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Nutrient-Independent and Nutrient-Dependent Factors Stimulate Protein Synthesis in Colostrum-Fed Newborn Pigs

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ABSTRACT

We hypothesized that nonnutrient components, including growth factors, present in colostrum contribute to the stimulation of protein synthesis in colostrum-fed neonatal pigs. We studied neonatal pigs fed mature milk, colostrum, or a formula containing a macronutrient composition comparable to that of colostrum for 24 h. We measured the circulating concentrations of insulin, insulin-like growth factor I, glucose, and amino acids at intervals throughout the 24-h period, after which we measured *in vivo* protein synthesis using a flooding dose of [^{3}H]phenylalanine. The rates of protein synthesis in several tissues measured after 24 h of feeding were greater than those we reported previously after 6 h of feeding. The acute (within 6 h) stimulation of protein synthesis in visceral and skeletal muscle tissues of neonatal pigs fed milk, colostrum, or formula was primarily influenced by nutrient intake and associated with rapid secretion of insulin. Indirect evidence suggests that intestinal absorption of ingested colostral insulin was minimal. However, the sustained increase in tissue protein synthesis between 6 and 24 h coincided with an increase in circulating insulin-like growth factor I. We found a novel, specific stimulation of skeletal muscle and jejunal protein synthesis in colostrum-fed pigs that can be attributed to some nonnutrient component of colostrum. (***Pediatr Res* 37: 593–599, 1995**)

Abbreviations

BW, body weight
IGF-I, insulin-like growth factor I
PCA, perchloric acid
ANOVA, anaylsis of variance

The relative rates of tissue and organ growth during the early neonatal period are more rapid than at any stage of postnatal development (1–3). Studies in which newborn animals have been fed various combinations of formula, mature milk, or colostrum suggest that some component of colostrum stimulates tissue growth, particularly the gastrointestinal tract and liver (4, 5). In addition, research with various mammalian species has identified a number of peptide growth factors, including insulin, IGF-I, and epidermal growth factor, that are present in higher concentrations in colostrum than in mature milk or formulas (6, 7). Given the anabolic nature of these growth factors, the prevailing hypothesis has been that their ingestion in colostrum enhances protein synthesis, thereby resulting in increased protein accretion and tissue growth, especially of the gastrointestinal tissues. However, few studies have demonstrated a specific effect of colostrum on protein synthesis in either gastrointestinal or peripheral tissues in the neonate.

In support of this hypothesis, our previous acute study with newborn pigs (8) demonstrated that after only 6 h of feeding, the rates of protein synthesis in the intestines, liver and extrasplanchnic tissues were significantly greater in pigs fed either mature milk or colostrum than in those receiving water. Furthermore, skeletal muscle protein synthesis in colostrum-fed pigs was 50% higher than in pigs fed mature milk. Given the relatively large increase in protein synthesis after only 6 h of colostrum feeding, we questioned whether the acute protein anabolic response could be sustained beyond the immediate perinatal period. Although our findings were consistent with an anabolic stimulus attributable to colostral growth factors, the concentrations of protein, energy and several other nutrients also are significantly higher in colostrum than in mature milk. Thus, it was conceivable that the enhanced protein anabolic response we observed in colostrum- *versus* milk-fed pigs could be attributed to differences in nutrient content of these two diets.

Insulin and IGF-I are two of the most well-characterized growth factors present in mammary secretions and are sever-

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alfold more concentrated in porcine colostrum than in mature milk (6, 7). Insulin (9) and IGF-I (10, 11) both have been shown to stimulate protein synthesis in a number of tissues, most notably in the skeletal muscle of young animals. A number of studies also have demonstrated that both insulin (12, 13) and IGF-I (14, 15), administered either orally or parenterally to neonatal animals, can stimulate gastrointestinal tissue growth and maturation. Furthermore, orally administered insulin (16) and IGF-I (17) appears to be absorbed intact from the neonatal intestine and thus could potentially affect peripheral tissues. Therefore, we hypothesized that insulin and IGF-I may mediate the rapid stimulation of protein synthesis in both colostrum- and milk-fed newborn pigs.

In this report we present findings from two studies. In study 1, our objective was to determine whether the tissue protein synthesis response we observed in neonatal pigs fed either mature milk or colostrum for only 6 h was sustainable for 24 h. In study 2, we determined the relative significance of colostral nutrients and nonnutrient components, such as growth factors, on the tissue protein synthesis response. In the latter study, we measured the tissue protein synthesis response in neonatal pigs fed colostrum, mature milk or a fortified formula composed of purified ingredients with a nutrient composition similar to that of colostrum but essentially devoid of growth factors.

METHODS

Animals and design. Six litters of conventional crossbred pigs (Texas A&M University, College Station, TX) were obtained immediately after birth (before suckling), weighed, and randomly assigned to receive their respective dietary treatment. Study 1 involved a total of 12 pigs taken from three litters and fed either porcine colostrum or mature milk for 24 h (six pigs/treatment group). Study 2 involved a total of 24 pigs taken from three litters and fed porcine colostrum, mature milk, or formula; a fourth treatment group was studied 2–3 h after birth, having never been fed. Before initiating the feeding protocol, the umbilical artery of each pig was catheterized with polyvinyl chloride catheters (Sherwood Medical, St. Louis, MO) under general isoflurane anesthesia (Aerrane, Anaquest, Madison, WI). The pigs were allowed to recover approximately 1 h before beginning the feeding protocol. Animals were housed in separate cages with a dry towel for bedding; ambient temperature was maintained at approximately 28–29°C. The protocol was approved by the Animal Care and Use Committee of Baylor College of Medicine and was conducted in accordance with the National Research Council's *Guide for the Care and Use of Laboratory Animals.*

Feeding and blood sampling protocol. The colostrum and mature milk were pooled samples collected from conventional sows; colostrum was obtained within the first 24 h and mature milk in the third week postpartum. The formula was composed of the following semipurified ingredients mixed in proportions necessary to equal the nutrient content of colostrum; (g/L) casein, 51.36; lactalbumin, 53.93; albumin, 23.11; lactose, 35.00; corn oil, 35.00; coconut oil, 35.00; mineral mix, 20.00; vitamin mix, 5.00 (U. S. Biochemical Corp., Cleveland, OH). The pigs were weighed, then bottle-fed (20 g/kg BW) hourly for 24 h an exclusive diet of mature milk, colostrum, or formula. Intakes were determined by weighing the bottles before and after each feeding. Aliquots of the colostrum, mature milk, and formula were frozen at −70°C for later analysis. In both study 1 and 2, arterial blood samples (2.0 mL) were collected from all pigs in each of the dietary treatment groups before feeding (fasted, time 0) and 2, 4, 12, and 24 h after initiating the feeding protocol. In study 1, at 2 and 4 h after initiating feeding, blood samples were obtained from only three animals within a treatment group.

Measurements of in vivo protein synthesis. Animals were infused slowly over 2 min with a flooding dose of L-[4-^{3}H] phenylalanine (37 MBq · kg BW^{-1}) in a 150 mM phenylalanine solution at a dose of 10 mL · kg BW^{-1} via the umbilical arterial catheter. At 5, 15, and 30 min after the midpoint of the infusion, arterial blood samples were collected in heparinized tubes and placed on ice for measurement of blood phenylalanine-specific radioactivity. Immediately after withdrawing the 30-min blood sample, animals were anesthetized with an i.v. dose of pentobarbital (50 mg/kg BW) and exsanguinated by withdrawing approximately 30 mL of blood. The abdomen was opened and flushed with ice-cold saline, and the small intestine, from the pylorus to the ileocecal junction, was removed, free of mesenteric tissue, and placed in ice-cold saline. The small intestine was flushed with cold saline. The duodenum was defined as the segment from the pylorus to the ligament of Trietz. The remaining small intestine, from the ligament of Trietz to the ileocecal junction, was divided in half, and the proximal and distal halves designated as jejunum and ileum, respectively. The stomach was removed and flushed of digesta with cold saline. The stomach and intestinal segments were weighed and frozen in liquid nitrogen. After the small intestine was removed, the liver, pancreas, stomach, and samples of the gastrocnemius and longissimus dorsi skeletal muscle were quickly removed and weighed, and a subsample of each was frozen in liquid nitrogen.

Blood and tissue analysis. Blood samples were centrifuged at 3000 × *g* for 20 min at 4°C, and plasma was collected. In plasma and diet samples, immunoreactive insulin and C-peptide were measured using a radioimmunoassay (Linco Research, St. Louis, MO). The human-specific antibody that was used in the insulin assay exhibited 100% cross-reactivity with porcine insulin and that in the C-peptide assay was porcine-specific. Inter- and intraassay coefficients of variation were 6.8 and 6.25% for insulin and 6.0 and 4.43% for C-peptide, respectively. Plasma total IGF-I was measured by radioimmunoassay after acidification (1 g/100 mL trifluoroacetic acid) and chromatography (Sep-Pack Plus C_{18}) to remove the binding proteins (18). IGF-I antiserum was obtained courtesy of Drs. L. Underwood and J. J. Van Wyk through the NIDDK and the National Hormone and Pituitary Program. The ^{125}I-IGF-I used was obtained from Amersham Corp. (Arlington Heights, IL). The inter- and intraassay coefficients of variation for the IGF-I assay were 7.59 and 8.45%, respectively.

Plasma glucose was measured enzymatically using hexokinase coupled with glucose-6-phosphate dehydrogenase (Roche, Nutley, NJ). Free amino acid concentrations were

determined in whole blood samples. An aliquot of whole blood was thawed and immediately deproteinized with 2 volumes 0.1 mol/L HCl containing 0.4 mmol/L methionine sulfone, which was used as an internal standard. The samples were combined with the acid-internal standard mixture, vortexed and placed on ice for 20 min. The samples were then transferred to an Ultrafree (Millipore, Milford, MA) sample filter (5,000 M_r exclusion) and centrifuged at 10,000 × g for 60 min at 4°C. The resulting deproteinized supernatant was immediately derivatized with phenylisothiocyanate in preparation for amino acid analysis using reverse-phase chromatography (Picotag, Millipore Corp., Milford, MA).

The specific radioactivity of [^{3}H]phenylalanine was determined in whole blood samples obtained 5, 15, and 30 min after infusion of the [^{3}H]phenylalanine and in tissue samples. The tissue samples were homogenized in 0.2 mol/L PCA as described previously (19). The PCA-soluble homogenate supernatants containing the tissue free amino acid pools were separated from the PCA-insoluble precipitates and neutralized. The PCA-insoluble precipitates were washed and solubilized. An aliquot of the solubilized pellet was assayed for protein as described by Lowry (20). The protein pellet was washed and hydrolyzed with 6 mol/L HCl. The protein hydrolysate, homogenate supernatant, and blood supernatant were vacuum-dried (Jouan Inc., Winchester, VA), washed three times with water, and resuspended in water for determination of phenylalanine specific activity. Phenylalanine was separated from the other amino acids using anion exchange chromatography. The radioactivity associated with the collected phenylalanine fraction was measured using liquid scintillation counting (LS5000TD, Beckman Instruments, Fullerton, CA).

Diet analysis. Aliquots of mature milk, colostrum and formula were analyzed for total nitrogen by Kjeldahl and total energy by bomb calorimetry. Measurements were made in triplicate.

Calculations. The rate of jejunal and ileal protein synthesis was estimated from the absolute rate of phenylalanine incorporation into PCA-insoluble tissue fractions as described previously, to avoid the confounding effect of endocytosis of colostral immunoglobulins (8). The rate of phenylalanine incorporation into tissue protein can be determined using the following equation:

$$dC/dt = V_s \cdot S_a - V_d \cdot S_b$$

where C is the radioactivity of phenylalanine in the PCA-insoluble or protein-bound pool (Bq), t is time of labeling in h, V_s is the rate of phenylalanine incorporation (μmol/h), S_a is the specific activity of the PCA-soluble or tissue free phenylalanine pool (Bq/μmol), V_d is the rate of phenylalanine appearance resulting from protein degradation (μmol/h), and S_b is the specific activity of the PCA-insoluble or protein-bound phenylalanine pool (Bq/μmol). This equation can be further simplified to the following:

$$V_s = C/(S_a \cdot t)$$

assuming that the term $V_d \cdot S_b$ becomes negligible. This assumption was made because the observed value for S_a was approximately 100-fold greater than the value for S_b. In addition, the value for S_b is likely to be confounded from dilution by phenylalanine in ingested proteins. The value used for S_a was corrected to represent the average tissue phenylalanine specific activity at the midpoint ($t_{1/2}$) of the 30-min labeling period. The corrected S_a for each pig was calculated by adding individual tissue S_a (Bq/μmol) after time (t) and the rate of change in blood S_a (Bq · μmol^{-1} · min^{-1}) estimated from the regression of 5-, 15-, and 30-min blood samples of all pigs within a treatment group as follows:

$$\text{corrected tissue } S_a = \text{tissue } S_{a(t)} + (\Delta \text{ blood } S_a \cdot t/2)$$

The absolute rate of phenylalanine incorporation calculated for the entire jejunal and ileal segments was expressed per unit of BW (μmol · h^{-1} · kg BW^{-1}). For all other tissues besides the jejunum and ileum, protein synthesis was calculated as a fractional rate (K_s, %/d) from the equation described by Garlick *et al.* (21):

$$K_s = (S_b/S_a) \times (1440/t) \times 100$$

where all parameters are as described above.

Statistics. Treatment means were analyzed by one-way ANOVA; dietary treatment was the main effect. Differences between treatments were determined by Fisher's LSD test. Analysis of plasma insulin, IGF-I, and C-peptide data were by repeated measures ANOVA with treatment and sampling time as main effects. Differences between sampling times within a treatment group were determined by t test. Results are presented as means with the pooled SEM from the one-way ANOVA. A probability value of less than 0.05 was considered significant.

RESULTS

The protein (nitrogen × 6.38) and gross energy content of the colostrum (118 g/L and 1434 kcal/L) was higher than that of mature milk (48 g/L and 980 kcal/L), but not different from that of the formula (122 g/L and 1362 kcal/L). The protein and gross energy intakes (g/kg) among all groups in both study 1 and study 2 were not significantly different (Table 1). In both studies, the protein and energy intakes of the colostrum-fed pigs were higher than in mature milk-fed pigs (Table 1). In study 2, protein and energy intakes in the colostrum- and formula-fed pigs were not significantly different. In study 1, BW gained (g/kg BW) during the 24-h feeding period in colostrum-fed pigs was greater than mature milk-fed pigs (182

Table 1. *Dietary protein and energy intakes in neonatal pigs fed mature milk, colostrum, and formula for 24 h*

Study*	Mature milk	Colostrum	Formula	Pooled SE
Protein, g/kg BW				
Study 1	21†	47‡		1
Study 2	23†	54‡	53‡	1
Energy, kcal/kg BW				
Study 1	421†	587‡		16
Study 2	460†	648‡	609‡	13

* Treatment means, $n = 6$.

†, ‡ Means within a row with different superscripts differ significantly $p < 0.01$.

± 18 *versus* 123 ± 2). Similarly, in study 2, BW gained (g/kg BW) during the 24-h feeding period in colostrum- and formula-fed pigs (173 ± 17 and 154 ± 22) was greater than mature milk-fed pigs (84 ± 14); BW gain in colostrum- and formula-fed pigs was not statistically different.

In study 1, the rates of protein synthesis in all tissues measured, except the stomach, were significantly higher in pigs fed colostrum than in those fed milk; the percentage increases ranged from 30% (pancreas) to 80% (ileum) (Table 2). In study 2, the rates of protein synthesis in all tissues measured were significantly higher in all three fed groups (*i.e.* mature milk, colostrum, and formula) than in the unfed newborn group (Table 2). Among the three fed groups, the protein synthesis rates in the jejunum and the longissimus dorsi and gastrocnemius muscles were higher in the colostrum-fed pigs than those fed either milk or formula. The liver protein synthesis rates in both colostrum- and formula-fed pigs were higher than in milk-fed pigs. There was no difference in the protein synthesis rates of the ileum, stomach, and pancreas among the three fed groups.

In study 1 and 2, the plasma glucose concentrations in all feeding groups measured 4, 12, and 24 h after the initiation of feeding were higher than in the fasted state (Table 3). During the 24-h period, the plasma glucose concentration in all feeding groups increased quadratically with most of the increase occurring in the first 4 h after feeding (data not shown).

In study 1, after 12 h of feeding, the circulating blood amino acid concentrations were increased in both the milk- and colostrum-fed groups (Table 3). After 12 h of feeding, the increase in the blood concentrations of essential and particularly branched-chain amino acids was significantly greater in colostrum-fed than in milk-fed pigs. In study 2, the blood amino acid concentrations were significantly increased in all three feeding groups (Table 3). Also in study 2, the increase in the concentrations of the essential and branched-chain amino acids with feeding was markedly higher in the formula- (5- to 6-fold) and colostrum-fed (3-fold) pigs than in the milk-fed pigs. Furthermore, the concentrations of essential and branched-chain amino acids in formula-fed pigs were higher than in colostrum-fed pigs.

In study 1, the plasma insulin concentrations in both feeding groups increased quadratically with most of the increase occurring during the first 4 h (Fig. 1). In study 2, plasma insulin concentrations in all three feeding groups also increased quadratically during the 24-h feeding period, again with the largest increase during the first 4 h of feeding (Fig. 1). In both study 1 and 2, there was a highly significant ($p < 0.001$) correlation between plasma C-peptide and insulin concentrations measured at all sampling times (C-peptide data not shown). The coefficients of determination (R^2) were 0.796 and 0.802 in study 1 and 2, respectively, indicating that approximately 80% of the variation in circulating insulin could be attributed to changes in the C-peptide concentration. In addition, in both study 1 and 2, the regression of circulating C-peptide and insulin concentrations was best described by quadratic equations.

In study 1 and study 2, the plasma concentrations of IGF-I in all treatment groups increased linearly during the 24-h feeding period (Fig. 2); the largest increases in plasma IGF-I occurred between 4 and 12 h after the initiation of feeding. In study 1, the plasma IGF-I concentration in colostrum-fed pigs tended ($p = 0.07$) to be higher than mature milk-fed pigs. In study 2, the plasma IGF-I concentration was not significantly difference among the three treatment groups.

Table 2. *Tissue protein synthesis rates in unfed newborn pigs or those fed mature milk, colostrum, or formula for 24 h*

Study*	Newborn	Mature milk	Colostrum	Formula	Pooled SE
Phenylalanine incorporation rate, $\mu mol \cdot h^{-1} \cdot kg\ body\ wt^{-1}$					
Jejunum					
Study 1		10.8[a]	15.9[b]		1.0
Study 2	4.0[a]	13.1[b]	16.1[c]	13.2[b]	0.8
Ileum					
Study 1		8.4[a]	15.2[b]		1.4
Study 2	3.9[a]	11.4[b]	12.7[b]	10.9[b]	1.0
Fractional synthesis rate, $\% \cdot d^{-1}$					
Stomach					
Study 1		46.7	48.0		3.5
Study 2	21.8[a]	57.0[b]	52.9[b]	54.9[b]	3.1
Liver					
Study 1		59.7[a]	90.4[b]		3.3
Study 2	30.0[a]	77.0[b]	93.4[c]	98.7[c]	3.2
Pancreas					
Study 1		110.2[a]	144.1[b]		10.0
Study 2	40.0[a]	142.1[b]	143.8[b]	147.2[b]	7.5
Longissimus dorsi					
Study 1		19.8[a]	31.0[b]		2.7
Study 2	8.9[a]	24.8[b]	32.7[c]	22.5[b]	1.3
Gastrocnemius					
Study 1		21.3[a]	31.9[b]		1.3
Study 2	11.4[a]	25.1[b]	35.5[c]	25.9[b]	1.4

* Treatment means, $n = 6$.

[abc] Means within a row with different superscript differ $p < 0.05$.

Table 3. *Changes in blood glucose and amino acid profiles after 12 h of feeding in neonatal pigs fed mature milk, colostrum or formula*

Study *	Fasted[1] (n = 18)	Δ Mature milk[2] (n = 6)	Δ Colostrum[2] (n = 6)	Δ Formula[2] (n = 6)	Pooled SE
Glucose, mM					
Study 1	4.0	5.0‡	5.0‡		0.7
Study 2	3.4	2.4‡	4.1‡	3.3‡	0.5
Total AA, μM					
Study 1	3180	1807‡[a]	3064‡[b]		93
Study 2	3422	1080†[a]	2709‡[bc]	3903‡[c]	290
Essential AA, μM					
Study 1	511	662‡[a]	1241‡[b]		23
Study 2	626	360†[a]	1208‡[b]	2269‡[c]	127
Nonessential AA, μM					
Study 1	2669	1145‡[a]	1823‡[b]		84
Study 2	2796	721†[a]	1502‡[ab]	1634‡[b]	197
Branched-chain AA, μM					
Study 1	237	225‡[a]	515‡[b]		20
Study 2	212	168‡[a]	559‡[b]	853‡[c]	52

* Treatment means, n = 6.

[1] Fasted values represent a pooled mean of (n = 12, study 1) or (n = 18, study 2) of the treatment groups.

[2] The Δ values for the mature milk, colostrum, and formula-fed groups represent the difference between the blood glucose and amino acid concentration measured after 12 h of feeding and the fasted baseline.

[abc] Treatment means within a row with different superscript differ $p < 0.05$ as determined by t test. The effect of feeding is indicated when the Δ values for the mature milk-, colostrum- and formula-fed groups were significantly different from zero † ($p < 0.05$), ‡ ($p < 0.01$) as determined by t test.

DISCUSSION

The most significant finding from our current studies was the demonstration of a novel and relatively specific stimulation of skeletal muscle and jejunal protein synthesis in colostrum-fed pigs that was independent of nutrient intake. The rates of protein synthesis in both the longissimus and gastrocnemius muscles and the jejunum were significantly higher in the colostrum-fed pigs than in either the mature milk- or formula-fed pigs. The additional nutrient intake in the formula group, above that provided in mature milk, increased the blood total amino acid concentration but did not affect skeletal muscle and jejunal protein synthesis. The nutrient intakes in formula- and colostrum-fed pigs were essentially equal based on the similarity in the circulating concentrations of glucose, amino acids and insulin, yet the formula-fed pigs still had lower rates of skeletal muscle and jejunal protein synthesis than colostrum-fed pigs. These results suggest that mature milk provided sufficient energy and amino acids to support the optimal protein anabolic response in skeletal muscle and jejunum. Therefore, the enhanced rate of skeletal muscle and jejunal protein synthesis observed in the colostrum-fed pigs would appear to be a consequence of either altered endocrine status or provision of some additional growth factor or nutrient that is not provided by either mature milk or formula.

Initially, we considered insulin as a primary factor responsible for the substantial protein anabolic response we observed in the colostrum-fed neonatal pigs. Indeed, the rather "acute" and substantial stimulation of tissue protein synthesis that we demonstrated previously (8) in response to feeding, *per se*, for 6 h could be explained by the rapid increase and plateau in circulating insulin concentration we observed 2–4 h after the onset of feeding. However, the evidence from study 2 suggests that insulin probably was not responsible for the differences in skeletal muscle protein synthesis among pigs fed milk, formula and colostrum, because the circulating insulin concentration in formula-fed pigs was equal to or greater than that in the colostrum-fed pigs. The results indicate that the circulating insulin concentration of approximately 140–175 pmol/L attained 2–4 h after feeding in all three treatment groups is a critical factor in the acute protein anabolic response to feeding, but is involved only permissively in the optimal skeletal muscle protein synthesis response observed in the colostrum-*versus* formula-fed pigs.

In addition to the endocrine role of insulin secretion as a stimulus in the protein anabolic response to feeding, we wished also to establish the potential significance of intestinal absorption of insulin from ingested colostrum and mature milk. Based on the high correlation between circulating insulin and C-peptide concentrations, endogenous insulin accounted for roughly 80% of variation in circulating insulin, suggesting a relatively small contribution from intestinal absorption. More importantly, however, we found similar circulating insulin concentrations in both the colostrum- and formula-fed pigs, despite the presence of barely detectable amounts of insulin (~35 pmol/L) in the formula compared with the colostrum (3844 pmol/L). Thus, factors such as proteolytic digestion in either the stomach or intestinal lumen or first-pass extraction by the liver appeared to limit the absorption of ingested milk- or colostral-borne insulin into the peripheral circulation in the neonatal pig. Despite the limited intestinal absorption of insulin, however, we found that feeding colostrum resulted in a specific stimulation of jejunal protein synthesis that was roughly 20% greater than in either mature milk- or formula-fed pigs. This latter finding could be attributed to either colostral insulin or perhaps IGF-I, because these two growth factors have been shown to promote intestinal growth when given orally (13–15).

It is possible that IGF-I also may be responsible for the specific stimulation of skeletal muscle protein synthesis in colostrum-fed pigs. The circulating concentration and tissue

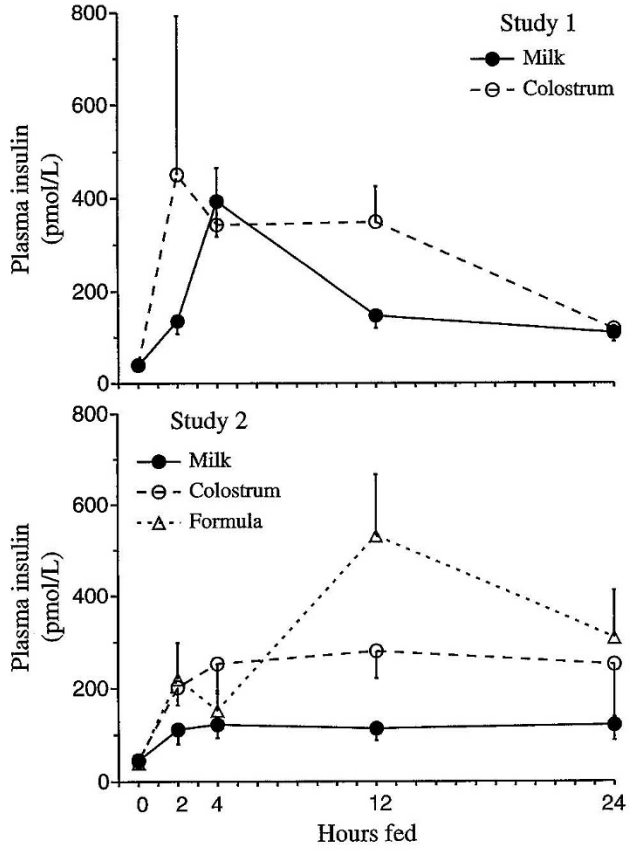

Figure 1. Mean (± SEM) plasma insulin concentrations (nmol/L) in neonatal pigs fed mature milk, colostrum or formula for 24 h (study 1 and 2). In both study 1 and 2, plasma insulin concentrations increased quadratically ($p < 0.05$) in all feeding groups during the 24-h feeding period as determined by repeated measures ANOVA.

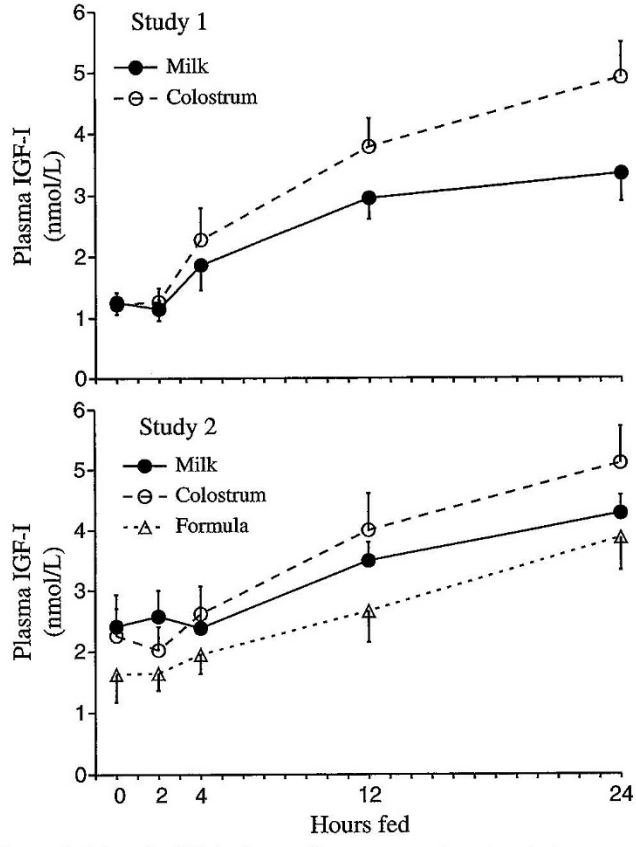

Figure 2. Mean (± SEM) plasma IGF-I concentrations (nmol/L) in neonatal pigs fed milk, colostrum, or formula for 24 h (study 1 and 2). In all treatment groups in both study 1 and study 2, plasma IGF-I increased linearly ($p < 0.05$) during the 24-h feeding period as determined by repeated measures ANOVA.

expression of IGF-I in neonatal animals has been shown to be directly influenced by nutrient intake, especially the quantity (22) and quality (23) of protein. In both studies, this important stimulus of nutrient intake was evident in the increased circulating IGF-I during the 24-h period in all feeding groups. Few studies have measured a specific effect of IGF-I on protein synthesis in neonatal animals, although administration of IGF-I has been shown to increase muscle protein synthesis in young growing animals (10, 11). Recently, we have demonstrated that chronic administration of IGF-I to neonatal pigs via osmotic pumps resulted in a modest increase in skeletal muscle, but not visceral organ protein synthesis (24). Our results suggest that the maximal stimulation of skeletal muscle protein synthesis in colostrum-fed pigs cannot be attributed to circulating IGF-I that may have originated from intestinal absorption of colostral-borne IGF-I or endogenous secretion.

In addition to the specific stimulus of nutrient-independent colostral factors on skeletal muscle and jejunal tissue, our results demonstrate that feeding or nutrient intake had the largest influence on the magnitude of the tissue protein synthesis response; this was particularly evident in the visceral tissue protein synthesis rates among the three feeding groups. In general, the protein synthesis rates in the small intestine, stomach and pancreas of pigs fed mature milk, colostrum or formula were not significantly different, with the exception of the higher rate of jejunal protein synthesis in colostrum *versus* either the mature milk or formula groups. In contrast to our findings in study 1, the rates of protein synthesis in the three gastrointestinal tissues in the colostrum- and mature milk-fed pigs were not significantly different in study 2. This conflicting result appeared to be due to the greater response of the milk-fed group in study 2, because the protein synthesis rates in the colostrum-fed pigs were similar in both studies. In agreement with study 1 and our previous report (8), the rate of liver protein synthesis in colostrum-fed pigs was higher than in milk-fed pigs. However, equalizing the nutrient intake between the formula- and colostrum-fed pigs resulted in similar rates of liver protein synthesis. Therefore, the results suggest that the ingestion and possible intestinal absorption of growth factors present in colostrum or mature milk had a relatively minor influence on the rate of protein synthesis in the liver and most gastrointestinal tissues, except for the jejunum. The predominant factor affecting the protein anabolic response to feeding in visceral tissues appeared to be nutrient intake and perhaps the associated physiologic processes associated with feeding, *per se*.

Our results indicate that the protein anabolic response to both colostrum and mature milk feeding was sustained for 24 h. In pigs fed either colostrum or milk for 24 h, the rates of protein synthesis in small intestine, stomach, pancreas, liver and both skeletal muscles were equal to or greater (20–60%)

than the respective tissue rates observed previously in pigs fed colostrum or milk for only 6 h (8). Similarly in study 2, regardless of whether pigs were fed mature milk, colostrum or formula for 24 h, the rates of tissue protein synthesis were 2- to 4-fold higher than in the unfed newborn group. These relative responses to hourly feeding for 24 h tended to be larger than the roughly 2-fold increase in protein synthesis we observed previously in pigs fed similarly for 6 h (8). The difference in the relative rates of tissue protein synthesis between 6 and 24 h may be related to the pattern of change in both circulating insulin and IGF-I concentrations. In both treatment groups, the plasma concentration of insulin rapidly increased with feeding and appeared to plateau after 2–4 h, whereas that of IGF-I increased gradually beginning 4 h after the onset of feeding. Thus, the stimulation of tissue protein synthesis observed previously after only 6 h of feeding (8) would appear to be associated with the acute increase in circulating concentrations of insulin and substrates, such as glucose and amino acids. However, the increase in tissue protein synthesis between 6 and 24 h did coincide with the increase in circulating IGF-I. These temporal changes in protein synthesis are consistent with the hypothesis that the anabolic response to nutrient intake is mediated acutely by insulin secretion, but is further increased with time by the gradual increase in circulating IGF-I.

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