimulation of brush-border IGF-I receptors (23, 25, 47) and/or transepithelial transport of IGF-I and subsequent activation of IGF-I receptors located on basolateral membranes. Future studies should focus on elucidating the cellular mechanisms of IGF-I action in the neonatal intestine.

We thank Nancy Sills and Yanira Oneill-Naumann for technical assistance and Dr. Tom Crenshaw for advice and assistance in piglet management.

This study was supported by United States Department of Agriculture Grant 93–37206–9222 (H. V. Carey), National Institute of Diabetes and Digestive and Kidney Diseases Grant F32-DK-09629 (A. N. Alexander), and grants from the University of Wisconsin-Madison Graduate School and the University of Wisconsin-Madison School of Veterinary Medicine.

Address for reprint requests and other correspondence: H. V. Carey, Department of Comparative Biosciences, Univ. of Wisconsin, 2015 Linden Drive West, Madison, WI 53706 (E-mail: careyh@svm.vetmed.wisc.edu).

Received 25 February 1999; accepted in final form 16 June 1999.

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