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Investigation of Three Doses of Oral Insulin-like Growth Factor-I on Jejunal Lactase Phlorizin Hydrolase Activity and Gene Expression and Enterocyte Proliferation and Migration in Piglets

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ABSTRACT

In a previous study, oral IGF-I at 65 nM increased lactase phlorizin hydrolase (LPH) activity and villus height in piglets, however, the mechanisms were unknown. Herein, the response to a range of doses of IGF-I was investigated and we hypothesized that LPH and villus height would respond to oral IGF-I in a dose-dependent manner. Two 14-d experiments were conducted using cesarean-derived piglets. In experiment 1, piglets ($n = 28$) were fed formula containing 0, 33, 65, or 131 nmol/L (0, 0.25, 0.5, or 1.0 mg/L) recombinant human IGF-I. In experiment 2, 5′-bromodeoxyuridine was administered to piglets fed formula alone ($n = 4$) or containing 131 nmol/L IGF-I ($n = 4$). IGF-I did not affect body weight gain or intestinal weight or length. Jejunal villus height and LPH activity were significantly greater in piglets fed 131 nmol IGF-I/L than control piglets. Villus height and lactase activity in piglets fed the 33 and 65 nmol/L IGF-I doses were similar and intermediate between control and 131 nmol IGF-I/L. Jejunal mRNA expression and LPH polypeptide abundance were investigated in piglets receiving 0 or 131 nmol/L IGF-I. Steady state LPH mRNA abundance was significantly higher ($p < 0.05$) in IGF-I-treated piglets. The relative abundance of proLPH$_h$ was not significantly increased ($p = 0.06$) by IGF-I treatment. Mucosal DNA content and DNA synthesis were greater in piglets receiving 131 nmol IGF-I/L than control, however, enterocyte migration and mucosal protein content were unaffected. Thus, oral IGF-I increased jejunal LPH activity and LPH mRNA abundance and stimulated intestinal cell hyperplasia in normal piglets. **(*Pediatr Res* 48: 497–503, 2000)**

Abbreviations

AP-1, activator protein-1
BrdU, 5′-bromodeoxyuridine
C/EBP, CCAAT/enhancer binding protein
EF1-α, elongation factor 1-α
FLE, foremost labeled enterocyte
HNF-1, hepatocyte nuclear factor-1
LPH, lactase phlorizin hydrolase
BB LPH, brush border lactase phlorizin hydrolase
proLPH$_h$, high mannose lactase phlorizin hydrolase precursor
proLPH$_c$, complex glycosylated lactase phlorizin hydrolase precursor
MAPK, mitogen activated protein kinase
NHS, normal horse serum
PI 3-kinase, phosphatidylinositol 3-kinase

The presence of IGF-I in colostrum and milk (1) and IGF receptors within the intestine (2) has led to the postulate that milk-borne IGF-I could play a role in neonatal intestinal development (3). Our group and others have shown that orally administered IGF-I stimulates intestinal villus growth (4–6) and LPH activity (E.C. 3.2.1.23–62) (6) in the neonatal piglet. However, the doses of IGF-I administered in these experiments (65–1300 nmol/L) were above the concentrations of IGF-I present in porcine colostrum (13–52 nmol/L) or mature milk (1.3–2.6 nmol/L) (7). It was unknown whether oral administration of lower doses of IGF-I would exert similar biologic effects within the neonatal intestine. Further, the mechanisms by which oral IGF-I up-regulated intestinal LPH activity and villus growth had not been described. Therefore, the goals of the current study were to determine the impact of a range of doses of oral IGF-I on intestinal villus growth and LPH activity and to investigate potential mechanisms underlying these ef-

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fects. Specifically, IGF-I-mediated effects on villus growth were investigated by measuring intestinal DNA synthesis and enterocyte migration. LPH biosynthesis is a multistep process involving significant post-translational processing of LPH, thus introducing the potential for transcriptional (8) and post-transcriptional regulation (8, 9). The first detectable product of LPH mRNA translation is a glycosylated protein, $proLPH_h$. This polypeptide, is transported to the Golgi wherein it is further glycosylated forming $proLPH_c$. $ProLPH_c$ is then translocated to the brush border (BB) membrane and proteolytically cleaved to form the mature BB LPH. Herein, the relative distribution of precursor and mature LPH polypeptides in jejunal mucosa were assessed by immunoprecipitation.

MATERIALS AND METHODS

Experimental design and sample collection. The animal protocol was approved by the Laboratory Animal Care Advisory Committee of the University of Illinois and was in compliance with National Research Council guidelines for the care and use of laboratory animals (10). Colostrum-deprived piglets ($n = 36$) were obtained by cesarean section from term pregnant Yorkshire-Duroc sows and were housed as previously described (6). Two experiments were conducted. In experiment 1, piglets ($n = 28$) were fed a commercial sow-milk replacer (Advance Baby Pig Liqui-Wean, Milk Specialties Company, Dundee, IL, U.S.A.) containing either 0, 33, 65, or 131 nmol/L (0, 0.25, 0.50, or 1.0 mg/L) recombinant human IGF-I (Genentech, Inc., So. San Francisco, CA, U.S.A.). Human and porcine IGF-I have 100% amino acid homology (11). Formula ($\sim$15 $mL \cdot kg^{-1} \cdot h^{-1}$) was provided three times daily and purified porcine IgG (American Protein Corp., Ames, IA, U.S.A.) was supplemented to the formula for the first 4 d. IGF-I was added to the formula immediately before feeding. Experiment 1 was conducted in two replicates 3 mo apart. In experiment 2, piglets ($n = 8$) were fed milk replacer containing 0 or 131 nmol/L IGF-I and supplemented with porcine IgG as described above for 14 d. Bromodeoxyuridine (50 mg/kg 5′-BrdU; Sigma Chemical Co., St. Louis, MO, U.S.A.) was administered intraperitoneally 48 h before termination. On d 14, piglets were killed and serum was collected. The small intestine from the pyloric sphincter to the ileocecal valve was excised, flushed with ice-cold saline, divided into 13 segments, weighed, and measured (6). A 1-cm sample of jejunum was fixed in formalin (Surgipath Medical Industries, Inc., Richmond, IL, U.S.A.) for 24 h for histomorphological analysis. Mucosa was scraped from each segment, weighed, and frozen in liquid nitrogen.

Serum IGF concentrations. IGF-I and IGF-II concentrations were determined by RIA as previously described (7). Samples were analyzed in a single assay with intra-assay coefficients of variation of 4% for IGF-I and 6% for IGF-II in experiment 1 and 6% for IGF-I and 5.2% for IGF-II in experiment 2.

Mucosal DNA and protein content. Mucosal samples (0.1 g) were homogenized in 1 mL buffer (0.45 M NaCl, 0.001 M phenylmethylsulfonylfluoride, 0.002 M iodoacetic acid), diluted into DNA buffer (2 mM EDTA, 2.0 M NaCl, 50 mM $NaPO_4$) and sonicated for 30 s (Fisher Scientific, Pittsburgh, PA, U.S.A.). DNA content was determined fluorometrically (excitation wavelength 365 nm and emission wavelength 460 nm; F-2000 Fluorescence Spectrophotometer, Hitachi Instruments, Inc., Chicago, IL, U.S.A.) using Hoechst H 33258 dye (2′-[4-Hydroxyphenyl]-5-[4-methyl-1-piperazinyl]-2,5′-bi-1H-benzimidazole) (Sigma Chemical Co.) (12). Protein content was measured by the Lowry method (13). Calf thymus DNA and BSA (Sigma Chemical Co.) were used as standards.

Intestinal histomorphology. Formalin-fixed, paraffin-embedded jejunal samples were sliced to approximately 5 μm with a microtome, mounted on slides, and stained with hematoxylin. Villus height and crypt depth were measured by using a Nikon microscope (Fryer Company Inc., Carpentersville, IL, U.S.A.) and Image 1 software (Universal Imaging Corp., Westchester, PA, U.S.A.) in 6–10 vertically well-orientated villi and crypts.

Intestinal immunohistochemistry. Incorporation of BrdU into nuclei was visualized by standard immunohistochemical techniques (14) using reagents purchased from Sigma Chemical Co., unless otherwise indicated. Formalin-fixed, paraffin-embedded jejunal samples were sliced to approximately 5 μm with a microtome, mounted on slides, and incubated overnight at room temperature in a humidified chamber with the primary antibody, a mouse monoclonal anti-BrdU IgG (1:1,000 in 3% NHS), or 3% NHS (control for nonspecific binding). Labeled nuclei were detected with a biotinylated universal secondary antibody (horse anti-mouse/rabbit IgG) (Vector Laboratories, Burlingame, CA, U.S.A.) and staining was achieved using a Vectastain Elite ABC (Avidin-Biotin horseradish peroxidase macromolecular complex) Kit and a DAB (3,3′-diaminobenzidine) Substrate Kit for Peroxidase (Vector). Slides were counterstained with hematoxylin and DNA synthesis was quantified by counting under a microscope the number of BrdU-labeled nuclei in 200x fields from 6 to 10 randomly chosen villi per segment. Enterocyte migration was expressed in three ways: (1) measuring the distance (μm) from the bottom of the crypt to the foremost labeled enterocyte (FLE); (2) expressing the migration distance as a percentage of total villus height; and (3) counting the number of cell positions from the bottom of the crypt to the FLE.

LPH activity. Mucosa (0.2 g) were homogenized in 2 mL of homogenization buffer containing protease inhibitors (0.45 M NaCl, 0.001 M phenylmethyl-sulfonylfluoride, 0.002 M iodoacetic acid). LPH activity was determined by the Dahlqvist method as previously described (6) and enzyme activities were expressed per gram of mucosal protein (11).

LPH steady state mRNA abundance. RNA was isolated by the guanidine-isothiocyanate-cesium chloride method from intact jejunal samples obtained from randomly selected piglets from experiment 1 fed formula containing 0 ($n = 6$) or 131 nmol/L IGF-I ($n = 6$). RNA was size fractionated by agarose electrophoresis (10 μg/lane) and northern blotted as described (8). The blot was hybridized by the method of Wahl *et al.* (15) using random-primed ^{32}P-labeled cDNA probes for rabbit LPH (RLac-8; 6.2 kb), generously provided by Dr. Ned Mantei (16), and mouse elongation factor-1α (EF-1α; 1.7 kb) generously provided by Dr. Susan Henning (Baylor College of Medicine, Houston, TX, U.S.A.). EF-1α is a ribosomal binding protein

that was used as a constitutive marker (17). Bands were visualized by autoradiography and quantified using an LKB Ultrascan XL densitometer (LKB, Bromma, Sweden). LPH mRNA abundance was expressed relative to EF-1α mRNA.

Immunoprecipitation of mucosal LPH. LPH polypeptides were isolated from jejunal mucosa of piglets from experiment 1 fed formula containing 0 ($n = 6$) or 131 nmol/L IGF-I ($n = 6$) exactly as described by Dudley *et al.* (9). Briefly, frozen mucosa was homogenized in PBS containing protease inhibitors. The mucosal homogenate was centrifuged at 40,000 rpm (70 ti rotor, Beckman Instruments, Palo Alto, CA, U.S.A.) for 40 min. The pellet, containing membrane protein, was solubilized and LPH isoforms were immunoprecipitated with a monoclonal anti-porcine lactase antibody (A. Quaroni, Division of Biologic Sciences, Cornell University, Ithaca, NY, U.S.A.) and visualized by SDS-PAGE and Coomassie blue staining. The relative abundance of $proLPH_h$ (the first detectable LPH precursor) and BB LPH were determined using densitometry.

Statistical analyses. Analyses were performed using the general linear model procedure within SAS (Version 6.09, SAS Institute, Cary, NC, U.S.A.) on untransformed data, nesting each segment within treatment and using least squares means to obtain a p value for comparison between control and treatment groups for each segment. Student's t test was used to compare LPH activity, LPH mRNA, and LPH polypeptide abundance within the jejunum of piglets fed formula containing 0 or 131 nmol/L IGF-I. Significance was assigned at $p < 0.05$ and p values between 0.05 and 0.10 are reported as trends. Data are expressed as means ± SEM.

RESULTS

Formula and IGF-I intake, serum IGF-I and -II, weight gain, and intestinal growth. Formula intake of piglets between d 2 and 14 postpartum was not significantly different between treatment groups in either experiment 1 or experiment 2 (Table 1). Mean IGF-I intake ranged from 10 to 43 nmol/kg/d (0.08–0.33 mg/kg/d). There were no significant differences in initial or final body weight (BW) or visceral organ weight (data not shown) among the treatment groups (Table 1) for either experiment. Serum IGF-I and -II concentrations were similar between experiments and were unaffected by oral IGF-I administration. Serum IGF-I and -II values averaged 5.2 ± 0.5 nmol/L and 22.8 ± 1.7 nmol/L, respectively, across all treatment groups. Intestinal weight (g/kg BW) and length (cm/kg) (Table 2) were not different among groups for either experiment 1 or experiment 2 and were comparable between experiments as well as with our previous study (6).

Mucosal DNA and protein content. Mucosal DNA and protein content were assessed in experiment 2. No effect of oral IGF-I on mucosal protein content was detected (Table 2). However, piglets fed formula supplemented with 131 nmol IGF-I/L had significantly higher jejunal mucosal DNA content compared with piglets fed formula alone (Table 2).

Histomorphology. Jejunal villus height and crypt depth are shown in Table 2. In experiment 1, piglets consuming formula containing 33 or 131 nmol/L had significantly longer villi than piglets fed formula alone. Piglets fed the highest dose of IGF-I also had significantly longer jejunal villi than piglets fed formula containing 65 nmol/L IGF-I ($p < 0.05$). In experiment 1, crypts within the jejunum of piglets fed formula containing 33 or 131 nmol/L were significantly deeper than piglets fed formula alone ($p < 0.05$). In experiment 2, villi and crypts were shorter than experiment 1; however, villi in piglets receiving 131 nmol/L IGF-I were significantly longer than piglets fed formula alone. Therefore, although absolute differences in villus height were observed between the two experiments, a consistent IGF-I treatment effect was still apparent.

LPH activity. The distribution of LPH activity (μmol glucose/min/g protein) throughout the small intestine of piglets fed formula containing 0 or 131 nmol/L IGF-I is illustrated in Figure 1*A*. LPH activity (μmol glucose/min/g protein) was greater throughout the duodenum and proximal and distal jejunum of piglets fed formula containing 131 nmol/L IGF-I compared with formula alone ($p < 0.05$). Based upon the distribution of enzyme activity shown in Figure 1*A*, data from intestinal segments representing the duodenum (segment 1), proximal jejunum (segments 2–7), distal jejunum (segments

Table 1. *Formula and IGF-I intake and whole body growth of piglets consuming formula with or without recombinant human IGF-I for 14 d*†*

	Formula IGF-I Concentration (nmol/L)			
	0	33	65	131
Experiment 1				
n	8	6	8	6
Birth wt (kg)	1.72 ± 0.10	1.63 ± 0.10	1.69 ± 0.12	1.64 ± 0.11
Body wt on d 14 (kg)	3.73 ± 0.11	3.87 ± 0.22	3.87 ± 0.11	3.83 ± 0.20
Formula intake (mL/kg/d)§	337.6 ± 17.7	309.7 ± 14.3	326.8 ± 15.9	331.2 ± 16.3
IGF-I intake (nmol/kg/d)§	0	10.0 ± 0.38	20.1 ± 0.13	41.2 ± 0.13
Experiment 2				
n	4			4
Birth wt (kg)	1.4 ± 0.28			1.6 ± 0.27
Body wt on d 14 (kg)	4.0 ± 0.17			4.0 ± 0.69
Formula intake (mL/kg/d)§	310.6 ± 34.0			300.2 ± 32.3
IGF-I intake (mmol/kg/d)§	0			37.5 ± 0.375

* Values are expressed as means ± SEM of the numbers of animals indicated in each column.

† No statistically significant differences between groups were noted by ANOVA.

§ Mean intake d 2–14 postpartum.

Table 2. *Intestinal weight, length, protein and DNA content, and jejunal histomorphology of piglets consuming formula with or without recombinant human IGF-I for 14 d*†*

	Formula IGF-I Concentration (nmol/L)			
	0	33	65	131
Experiment 1				
Intestinal wt (g/kg BW)	42.6 ± 5.5	42.7 ± 5.7	43.8 ± 6.7	44.0 ± 6.0
Intestinal length (cm/kg BW)	162.9 ± 32.1	170.8 ± 30.9	165.9 ± 32.7	162.0 ± 29.3
Villus height (μm)	1147 ± 130[a]	1489 ± 136[bc]	1332 ± 133[ab]	1767 ± 160[c]
Crypt depth (μm)	147 ± 6[a]	169 ± 9[b]	155 ± 6[ab]	164 ± 8[b]
Experiment 2				
Intestinal wt (g/kg BW)	47.0 ± 4.2			48.0 ± 6.0
Intestinal length (cm/kg BW)	140.9 ± 8.1			166.0 ± 28.3
Mucosal protein (mg/cm)	14.1 ± 3.5			13.9 ± 4.2
Mucosal DNA (μg/cm)	44.2 ± 8.7[a]			82.8 ± 9[b]
Villus height (μm)	706.5 ± 44.5[a]			880 ± 43.9[b]
Crypt depth (μm)	69 ± 1.3			73 ± 6.9

* Values are expressed as means ± SEM of the numbers of animals indicated in each column.
† Different superscripts indicate significant differences at $p < 0.05$.
BW, body weight.

8–11), and ileum (segments 12 and 13) were pooled. Mean LPH activity within these four regions of the small intestine are shown in Figure 1*B*. Piglets fed formula containing 131 nmol/L IGF-I had higher LPH activity within the duodenum and proximal and distal jejunum than piglets fed formula alone ($p < 0.05$). Within the proximal and distal jejunum, LPH activity of piglets fed formula containing 33 or 65 nmol/L were intermediate between the formula alone and 131 nmol/L IGF-I groups.

Jejunal LPH mRNA expression. Steady state LPH and EF-1α mRNA expression within the jejunum of piglets fed formula containing 0 or 131 nmol/L IGF-I were determined by Northern analysis (Fig. 2). No difference in EF-1α abundance was observed between piglets fed formula containing 0 or 131 nmol/L IGF-I (668 ± 88 *versus* 781 ± 109 arbitrary densitometric units, respectively), therefore LPH mRNA expression was normalized by EF-1α mRNA expression for each sample to correct for any differences in loading. Piglets fed formula containing 131 nmol/L IGF-I had a 40% greater steady state LPH mRNA abundance than piglets fed formula alone ($p < 0.05$) (Table 3).

Jejunal LPH polypeptide relative abundance. To investigate potential differences in post-translational processing, mucosal precursor and mature BB LPH polypeptides were immunoisolated and separated by SDS-PAGE (Fig. 3). Three bands are apparent on the Coomassie-stained gel corresponding to mature BB LPH (~160 kD), proLPH$_h$ (~200 kD), and a dimer of mature BB LPH (~240 kD), which has been reported in immunoprecipitates of piglet intestinal mucosa (13). The relative abundance of the intermediate precursor (proLPH$_c$) was below the level of detection. No statistically significant ($p = 0.06$) difference in LPH polypeptide abundance was observed with IGF-I treatment (Table 3).

BrdU incorporation and enterocyte migration. Sections of jejunum from 14-d-old piglets fed formula alone (Fig. 4, *A* and *B*) or formula containing 131 nmol/L IGF-I (Fig. 4, C and D) are shown. Sections in Figure 4 *A* and *C* were incubated with the primary antibody (mouse monoclonal anti-BrdU), whereas sections in Figure 4 *B* and *D* were incubated with normal horse serum in place of the primary antibody. BrdU-labeled nuclei are apparent as black dots in Figure 4 *A* and *C*. DNA synthesis was 38% higher in the jejunum of 14-d-old piglets fed formula supplemented with 131 nmol/L IGF-I compared with controls (Table 4). There was no effect of IGF-I on enterocyte migration when measured in μm or when expressed as the number of cell positions (Table 4). The FLE had migrated approximately 25% of the villus height in 48 h, suggesting an enterocyte turnover rate of approximately 8 d.

DISCUSSION

Previous studies in neonatal rats and piglets have demonstrated that orally administered IGF-I retains both structural and functional activity within the gastrointestinal tract (18, 19) and exerts physiologic effects on gut morphology (4–6, 20) and LPH activity (6, 20–22). The stability of IGF-I within the neonatal gastrointestinal tract is likely due to lower proteolytic enzyme secretion, coupled with the fact that the milk protein casein may help protect IGF-I from digestion (23). Lactose is the primary carbohydrate source in infant diets. Therefore, the ability of IGF-I to up-regulate LPH activity may be of clinical importance in infants at risk for low LPH activity secondary to premature delivery, prolonged support on total parenteral nutrition (TPN), or villus damage secondary to diarrhea or inflammation. Indeed, we have recently shown that orally administered IGF-I markedly enhanced LPH activity in parenterally fed piglets (24). The fact that IGF-I does not appear to be absorbed (19) and does not exert whole body and extraintestinal organ growth responses suggests that the oral route of IGF-I administration may be desirable for patients with compromised intestinal health (25).

Our first aim was to investigate effects of different concentrations of oral IGF-I on jejunal LPH activity. We had previously demonstrated that 65 nmol/L IGF-I significantly increased intestinal LPH activity, herein we also investigated the effect of 33 and 131 nmol/L concentrations of IGF-I. Consistent with our previous study (6), oral IGF-I up-regulated small intestinal LPH activity. This effect was detected at all three

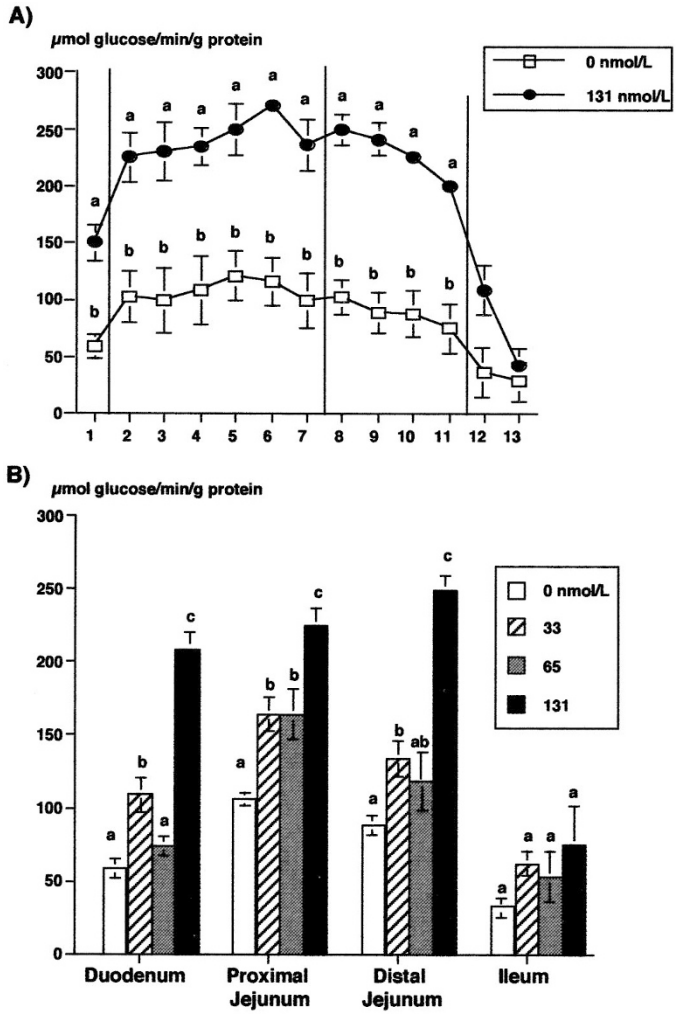

Figure 1. Small intestinal LPH activity (μmol glucose/min/g protein) of 14-d-old piglets fed formula containing 0, 33, 65, or 131 nmol/L recombinant human IGF-I. (*A*) Distribution of LPH activity (μmol glucose/min/g protein) throughout the small intestine of piglets fed formula containing 0 or 131 nmol/L IGF-I for 14 d. Different letter superscripts are significantly different at $p < 0.05$ by *t* test. (*B*) Based upon this distribution of activity, LPH activity in segments representing the duodenum (segment 1), proximal jejunum (segments 2–7), distal jejunum (segments 8–11) and ileum (segments 12 and 13) were pooled. Values are means ± SEM. Bars not sharing the same letter superscripts are significantly different at $p < 0.05$ by ANOVA.

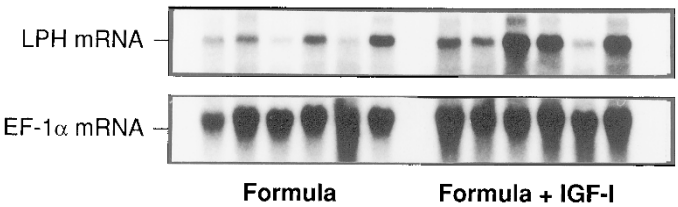

Figure 2. Jejunal LPH and EF-1α mRNA expression in 14-d-old piglets fed formula containing either 0 or 131 nmol/L recombinant human IGF-I. An autoradiogram of a Northern blot is shown. EF-1α is the standard for equal loading. Results of densitometric analyses are summarized in Table 3.

concentrations administered, suggesting that concentrations of IGF-I within the range observed in porcine colostrum (33–65 nM) are sufficient to increase intestinal LPH activity. Previous experiments have demonstrated that diet-induced changes in LPH activity are associated with alterations in LPH mRNA abundance (8, 26, 27). Herein, steady state mRNA abundance within the jejunum of piglets fed formula containing 131 nmol/L IGF-I was significantly greater than that observed in piglets fed formula alone. There were no significant differences in the relative abundance of LPH polypeptides, suggesting that the major site at which IGF-I regulates LPH activity is at the level of transcription.

The cellular mechanism(s) by which IGF-I increases steady state LPH mRNA remains to be elucidated, but may result from either increased gene transcription or reduced mRNA turnover. In support of the first possibility, the promoter region of the LPH gene contains putative binding sites for a number of transcription factors including AP2, C/EBP, CTF/NF (27), and HNF-1 (28, 29). Studies in fetal rats have linked increased C/EBP-alpha mRNA expression to intestinal differentiation, specifically LPH mRNA expression (30). Studies using cultured brown adipose tissue demonstrated a link between IGF-I treatment, markers of cellular differentiation, and C/EBP alpha (31). Further, this effect has been shown to occur through AP-1 activity in a PI-3 kinase and RAS/MAP kinase-dependent

Table 3. *LPH-specific activity, mRNA abundance, and relative proportions of LPH polypeptides in the jejunum of piglets consuming formula containing 0 or 131 nmol/L recombinant human IGF-I for 14 d*†*

	Formula IGF-I Concentration (nmol/L)	
	0	131
LPH specific activity (μmol glucose/min/g protein)	151.1 ± 25.8[a]	249.7 ± 32.0[b]
LPH mRNA abundance (LPH mRNA/EF-1α mRNA)‡	0.36 ± 0.04[a]	0.51 ± 0.07[b]
Relative abundance of proLPHh and (% total)§		
$proLPH_h$ (200 kD)	2.0 ± 0.29[a]	3.2 ± 0.29[a]¶
mature brush border LPH (160 kD)	98.0 ± 0.29[a]	96.8 ± 0.29[a]

* Values are mean ± SEM; $n = 6$ per treatment group.
† Different superscripts indicate significant differences at $p < 0.05$.
‡ Data derived from densitometric analysis of Northern blot shown in Figure 2.
§ Amount of the individual LPH polypeptide relative to total immunoisolated LPH protein separated by SDS-PAGE (Figure 3).
¶ $p = 0.06$.

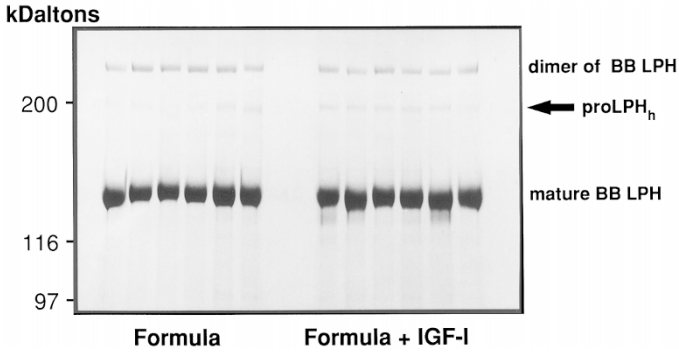

Figure 3. Jejunal LPH polypeptides isolated from 14-d-old piglets fed formula containing either 0 or 131 nmol/L recombinant human IGF-I. A Coomassie blue-stained 5% SDS-PAGE gel of mucosal brush border membranes immunoprecipitated with a lactase antibody is shown. Mature BB LPH (~160 kD), $proLPH_h$ (~200 kD), and dimerized mature BB LPH (~240 kD) are shown. Results of densitometric analyses are summarized in Table 3.

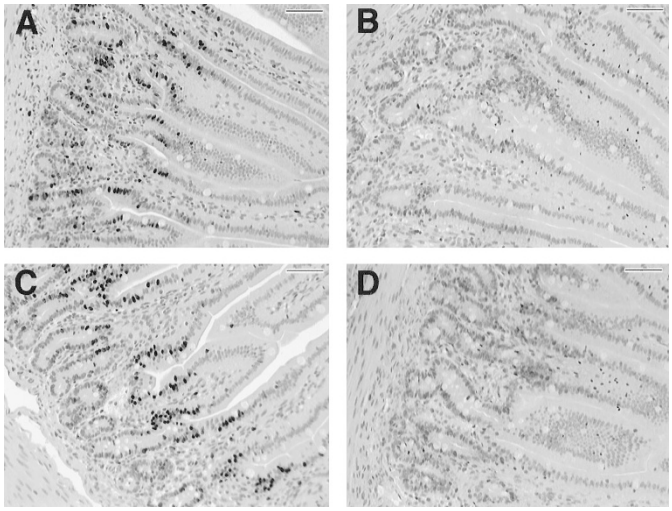

Figure 4. Immunohistochemical detection of bromodeoxyuridine incorporation into jejunal enterocytes. *A* and *B* illustrate jejunal section (5 μm) from a 14-d-old control animal (0 nmol/L IGF-I). *C* and *D* illustrate jejunal section (5 μm) a 14-d-old piglet who received formula supplemented with 131 nmol/L IGF-I. Piglets were injected intraperitoneally with 50 mg/kg of BrdU 48 h before intestinal sampling. BrdU incorporation was detected immunohistochemically (*A* and *C*) and the slides were counterstained with hematoxylin. *B* and *D* show intestinal sections incubated with NHS instead of primary antibody. The bar is 50 μm in length.

Table 4. *Enterocyte proliferation and migration in the jejunum of piglets consuming formula containing 0 or 131 nmol/L recombinant human IGF-I for 14 d*†*

	Formula IGF-I Concentration (nmol/L)	
	0	131
Proliferation‡	444.8 ± 31.9[a]	590.3 ± 50.5[b]
Migration		
Distance (μm)§	193.5 ± 22.8	205 ± 8.8
% of villus height¶	27 ± 1.8	24 ± 3.1
Cell positions‖	68 ± 5	64 ± 8

* Values are mean ± SEM; $n = 4$ per treatment group.

† Different superscripts indicate significant differences at $p < 0.05$.

‡ Number of BrdU-labeled nuclei in 6 to 10 200× microscope fields per piglet.

§ Distance (μm) from the bottom of the crypt to the FLE.

¶ Distance (μm) from the bottom of the crypt to the FLE relative to total villus height (Table 3).

‖ Number of cell positions from the bottom of the crypt to the FLE.

manner (32). IGF-I has been shown to stabilize FSH receptor mRNA (33) and LDL receptor mRNA (34), suggesting this as a potential mechanism for LPH up-regulation. Alternatively, IGF-I could potentially increase mRNA abundance by a combination of transcriptional activation and enhanced mRNA stability (34). Future studies are needed to establish the underlying mechanism(s) of IGF-I up-regulation of LPH mRNA abundance.

Our second aim was to investigate effects of oral IGF-I on enterocyte proliferation and migration. IGF-I has previously been shown to increase proliferation of intestinal epithelial cells in culture (35) and migration of piglet esophageal cells (36) and rat intestinal cells (20). Positive effects of IGF-I on either parameter could increase villus height. Additionally, increasing intestinal cell number and migration rate could also indirectly increase LPH activity. We observed a 40% increase in jejunal DNA content in piglets fed formula containing 131 nmol/L IGF-I. In addition, DNA synthesis, as assessed by *in vivo* BrdU incorporation, was greater in IGF-I-treated piglets. Xu *et al.* (4) also reported greater *in vivo* BrdU incorporation into intestinal cells of newborn piglets fed formula containing 262 nmol IGF-I/L for 24 h. However, there were no differences in jejunal protein content or total intestinal weight. Using protein-to-DNA ratio as an index of cell size, cells within the intestinal mucosa of piglets fed formula containing 131 nmol/L IGF-I were approximately 50% smaller than cells from piglets fed formula alone (data not shown). These data suggest that at the concentrations of IGF-I investigated herein, there was a greater effect on enterocyte hyperplasia than hypertrophy. The presence of a greater number of smaller enterocytes would be consistent with the relatively modest effects of oral IGF-I on villus height (20–40% increases over formula alone) observed in this and our previous studies (6). However, Burrin *et al.* (5) demonstrated that if piglets are fed formula containing a pharmacological concentration of IGF-I (~1.3 mM) significant increases in mucosal protein content, mucosal mass, and villus height can be achieved.

Although a significant increase in BrdU incorporation was noted in crypt cells, no effect of oral IGF-I on enterocyte migration was observed. Our enterocyte turnover rate of approximately 8 d is within the 7–10 d range observed in neonatal piglets (37). Other studies have shown that enhanced crypt cell proliferation does not drive enterocyte migration (38), therefore the two processes may be differentially impacted by oral IGF-I. Our data do, however, differ from those of Phillips *et al.* (20), who reported that migration was increased by 50% within the proximal jejunum of rats pups fed a milk substitute containing IGF-I compared with milk substitute alone. It is unknown why our data differ from theirs, however, it may be related to species as Peterson *et al.* (39) also showed in rats that enterocyte migration is increased in response to systemically administered IGF-I. In addition to modulating enterocyte proliferation, IGF-I could increase villus height by inhibiting enterocyte programmed cell death (40, 41). Enterocyte apoptosis can be regulated by dietary components, however, the basal level of intestinal apoptosis is low in the pig and the degree of apoptosis is correlated with mitogenesis (42). Inhibition of apoptosis of enterocytes at the tips of the villi would lead to retention of functionally mature enterocytes, and could provide a common mechanism by which the villi of IGF-I-treated piglets are longer and lactase activity is increased.

In conclusion, we have confirmed that orally administered IGF-I up-regulates intestinal LPH activity in the neonatal piglet and demonstrated that the mechanism appears to be *via* increased LPH mRNA abundance. Although the greatest response in LPH activity was observed with 131 nmol/L, a concentration as low as 33 nmol/L was effective. In addition, oral IGF-I at 131 nmol/L increased enterocyte proliferation and mucosal DNA content, but did not affect enterocyte migration. The fact that IGF-I appeared to stimulate both the processes of proliferation and differentiation (increased LPH activity) in piglets, may appear paradoxical. However, depending upon the cell type and experimental design, IGF-I has been shown to

stimulate multiple responses associated with cellular proliferation, as well as promote cellular differentiation and inhibit apoptosis (40). We are speculating that the effect of oral IGF-I in the neonatal piglet intestine may differ depending upon the target cell (*e.g.* a proliferating crypt stem cell *versus* a differentiating villus enterocyte).

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