Nutritional Sciences 3(2), November, 2000, pp. 57-65
© 2000 The Korean Nutrition Society

Oral Insulin-like Growth Factor-I Combined Alters Intestinal Protein Synthesis in Parenterally-fed Piglets

Yoo Kyoung Park[§] and Sharon M. Donovan

Division of Nutritional Sciences, University of Illinois, Urbana, IL, 61801, USA

ABSTRACT

Background: Partial enteral nutrition (PEN) supplemented with insulin-like growth factor-I (IGF-I) to neonatal piglets receiving parenteral nutrition increases lactase-phlorizin hydrolase (LPH) activity, but not LPH mRNA. The goal of the current study was to investigate the mechanism by which IGF-I up-regulates LPH activity. We hypothesized that IGF-I regulates LPH synthesis post-transcriptionally. **Methods**: Newborn piglets (n = 15) received 100% parenteral nutrition (TPN), 80% parenteral nutrition + 20% PEN (PEN), or PEN + IGF-I (1.0 mg/kg/d). On day 7, two stable isotopes of leucine, [$^{2}H_{3}$]-leucine and [$^{13}C_{1}$]-L-leucine were intravenously administered to measure mucosal protein and brush border LPH (BB LPH) synthesis. **Results**: Weight gain, nutrient intake and jejunal weight and length were similar among the treatment groups. PEN increased mucosal weight, villus width and cross-sectional area, LPH activity, mRNA expression and the abundance of proLPHh compared to 100% TPN ($p < 0.05$). IGF-I further increased mucosal weight, LPH activity and LPH activity per unit BB LPH ~2-fold over PEN alone ($p < 0.05$), but did not affect LPH mRNA or the abundance of proLPHh or mature LPH. Isotopic enrichment of [$^{2}H_{3}$]-leucine and [$^{13}C_{1}$]-L-leucine in plasma, mucosal protein and LPH precursors, and the fractional and absolute synthesis rates of mucosal protein and LPH were similar among the treatment groups. Total mucosal protein synthesis was increased 60% ($p < 0.05$) and LPH synthesis tended ($p = 0.14$) to be greater in the IGF-I treated animals compared to the other two groups. **Conclusions**: The primary mechanism by which IGF-I up-regulates LPH may be post-translational, either via reducing LPH turnover, or by specifically altering LPH activity.

KEY WORDS: TPN, IGF-I, intestine, lactase, sucrase.

INTRODUCTION

Total parenteral nutrition (TPN) results in villus atrophy (Duersken *et al.*,[1] Park *et al.*[2]) and compromises intestinal functional development (Gall *et al.*,[3] Wykes *et al.*[4]). The villus flattening results in reduced abundance of digestive enzymes which are primarily distributed on the upper part of the villus along the luminal border of the microvilli (Van Beers *et al.*[5]). One of the digestive enzymes that is compromised with parenteral nutrition is lactase-phlorizin hydrolase (LPH). LPH is the disaccharidase responsible for the hydrolysis of lactose, and thus, is an essential enzyme in early life since lactose is the main dietary source of carbohydrate in newborn mammals. Therefore, recovery of the synthesis and abundance of this enzyme is likely of critical importance to neonates, particularly during the transition from TPN to enteral support.

Thus, it is important to investigate potential clinical therapies which will induce intestinal digestive and absorptive function in parenterally-fed neonates. We have demonstrated that provision of 20% partial enteral nutrition (PEN) to piglets maintained on TPN increased mucosal weight and LPH activity (Park *et al.*[6]). The increase in LPH enzymatic activity in response to 20% PEN was proportional to the increase in mucosal weight (e.g. LPH activity per unit DNA were comparable). In addition, the increase in LPH activity in response to 20% PEN was associated with an increase in LPH steady-state mRNA abundance, suggesting regulation at the level of transcription (Park *et al.*[6]). In contrast, supplementing 20% PEN with insulin-like growth factor-I (IGF-I) increased mucosal LPH activity ~2-fold over 20% PEN alone, with no further increase in LPH mRNA expression. Therefore, we hypothesized that the mechanism by which IGF-I induces intestinal LPH activity in parenterally-fed piglets is post-transcriptional.

Intestinal LPH is synthesized as a precursor protein which requires significant post-translational modification prior to insertion into the brush border (BB) membrane in its active form (Dudley *et al.*[7]). Its complex synthesis introduces the possibility for regulation of enzyme activity at the levels of transcription, translation, post-translation modification or enzyme stability. Over the past several

Accepted : November 13, 2000
[§]To whom correspondence should be addressed.

years, accurate determination of *in vivo* LPH synthesis has been achieved using isotopically labeled amino acids (Dudley *et al.*[8-10]). The objective of this experiment was to determine the effect of enterally administered IGF-I on intracellular LPH processing utilizing the continuous isotope infusion strategy. Continuous isotope infusion protocol, coupled with a priming isotope dose, brings the LPH precursors to isotopic equilibrium rapidly and maintains this steady state for a sufficient time to allow for processing and translocation of LPH precursors to the BB membrane (Dudley *et al.*[10]).

MATERIALS AND METHODS

1. Experimental protocol

Newborn piglets were obtained (n = 15) and handled as described previously (Park *et al.*[2]). The piglets were randomized into three groups. One group was assigned to receive 100% of their energy needs parenteral nutrition to serve as a TPN control group (n = 3, TPN). The remaining piglets were randomized to receive 80% parenteral nutrition + 20% PEN (n = 6, PEN): or PEN supplemented with 1.0 mg/kg/d IGF-I (n = 6, PEN + IGF-I). A catheter for TPN infusion was inserted surgically through the umbilical artery and advanced ~22 cm into the blood vessel until the tip reached the aortic arch, as previously described (Park *et al.*[6]). A second catheter was inserted ~18 cm into the umbilical artery for subsequent blood sampling during the stable isotope infusion. PEN was provided as a commercial sow milk replacer which was offered twice daily (Park *et al.*[6]). Piglets on TPN were provided with a nutritionally complete intravenous solution by 24 hour continuous infusion. Animal housing, TPN solution composition and administration, and IGF-I supplementation into the formula were as described in Park *et al.*[6]

2. Isotope infusion

On day 7, piglets were administered intravenously a dual isotope infusion of [3,3,3-$^{2}H_3$]-leucine ([$^{2}H_3$]-leucine) and [$^{13}C_1$]-L-leucine (Cambridge Isotope Laboratories, Andover, MA) as described by Dudley *et al.*[7] The dual infusion technique allows us to document that proLPHh reaches isotopic steady state over a defined period of time. Both isotopes were prepared in a sterile saline solution and were infused as part of the TPN solution. A priming dose of 30 μmol/kg of [$^{2}H_3$]-leucine was administered via the umbilical catheter followed by a constant infusion of 30 μmol/kg/h for 6 hours (Dudley *et al.*[11]). After two hours of infusion of the first isotope, a primed infusion (30 μmol/kg) of the second isotope, [$^{13}C_1$]-leucine, was initiated followed by a constant infusion at 30 μmol/kg/h for 4 hours. In a previous study by Dudley *et al.*[12]), isotopic steady state was achieved within 90 minutes of infusion. In order to maintain luminal nutrition throughout the 6 hr isotope infusion, piglets on partial enteral nutrition were administered by oro-gastric gavage one fourth of their daily intake (15 - 20 mL/kg) at two hour intervals.

Blood samples were collected at baseline, 15, 30, 60, 120, 240, and 360 min into the infusion. Serum was obtained after centrifugation at 4,000 × g for 15 minutes at 4℃. At the end of the infusion, pigs were killed with an intravenous injection of pentobarbital sodium (~80 mg/kg). The abdomen was opened and the small intestine from pyloric sphincter to ileocecal valve was removed and divided into 13 approximately equal length segments which were flushed with cold saline, weighed and measured as described by Park *et al.*[2] A 2 cm section from segments 3-9, representing jejunum, were processed for histomorphology as described by Park *et al.*[2] and mucosal samples were collected from the jeunum by opening each segment longitudinally and scraping the luminal surface with a glass slide. All samples were stored at −70℃ prior to analysis.

3. Sample analyses

1) Mucosal protein content and intestinal histomorphology

Mucosal protein content was measured as described previously (Park *et al.*[2]) and data were expressed as mg protein/g mucosa. Villus height, width cross-sectional area and crypt depth were determined as described by Park *et al.*[6]

2) LPH enzyme activity

LPH enzyme activity was measured at a 1 : 30 dilution of the original homogenate as previously described (Park *et al.*[6]). Enzyme activity was expressed as specific activity (mol glucose liberated/min/g protein), total mucosal activity (activity per g mucosa * mucosal weight) and per unit mucosal BB LPH (specific activity ± density of BB LPH from SDS-PAGE).

3) Mucosal free amino acid pools and total mucosal protein

Approximately 100 mg of mucosa scraping was homo-

genized with 5% trichloroacetic acid (TCA) to precipitate the protein, which was then spun down by centrifugation (3000 × g, 15 min) The supernatant, which contained the mucosal free amino acids, was applied to a cation exchange column (Dowex AG50, 8% cross-linked, 1.5 mL bed volume, H^+), and the amino acids were eluted and dried under nitrogen gas for further GC/MS analysis. The TCA precipitates, containing mucosal protein, were washed three times with deionized water by repeated centrifugation (12,000 × g, 30 minutes) and finally the purified pellet was hydrolyzed with 5.4 M HCl at 110℃ for 24 hours. The pellets were dissolved in 1 M glacial acetic acid and were applied to the cation exchange column prepared as described above. Final amino acids were eluted with 2 mL of 3M NH_4OH and dried under nitrogen for further processing.

The samples were then esterified and derivitized for GC/MS analysis as described by Dudley *et al.*[10] Briefly, 0.5 mL of esterification solution (isopropanol: acetyl chloride 5 : 1 [v/v]) was added to the dried amino acids, which were then incubated at 60℃ for two hours followed by drying under nitrogen gas. Derivatization was performed by adding 100 μL of HFBA (heptafluorobutric anhydride) to each previously dried sample and followed by heating for 20 min at 60℃. To each tube, 400 μL of ethyl acetate was added prior to analysis by negative chemical ionization GC/MS with methane as the carrier gas on a Hewlett-Packard 5988A mass spectrometer (Hewlett-Packard, Palo Alto, CA) linked to a HP 5890II gas chromatograph (Dudley *et al.*[10]). GC/MS analyses were performed by Dr. Douglas Burrin at the Children's Nutrition Research Center (Houston, TX).

4. Immunoisolation and purification of LPH polypeptides

Approximately 1 gm of scraped mucosa was homogenized in 10 mL of 1X PBS containing phenylmethylsulfonyl fluoride (PMSF, 2 mM), leupeptin (52 mM), trypsin inhibitor (10 U/L), aprotinin (35 mM), antipain (50 U/L) and streptomycin (58 mg/L) as described by Dudley *et al.*,[10] which were all obtained from Sigma Chemical Company (St. Louis, MO). The mucosal homogenate was centrifuged at 40,000 rpm (70 ti rotor, Beckman Instruments, Palo Alto, CA) for 40 minutes at 4℃. The pellet, which contained the membrane protein, was solubilized in 10 mL resuspension buffer (1X PBS, containing 2 mM PMSF, 10% Triton X-100 (Sigma) and protease inhibitors) and was rocked overnight at 4℃. The suspension was spun at 40,000 rpm for another 40 minutes. The supernatant were applied onto Sepharose beads to which LPH monoclonal antibodies had been immobilized as previously described (Dudley *et al.*[7]), which were provided by Dr. Mary Dudley (University of Medicine and Dentistry at New Jersey, Newark, NJ). The antibody/antigen bead complexes were washed several times with phosphate buffer, and then the antigens were released from the antibody by adding phosphate buffered saline containing 5% -mercaptoethanol. The mixture was heated for 4 min at 95℃ followed by centrifugation for 5 min at 3000 × g. The solution was stored at −20℃ prior to gel separation.

5. Separation of LPH precursor forms by SDS-PAGE

Immunoprecipitated LPH isoforms were separated on a 6% acrylamine SDS-PAGE gel at 30 mA for 4-5 hours. An equivalent concentration of mucosal protein for each animal was loaded onto the gel and molecular weight standards (Bio-Rad Laboratories, Hercules, CA) were included on all gels. Following electrophoresis, gels were stained overnight with 0.02% Commassie blue R-250 and destained with 7% acetic acid solution. The density of each band was determined (Fotodyne, New Berlin, WI). The relative abundance of each LPH polypeptide is expressed as a proportion of the total LPH protein in the sample (sum of proLPHh, proLPHc, proLPHi, BB LPH and dimer of BB LPH). The bands were excised for hydrolysis of the proteins to amino acids for GC/MS. Ultrapure hydrochloric acid (12 N, ~500 μL) was added to each gel slice in a glass hydrolysis tube and tubes were placed in a 110℃ oven for 24 hours. Samples were dried under nitrogen first and then ~16 mL deionized water was added to the test tube. The hydrolysates were dried once more and 1.25 mL 1 N acetic acid was added. The amino acid mixture was then isolated by Dowex 50 ion-exchange chromatography as described by Dudley *et al.*[10] The isotopic enrichment into each LPH polypeptide was measured by GC/MS analysis after processing as described above for mucosal protein.

6. Calculation of fractional synthesis rates (Ks) of mucosal protein and mature BB LPH

The fractional synthesis rates were calculated based upon two assumptions. First, that isotopic equilibrium was achieved rapidly in both the mucosal free amino acid pool and proLPHh; and second, that once the isotopic equilibrium is achieved in proLPHh, the isotopic incorporation into the LPH protein products is linear. The frac-

tional synthesis rate (FSR) of total mucosal protein and BB LPH were estimated using the following equations (Dudley *et al.*, 1995).

$$\text{protein FSR (day)} = \frac{\text{isotopic enrichment of protein}}{\text{isotopic enrichment of proLPHh}} \times \frac{24\text{ h}}{\text{hr of infusion}}$$

$$\text{BB LPH FSR (day)} = \frac{\text{isotopic enrichment of BB LPH}}{\text{isotopic enrichment of proLPHh}} \times \frac{24\text{ h}}{\text{hr of infusion}}$$

For all the calculations, only [$^2H_3$]-leucine values were used, as enrichment of [$^{13}C_1$]-L-leucine into BB LPH was very low compared to [$^2H_3$]-leucine. This is likely due to the shorter duration of infusion (4 vs. 6 hours) and the higher natural abundance of [$^{13}C_1$]-L-leucine compared to [$^2H_3$]-leucine (baseline tracer-to-tracer ratio: 15.5 vs. 0.19). Considering the higher natural abundance, the infusion concentration (30 μmol/kg) might have been insufficient.

7. Calculations of mucosal protein synthesis

Absolute jejunal protein synthesis rate was calculated as the product of the abundance of the protein per gram of mucosa and the FSR. Total jejunal protein synthesis rate was calculated by multiplying the absolute synthesis rate and the mucosal weight of the segment (Dudley *et al.*[10]).

8. Calculations of BB LPH synthesis

The abundance of mature BB LPH was estimated by dividing the LPH enzyme activity (mol glc/min/g mucosa) by the relative abundance of mucosal BB LPH protein, estimated from the density of the BB LPH band acquired from the SDS-PAGE separation. As a result, the values obtained represent an arbitrary unit of the total quantity of LPH in 1 g mucosa (Dudley *et al.*[10]). Absolute synthesis rate was calculated as the product of the abundance of LPH derived from above and the FSR for BB LPH. The total synthesis rate was calculated as the product of its absolute synthesis rate multiplied by the mucosal weight of the jejunal segment (Dudley *et al.*[10]).

9. Statistical analyses

Analyses were performed applying the General Linear Model technique with randomized complete-block design (groups blocked by litters) as described in Chapter 2. Least Significant Difference (LSD) technique was applied to compare differences among the three treatment groups. Data were considered to be significantly different at $p < 0.05$. Trends are reported as $p < 0.1$. Data are expressed as means ° æ SEM.

RESULTS

1. Nutrient intake and weight gain

There were no differences in initial or final body weights or daily weight gain among the treatment groups (Table 1).

2. Jejunal weight and length, mucosal weight and protein content, and histomorphology

Jejunal weight and length per kg BW were not affected by treatment, however, mucosal weight per kg BW was increased ~30% with provision of 20% partial enteral nutrition compared to piglets received 100% TPN alone (Table 2). Mucosal protein content was significantly lower in 100% parenterally-fed piglets compared to the piglets fed 20% partial enteral nutrition. There was no additional effect of enteral IGF-I on either mucosal weight or mucosal protein content above that observed with 20%

Table 1. Body weight gain and nutrient intake

	100% TPN	80 TPN/20 PEN	80 TPN/20 PEN + 1 mg/kg IGF-I
Initial body weight (kg)	1.41 ± 0.06	1.50 ± 0.05	1.56 ± 0.03
Final body weight (kg)	2.19 ± 0.06	2.26 ± 0.07	2.28 ± 0.03
Weight gain (g/kg/d)	75 ± 2.4	84 ± 3.0	79 ± 2.5
Energy intake (kcal/kg/d)			
Parenteral	194 ± 1.56	142 ± 0.33	153 ± 1.5
Enteral	0	44.9 ± 0.23	45.2 ± 0.23

Mean ± SEM
Different letter superscripts indicate a significant difference among groups at $p < 0.05$

Table 2. Jejunal weight and length, mucosal weight and protein content and histomorphology

	100% TPN	80 TPN/20 PEN	80 TPN/20 PEN + 1 mg/kg IGF-I
Length (cm/kg)	97.3 ± 5.1	102.2 ± 3.1	103.1 ± 2.4
Weight (g/kg)	12.7 ± 0.2	14.5 ± 0.4	15.5 ± 0.5
Mucosal weight (g/kg)	1.6 ± 0.02[a]	2.1 ± 0.3[b]	2.6 ± 0.2[b]
Mucosal protein (mg/g mucosa)	96.3 ± 1.1[a]	108.8 ± 0.9[b]	108.8 ± 1.3[b]
Villus height (μm)	720.9° ± 79.2	906.3 ± 58.8	862.1 ± 68.0
Villus width (μm)	45.7 ± 1.6[a]	78.4 ± 5.1[b]	81. ± 3.9[b]
Villus cross-sectional area (mm^2)	32.5 ± 3.4[a]	72.3 ± 8.7[b]	71.0 ± 8.8[b]
Crypt depth (μm)	86.0 ± 10.5	81.4 ± 4.2	87.6 ± 5.3

Mean ± SEM
Different letter superscripts indicate a significant difference among groups at $p < 0.05$

PEN alone (Table 2). Villus height and crypt depth were similar among the groups. Villus width and cross-sectional area were increased by 20% PEN compared to 100% TPN, with no further effect of IGF-I (Table 2).

3. LPH enzymatic activity

Jejunal LPH activity is shown in Table 3. LPH specific activity (μmol glc/min/g prot) was significantly greater in piglets receiving 20% PEN + IGF-I than piglets receiving 100% TPN or 20% PEN alone. Total mucosal LPH activity was significantly greater in piglets receiving 20% PEN compared to 100% TPN.(Table 3). Supplementation of 20% PEN with IGF-I further increased total mucosal LPH activity. In addition, LPH activity relative to BB LPH abundance in IGF treated piglets was twice that of the other two groups.

4. Relative abundance of LPH precursor forms

A representative SDS-PAGE separation of LPH polypeptides immunoisolated from the mucosa is shown in Fig. 1. Five distinct bands (BB LPH, 160 kDa; proLPHi, 180 kDa; proLPHh, 200 kDa; proLPHc, 220 kDa; dimer of BB LPH, ~240 kDa) were observed, which were identical to those described by Dudley *et al.*[7] The first synthesized precursor is the high mannose form, proLPHh, and then the complex glycosylated precursor proLPHc to proLPHi. Processing from the intermediate form proLPHi into mature BB LPH is rather fast, hence, proLPHi band was not easily detectable. The relative abundance of the individual LPH polypeptides (the proportional percentage density of the bands as proportion of the sum of all density of bands) are shown in Table 4. In all treatment groups, the 160 kDa mature LPH band constituted the largest proportion of the immunoprecipitated lactase isoforms. 100% parenterally fed piglets had a significantly lower abundance of proLPHh and higher abundance of BB LPH than piglets provided 20% partial nutrition. There were no significant differences in the relative abundance of LPH precursors and mature BB LPH between 20% PEN alone or with IGF-I.

Table 3. Jejunal LPH activity and steady state mRNA expression

	100% TPN	80 TPN/20 PEN	80 TPN/20 PEN +1 mg/kg IGF-I
Specific activity (μmol glc/min/g prot)	248.3 ± 14.8[a]	183.3 ± 19.2[a]	312.4 ± 16.5[b]
Total mucosal activity (μmol glc/min)° □	301.7 ± 45.7[a]	639.6 ± 90[b]	995.6 ± 118[c]
LPH activity per unit BB LPH*	27.4 ± 3.4[a]	22.3 ± 4.2[a]	44.7 ± 2.6[b]
LPH mRNA	2.0 ± 0.25[a]	3.0 ± 0.4[b]	3.1 ± 0.9[b]

Mean ± SEM

Different letter superscripts indicate a significant difference among groups at $p < 0.05$

° □ : Activity per g mucosa * mucosal weight

*: LPH specific activity densitometric value for immunoprecipitated BB LPH from SDS-PAGE (see Table 4.4).

5. Isotopic enrichment of leucine in plasma and mucosal free amino acid pools, mucosal bound protein and precursor forms

The tracer- to-tracee ratios of both [$^2H_3$]-leucine and [$^{13}C_1$]-L-leucine in plasma reached equilibrium within 2 hours of infusion in all the treatment groups (Fig. 2). The mean tracer-to-tracee ratio of [$^2H_3$]-leucine in the plasma free pool appeared lower in the 20% PEN group, however the data were not statistically different (Table 5). This likely did not affect the availability of [$^2H_3$]-leucine for protein synthesis as the tracer-to-tracee ratios of [$^2H_3$]-leucine in the mucosal free amino acid pool and in proLPHc and proLPHh at steady state were not significantly dif-

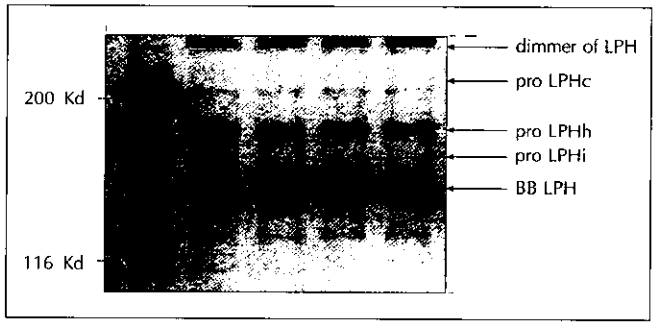

Fig. 1. Representative SDS-PAGE separation of LPH polypeptides. Immunoisolated mucosal samples were separated by SDS-PAGE. Five identifiable bands (BB LPH, 160 kDa; proLPHi, 180 kDa; proLPHh, 200 kDa; proLPHc, 220 kDa; dimer of BB LPH, ~240 kDa) were visualized after Commassie blue-stained gel.

Table 4. Abundance of LPH polypeptides in jejunal mucosa

	100% TPN	80 TPN/20 PEN	80 TPN/20 PEN +1 mg/kg IGF-I
Density of bands:			
proLPHh	16.1 ± 2.3	17.8 ± 1.1	17.0 ± 0.7
proLPHc	13.0 ± 1.1	10.9 ± 0.8	8.5 ± 1.0
proLPHi	6.1 ± 0.08	5.9 ± 0.1	3.5 ± 0.2
BB LPH*	176 ± 29	171 ± 19	148 ± 22
Relative Abundance° □ :			
proLPHh	6.9 ± 0.01[a]	8.7 ± 0.00[b]	9.5 ± 0.01[b]
proLPHc	6.2 ± 0.01	7.2 ± 0.00	6.7 ± 0.01
proLPHi	2.3 ± 0.00	2.9 ± 0.00	1.9 ± 0.00
BB LPH*	86.2 ± 0.01[a]	83.1 ± 0.00[b]	83.7 ± 0.00[b]

Mean ± SEM

Different letter superscripts indicate a significant difference among groups at $p < 0.05$.

° □ : Data are expressed as a % of the total LPH polypeptides present in the mucosa.

*: Sum of BB LPH and dimer LPH

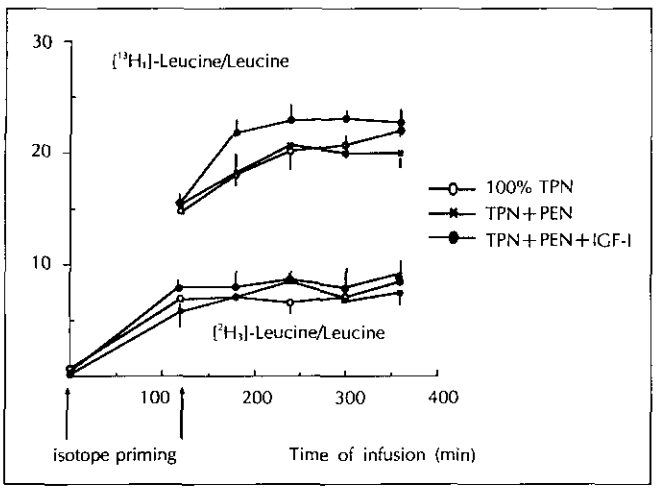

Fig. 2. Tracer-to-tracee ratio of leucine in the plasma pool over time. Plasma free leucine pools of both isotopes ([$^2H_3$]-leucine) and [$^{13}C_1$]-l-Leucine) reached steady state within 2 hours of infusion in all treatment groups.

ferent among the treatment groups (Table 5). Likewise, the tracer-to tracee ratio of the mucosal free leucine relative to the plasma tracer-to tracee ratio was similar in all groups, suggesting that uptake of labeled leucine from the plasma into the mucosa was similar in all treatment groups. The tracer-to-tracee ratios of labeled leucine in the individual precursor LPH forms were also similar among the treatment groups (Table 5). However, when the tracer-to-tracee ratio of proLPHc when expressed as a percentage of proLPHh, parenterally fed animals had significantly higher (92.2 ± 0.1%) values than those provided 20% PEN (76.1 ± 0.1% and 70.9 ± 0.3%), suggesting that the initial processing rate from proLPHh to proLPHc is higher in the 100% parenterally-fed piglets.

6. Fractional (Ks), absolute and total synthesis rates of mucosal protein

The fractional and absolute synthesis rates (represents the amount of protein synthesized per gram of mucosal tissue) were unaffected by treatment (Table 6). However the total protein synthesis rate (protein synthesized in the jejunal section) was nearly 50% greater in animals receiving 20% PEN + IGF-I compared to the other two treatment groups.

7. Fractional (Ks), absolute and total synthesis rates of BB LPH

The fractional synthesis rate of BB LPH was similar in all three treatment groups (Table 6). The LPH absolute synthesis, calculated as the product of the abundance of LPH immunoprecipitated from the mucosa and the fractional synthesis rate of LPH, was also not affected by provision of 20% enteral nutrition or 1.0 mg/kg enteral IGF-I. The total BB LPH synthesis rate was nearly 2-fold greater in piglets receiving 20% PEN + IGF-I compared to 20% PEN alone, however, data were not significantly *different due to animal variation* (p = 0.14).

Table 5. Tracer-to-tracee ratio of [$^2H_3$]-leucine in plasma and mucosal free amino acid pools, mucosal protein and LPH

	100% TPN	80 TPN/20 PEN	80 TPN/20 PEN +1 mg IGF-I
Plasma free	8.30 ± 0.8	4.99 ± 1.1	6.06 ± 0.9
Gut free	1.31 ± 0.2	1.15 ± 0.1	1.16 ± 0.1
Mucosal protein	0.44 ± 0.01	0.30 ± 0.02	0.35 ± 0.05
LPH polypeptides° □			
Pro LPHh	1.42 ± 0.13	1.47 ± 0.04	1.86 ± 0.28
Pro LPHc	1.31 ± 0.18	1.13 ± 0.11	1.32 ± 0.37
BB LPH	0.43 ± 0.02	0.33 ± 0.02	0.48 ± 0.06

Mean ± SEM
Different letter superscripts indicate a significant difference among groups at $p < 0.05$
° □ : n = 3 randomly selected piglets per treatment group

Table 6. Synthesis of mucosal protein and LPH in the jejunum

	100% TPN	80 TPN/20 PEN	80 TPN/20 PEN +1 mg/kg IGF-I
Mucosal Protein			
Ks/day	1.4 ± 0.1	1.2 ± 0.2	1.2 ± 0.1
Absolute synthesis° □	134.7 ± 9.0	120.4 ± 14.3	134.7 ± 13.2
Total synthesis*	501.5 ± 32.7[a]	534.7 ± 39.5[a]	816.9 ± 81.1[b]
LPH			
Ks/day	1.31 ± 0.21	1.02 ± 0.06	1.18 ± 0.18
Absolute synthesis	35.4 ± 5.7	23.1 ± 4.2	53.7 ± 11.8
Total synthesis	137.66 ± 27.1	119.1 ± 24.1	329.7 ± 73.2

Mean ± SEM
Different letter superscripts indicate a significant difference among groups at $p < 0.05$
° □ : Absolute synthesis rate (Ks * mucosal protein content)
*: Total synthesis rate (Absolute synthesis rate * mucosal weight), p = 0.14

DISCUSSION

Many studies, particularly those focusing on developmental changes, have shown that LPH activity is regulated primarily at the level of transcription (Van Beers *et al.* [8]). Indeed, our laboratory has previously shown that IGF-I up-regulated both LPH activity and mRNA expression in suckling pigs (Houle *et al.*[13]). In contrast, data from the current study and that of Dudley *et al.*[10] suggest that LPH is regulated post-transcriptionally in parenterally-fed piglets. Dudley *et al.*[10] studied neonatal piglets which were provided with the same diet either enterally or parenterally. LPH activity was significantly reduced in piglets

fed parenterally, however, LPH mRNA abundance was similar in both groups.

Based on these observations, the focus of the current study was to determine the effect of enteral IGF-I on mucosal protein synthesis, using two stables isotopes of leucine, and LPH synthesis and processing *in vivo*. LPH polypeptide synthesis and processing were assessed by immunoprecipitating precursor and mature forms of LPH from the jejunal mucosa, separating them by SDS-PAGE, and determining the isotopic enrichment of each protein. LPH within the mucosa is present in four distinct forms: three precursors, proLPHh, proLPHc and proLPHi, and mature BB LPH (Dudley *et al.*[7]). LPH is first synthesized within the rough endoplasmic reticulum as proLPHh, a high mannose precursor form, which is then transported to the Golgi apparatus where it is converted to a complex glycosylated form, proLPHc. ProLPHc then undergoes enzymatic cleavage prior to insertion into the brush border membrane. During this processing, proLPHi is formed as an intermediate precursor between proLPHc and BB LPH (Van Beers *et al.*[5]).

Work in our laboratory by Houle *et al.*[14] as well as others (Burrin *et al.*[15]; Dudley *et al.*[9]) have demonstrated that the relative proportions of the LPH polypeptides can be influenced by diet. Burrin *et al.*[15] compared LPH activity and processing in piglets fed colostrum vs. water for 6 h postpartum. Colostrum increased the total jejunal lactase activity (Burrin *et al.*[15]), however, piglets fed colostrum had lower abundance of mature LPH (160 kDa) and higher abundance of proLPHi (180 kDa, immediate precursor of the final isoform) compared to those of who were fed water (Burrin *et al.*[15]). The authors speculated that macromolecular uptake of colostral IgG into the enterocyte may have provided a physical barrier to the final post-translational processing of LPH from the 180 kDa (proLPHi) to 160 kDa (BB LPH) proteins. Using the same analytical techniques in a different animal model, Dudley *et al.*[9] compared LPH processing in piglets fed protein-deficient vs. protein adequate diets. The total abundance of proLPHh was reduced ~22% (9.7 $\pm$ 1.9 vs. 7.5 $\pm$ 0.6) and the LPH synthesis rate was reduced by 34% in the protein-malnourished piglets compared to protein adequate piglets. Lastly, Houle *et al.*[13] in our laboratory demonstrated that enteral IGF-I increased the relative abundance of proLPHh and decreased the relative abundance of mature BB LPH, suggesting that IGF-I was affecting the post-transcriptional processing of LPH. LPH activity was greater in piglets receiving enteral IGF-I even though the relative abundance of mature BB LPH was lower (Houle *et al.*[13]). These observations led us to hypothesize that enterally administered IGF-I would alter some aspect of LPH precursor post-translational processing in parenterally-fed piglets.

In the current study, the relative proportion of mature BB LPH was significantly higher in the piglets fed 100% TPN vs. those receiving 20% partial enteral nutrition. As the data are expressed as a relative proportion of total LPH polypeptides, there was a concomitant decrease in the relative abundance of 200 kDa proLPHh precursor in 100% TPN piglets compared to the 20% PEN piglets. In contrast, to the observations of Houle *et al.*,[13] no effect of IGF-I supplementation on the relative LPH polypeptide abundance was observed. It is important to note, that the estimates of each polypeptide abundance obtained by immunoisolation are only a relative qualitative measurements of the post-translational events at the cellular level (Dudley *et al.*[11]), Consequently, it is difficult to draw conclusions from the relative abundance of all the precursors, however, we found that the ratio of proLPHh to BB LPH was greater in 100% TPN piglets than in the PEN piglets (12.5 vs. 9.2). This relative accumulation of proLPHh within the enterocyte suggests the processing of proLPHh to the mature LPH was affected in the direction of slowing down the enzyme processing. We have found previously that TPN significantly decreased the enterocyte migration rate (Park *et al.*[6]), which supports this reduced turnover rate of LPH in the enterocytes of the TPN piglets.

Previous studies have shown that intestinal protein synthesis is depressed in parenterally vs. enterally-fed piglets (Dudley *et al.*[10]) and rats (Lo & Ney[16]). In the current study, we did not include an enterally-fed control group, therefore, we can only compare the effects of enteral nutrition and IGF-I relative to 100% TPN. Protein synthesis data were expressed three ways. First, we determined the fractional synthesis rate, which reflects the rate of protein synthesis per unit time. There were no differences among the treatment groups in the FSR for either mucosal protein or BB LPH, suggesting that the rate of protein synthesis was similar in all three groups. Since protein accretion was greater in the piglets receiving 20% PEN, it is possible that the rate of protein degradation or turnover was lower in piglets receiving PEN. In addition, there were no differences among the treatment groups in the absolute mucosal protein synthesis rate, which is the product of the fractional synthesis rate and mucosal protein abundance, respectively. It is possible that we did not provide enough enteral nutrition (~55 mL/kg/d) to observe

differences in absolute synthesis rates, since differences in absolute mucosal protein synthesis have been shown between 100% enterally- vs. 100% -parenterally-fed piglets (Dudley *et al.*[11]). Lastly, no effect of 20% PEN alone on total mucosal protein synthesis, the product of the absolute synthesis and mucosal weight, was observed; however, total mucosal protein synthesis was significantly greater in piglets receiving 20% PEN + IGF-I. This IGF effect is likely partially attributable to the greater mucosal weight in the IGF-I treated piglets. It is also possible that there was an interaction between the presence of PEN and IGF-I within the intestine, however, we can not prove that since we did not have a group of piglets which received only enteral IGF. Intravenously administered IGF-I has also been shown to increase mucosal protein synthesis in rats on TPN (Lo & Ney[17]).

IGF-I supplemented to 20% PEN increased both LPH specific activity and total mucosal LPH activity. In addition, there was a numerical increase in both the absolute (N.S.) and total synthesis ($p = 0.14$) of BB LPH in IGF-I treated piglets, although neither reached statistical significance due to inter-animal variation. It is possible that statistical significance would be achieved with a greater number of animals.

These data also support the possibility that stimulation of LPH synthesis may not be the primary mechanism by which IGF-I up-regulates LPH. We would like to propose two possible explanations. Firstly, that IGF-I is in someway altering the hydrolytic capacity of the enzyme. This is supported by the observation that LPH enzymatic activity normalized by BB LPH is nearly twice that of piglets receiving 20% PEN alone. LPH contains about 10% *N*-glycosylation (Van Beers *et al.*[5]) and a combination form of *N*- and *O*-glycosylation has been observed for most animal species (Bueller *et al.*[18][19]). In human intestine, both the *N*-,*O*-glycosylated and *N*-glycosylated mature LPH was isolated from the same tissue samples suggesting that LPH is heterogeneous in respect to glycosylation (Naim & Lentze[20]). There were no differences in the binding affinity towards lactose (14 mM) of the two isoforms, but, the presence of *O*-glycans on the mature enzyme increased V_{max} four-fold over the enzyme that contained no *O*-glycans. Therefore we postulate that oral IGF-I may alter the glycosylation pattern of LPH resulting in a greater proportion of O-glycosylation and thus, increasing the V_{max} of the enzyme. Secondly, it is possible that IGF-I affects LPH turn-over by inhibiting LPH degradation. Tsuboi *et al.*[21] showed that the maturational decline in LPH activity in rats was associated with increased turnover of the enzyme, based on the disappearance rate of labeled leucine from pre-labeled LPH pools, while the rate of LPH synthesis was unaffected. In addition, in a recent study by Fang *et al.*,[22] IGF-I inhibited protein breakdown in a dose-dependent fashion in muscles from burned rats. Ubiquitin mRNA (protein that signals degradation) was also reduced with coincubation of IGF-I in those muscle tissue.

In summary, we have demonstrated that enteral IGF-I administration combined with 20% PEN increases mucosal weight and total mucosal protein synthesis. In addition, enteral IGF-I increases LPH activity, however, we were unable to attribute this effect to either an increase in steady state LPH mRNA expression or LPH synthesis. Thus, based on our findings, it appears to be that IGF-I may regulate LPH activity by a combination of post-translational events which remain to be determined.

Literature cited

1) Duersken DR, Van Aerde JE, Chan G, Thomson ABR, Jewell LJ, Clandinin MT. Total parenteral nutrition impairs bile flow and later bile composition in newborn piglet. *Dig Dis Sci* 41: 1846-1870, 1996
2) Park YK, Monaco MH, Donovan SM. Delivery of total parenteral nutrition (TPN) via umbilical catheterization: Development of a piglet model to investigate therapies to improve gastrointestinal structure and enzyme activity during TPN. *Biol Neonate* 73: 295-305, 1998
3) Gall DG, Chung M, O'Loughlin EV, Zahavi I, Opleta K. Effects of parenteral and enteral nutrition on postnatal development of the small intestine and pancreas in the rabbit. *Biol Neonate* 51: 286-296, 1987
4) Wykes LJ, Ball RO, Pencharz PB. Development and validation of a total parenteral nutrition model in the neonatal piglets. *J Nutr* 123: 1248-1259, 1993
5) Van Beers EH, Buller HA, Grand RJ, Einerhand WC, Dekker J. Intestinal brush border glycohydrolases: Structure, function, and development. *Critical Reviews in Biochemistry and Molecular Biology* 30: 197-262, 1995
6) Park YK, Monaco MH, Donovan SM. Orally administered insulin-like growth factor-I stimulates intestinal disaccharidase acitivity in piglets on total parenteral nutrition. *J Pediatr Gastroenterol Nutr* 29: 198-206, 1999
7) Dudley MA, Nichols BL, Rosenberger J, Perkinson S, Reeds PJ. Feeding status affects *in vivo* prosucrase-isomaltase processing in rat jejunum. *J Nutr* 122: 528-534, 1992
8) Dudley MA, Burrin DG, Quaroni A, Rosenberger J, Cook G, Nichols BL, Reeds PJ. Lactase phlorizin hydrolase turnover *in vivo* in water-fed and colostrum-fed newborn pigs. *Biochem J* 320: 735-743, 1996
9) Dudley MA, WykesLJ, Dudley AW Jr, Fiorotto M, Burrin DG, Rosenberger J, Jahoor Farook, Reeds PJ. *J Nutr* 127: 687-693, 1997
10) Dudley MA, Burrin DG, Wykes LJ, Toffolo G, Cobelli C, Nichols BL,

Rosenberger J, Jahoor F, Reeds PJ. Protein kinetics determined *in vivo* with a multiple-tracer single-sample protocol: application to lactase synthesis. *Am J Physiol* 274: G591-G598, 1998a

11) Dudley MA, Jahoor F, Burrin DG, Reeds PJ. Brush-border disaccharidase synthesis in infant pigs measured *in vivo* with [$^2H_3$] leucine. *Am J Physiol* 267: G1128-G1134, 1994

12) Dudley MA, Wykes LJ, Dudley AJ Jr, Burrin DG, Nichols BL, Rosenberger J, Jahoor F, Heird WC, Reeds PJ. Parenteral nutrition selectively decreases protein synthesis in the small intestine. *Am J Physiol* 274: G131-G137, 1998b

13) Houle VM, Dudley MA, Donovan SM. Oral insulin-like growth factor-I upregulates intestinal lactase phlorizin hydrolase activity and messenger ribonucleic acid in piglets.

14) Houle VM, Park YK, Laswell SC, Freund GG, Dudley MM, Donovan SM. 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. *Pediatr Res* 48(4): 497-503, 2000

15) Burrin DG, Dudley MA, Reeds PJ, Shulman RJ, Perkinson S, Rosenberger J. Feeding colostrum rapidly alters enzymatic activity and the relative isoform abundance of jejunal lactase in neonatal pigs. *J Nutr* 124: 2350-2354, 1994b

16) Naim HY, Lentze MJ. Impact of O-glycosylation on the function of human intestinal lactase-phlorizin hydrolase: Characterization of glycoforms varying in enzyme activity and localization of O-glycoside addition. *J Biol Chem* 267(35): 25494-25504, 1992

17) Lo H-C, Ney DM. GH and IGF-I differentially increase protein synthesis in skeletal muscle and jejunum of parenterally fed rats. *Am J Physiol* 271: E872-878, 1995

18) Bueller HA, Rings EHHM, Montgomery RK, Sasak WV, Grand RJ. Further studies of glycosylation and intracellular transport of lactase phlorizin hydrolase in rat small intestine. *Biochem J* 263: 249-254, 1989a

19) Bueller HA, Rings EHHM, Montgomery RK, Sybicki MA, Grand RJ. Suckling rat colon synthesizes and processes active lactase phlorizin hydrolase immunologically identical to that from jejunum. *Ped Res* 26: 232-236, 1989b

20) Burrin DG, Shulman RJ, Langston C, Storm MC. Supplemental alanylglutamine, organ growth and nitrogen metabolism in neonatal piglets fed by total parenteral nutrition. *J Parent Ent Nutr* 18: 313-319, 1994a

21) Tsuboi KK, Kwong LK, Sunshine P, Castillo RO. Mechansim of maturational decline of rat intestinal lactase-phlorizin hydrolase. *Biochem J* 282: 107-113, 1992

22) Fang CH, Li BG, Wang JJ, Fischer JE, Hasselgren PO. Insulin-like growth factor 1 stimulates protein synthesis and inhibits protein breakdown in muscle from burned rats. *JPEN J Parenter Enteral Nutr* 21(5): 245-51, 1997