Biochemical and Molecular Roles of Nutrients

Colostrum Enhances the Nutritional Stimulation of Vital Organ Protein Synthesis in Neonatal Pigs$^{1,2,3}$

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ABSTRACT Our objective was to determine the relative importance of the macronutrient components of colostrum in the stimulation of vital organ protein synthesis in neonatal pigs. We studied colostrum-deprived newborn pigs within 4–6 h after birth (unfed) and three groups fed for 24 h mature milk, colostrum, or a formula containing a macronutrient composition comparable to that of colostrum. We measured protein synthesis in vivo using a flooding dose of $^3$H-phenylalanine. The fractional rates of protein synthesis ($K_s$) in the brain, heart, lung, kidney and spleen were significantly higher in all fed groups than in the unfed newborns. Among the three fed groups, brain and heart protein synthesis rates were greater in colostrum-fed than in either milk- or formula-fed pigs. Kidney and spleen protein synthesis rates in colostrum- and formula-fed pigs were not significantly different, but both were higher than in milk-fed pigs. The stimulation of kidney protein synthesis in response to feeding was primarily a consequence of greater protein synthetic efficiency; however, protein synthetic capacity in the heart, lung and spleen was generally greater in colostrum- and formula-fed pigs than in unfed newborns. Our results suggest that the predominant stimulus for vital organ protein synthesis in colostrum-fed neonatal pigs is nutrient intake. However, there was a specific stimulation of both brain and heart protein synthesis in colostrum-fed pigs that cannot be attributed to macronutrients. J.Nutr. 127: 1284–1289, 1997

KEY WORDS: • pigs • colostrum • protein synthesis • organs • growth factors

During the early neonatal period, there is a several-fold increase in organ growth and accelerated development associated with the transition from intrauterine to extrauterine life (Widdowson et al. 1976, Widdowson and Crab 1976). Much of the growth stimulus for many organs in the newborn undoubtedly results from the increased demands associated with physiological functions such as pulmonary respiration, thermogenesis (Herpin and LeDividich 1995) and gluconeogenesis (Girard 1986). The diet ingested by the newborn, namely colostrum, contains a rich source of nutrients that not only support these metabolic needs, but are critical for the rapid rate of organ growth and development. We have previously shown that the rate of protein synthesis in a number of organs is increased markedly when newborn pigs ingest either colostrum or mature milk (Burrin et al. 1992). However, in some organs, the proportional stimulation of protein synthesis is greater when pigs are fed colostrum rather than mature milk. These differences in the protein anabolic stimulus between colostrum and mature milk may be due to the differences in the concentration of either nutrients or non-nutritive components such as growth factors. Several researchers have identified a number of peptide growth factors, including insulin, insulin-like growth factor-I (IGF-I) and epidermal growth factor (EGF) that are present in higher concentrations in colostrum than in mature milk (Donovan et al. 1994, Jaeger et al. 1987, Simmen et al. 1988). Because of the mitogenic or trophic potential of these growth factors, it has been hypothesized that their ingestion via colostrum enhances tissue growth and development of the neonate.

In this report we present additional information derived from a previously reported experiment (Burrin et al. 1995) by determining the effects of colostrum ingestion on protein synthesis in vital organs, including brain, heart, lung, kidney and spleen of newborn pigs. We tested the hypothesis that

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$^6$ Abbreviations used: BW, body weight; DHA, docosahexanoic acid; EGF, epidermal growth factor; IGF-I, insulin-like growth factor-I; PCA, perchloric acid.
components of colostrum other than macronutrients are, in part, responsible for the marked stimulation of vital organ protein synthesis in newborn pigs. To test this hypothesis, we measured the rates of brain, heart, lung, kidney and spleen protein synthesis in newborn pigs fed colostrum, mature milk or a fortified formula having a macronutrient composition similar to that of colostrum but devoid of growth factors.

METHODS

Animals and design. The results reported here were derived from analysis of tissues collected from an animal experiment described as “Study 2” in the methods section of a previous report (Burrin et al. 1995). Briefly, three litters of conventional crossbred pigs (Texas A & M University, College Station, TX) were obtained immediately after birth (prior to suckling), weighed and randomly assigned to their respective treatment groups. A total of 23 pigs from three litters were assigned to one of four groups and fed porcine colostrum, mature milk or formula; the fourth treatment group was studied 4–6 h after birth, having never been fed. Before initiating the feeding protocol and within 1–2 h after birth, the umbilical artery of each pig was catheterized with polyvinyl chloride catheters (Sherwood Medical, St. Louis, MO) under general isoflurane anesthesia (Aerane, Anaquest, Madison, WI). The duration of anesthesia required for this procedure was typically less than 15–20 min. The pigs were allowed to recover at least 1–2 h before the feeding protocol which occurred approximately 4–6 h after birth. Therefore, the unfed newborn group was studied at approximately the same time the feeding protocol began in the groups fed colostrum, formula or milk. Pigs were housed in separate cages with a dry towel for bedding, and ambient temperature was maintained at ~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 each 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 concentration 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 (ICN Pharmaceuticals, Costa Mesa, CA). The pigs were weighed, then bottle fed hourly for 24 h an exclusive diet of mature milk, colostrum or formula at a rate of 20 g/kg body weight (BW). 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.

Measurements of in vivo protein synthesis. Pigs were administered a flooding dose of [1-14C]phenylalanine (37 MBq/kg BW) in a phenylalanine solution (150 mmol/L) at a dose of 10 mL/kg BW via the umbilical arterial catheter, which was then flushed with sterile saline. The phenylalanine solution was made sterile and warmed and passed through a 0.2 μm filter. At 5, 15 and 30 min after the midpoint of the infusion, arterial blood samples (0.5 mL) were collected and frozen for measurement of blood phenylalanine specific radioactivity. Blood samples were collected via the umbilical arterial catheter, which was then flushed with sterile saline. The phenylalanine solution was made in sterile water and multiplying the fractional synthesis rate (K, %/d) by the ratio of protein to RNA to yield grams of protein synthesized per gram of total RNA per day.

Statistics. Treatment means were analyzed by one-way ANOVA; dietary treatment was the main effect. When a significant difference among the four treatment groups was first detected by one-way ANOVA, then differences between treatments were determined by a Fisher’s LSD test (Milliken and Johnson, 1984) using the pooled error term derived from the one-way ANOVA. Results are presented as means with the pooled standard deviation (SD) from the one-way ANOVA. A probability value < 0.05 was considered statistically significant. Values in the text are means ± SD.

RESULTS

Summarized below are the nutrient intakes and body weight changes during the 24-h feeding period reported previously (Burrin et al. 1995). Protein (N × 6.38) and gross energy concentrations of colostrum (118 ± 12 g/L and 6.0 ± 3 MJ/L) were anesthetized with an intravenous 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. The spleen, kidney, lung, heart, and brain were quickly removed and weighed, and a sub-sample of each was frozen in liquid nitrogen.

The specific radioactivity of H-phenylalanine was determined in tissue samples and in whole blood samples obtained 5, 15, and 30 min after infusion of the H-phenylalanine. The tissue samples were homogenized in 0.2 mol perchloric acid/L (PCA) as described previously (Burrin et al. 1991). The PCA-soluble homogenate supernatants containing the tissue free amino acid pools were separated from the PCA-insoluble precipitates by centrifugation. The PCA-insoluble precipitates were neutralized, washed and solubilized in 0.3 mol NaOH/L for 1 h at 37°C. Aliquots of the NaOH solutions were assayed for protein as described by Lowry et al. (1951). The remainder of the solutions were reacidified with 2 mol PCA/L and, after precipitation at 4°C and centrifugation at 3000 × g for 15 min, the acid-soluble supernatant fractions were assayed for total RNA by the method of Munro and Fleck (1969). The acid-insoluble pellets, containing protein, were hydrolyzed with 6 mol HCl/L for 24 h at 110°C and then vacuum-dried in a Speed-Vac concentrator (Jouan, Winchester, VA). The dried residues from the protein hydrolysates were solubilized with 4 mL of water and vacuum-dried twice to remove any residual HCl. The protein hydrolysates, homogenate supernatants and blood supernatants were vacuum-dried and resuspended in water for determination of phenylalanine specific activity. Phenylalanine was separated from the other amino acids using anion exchange chromatography (AminoPac column, Dionex, Sunnyvale, CA). The radioactivity associated with the phenylalanine peak was collected automatically (Model 202, Gilson Medical Electronics, Middleton, WI) and measured using liquid scintillation counting (LS5000TD, Beckman Instruments, Fullerton, CA).

Calculations. Protein synthesis was calculated as a fractional rate (K, %/d) from the equation described by Garlick et al. (1980):

\[ K = \left( \frac{S}{S_0} \right) - \left( \frac{1}{440/t} \right) \times 100 \]

where \( S_0 \) is the specific activity of the PCA-insoluble or protein-bound phenylalanine pool (Bq/μmol), \( S \) is the specific activity of the PCA-soluble or tissue free phenylalanine pool (Bq/μmol), and \( t \) is time of labeling in min. The value used for \( S_0 \) was corrected to represent the tissue phenylalanine specific activity at the half-time of the 30-min labeling period. The corrected \( S_0 \) for each pig was calculated by adding individual tissue \( S \) (Bq/μmol) after time \( t \) and the rate of change in blood \( S \) (Bq/μmol⋅min^-1⋅min^-1) estimated from the regression of 5-, 15-, and 30-min blood samples of all pigs within a treatment group as described previously (Burrin et al. 1995):

\[ S = S(t) + (\Delta S \cdot t) / 2 \]

Because most of the RNA in tissues is ribosomal, the RNA-to-protein ratio is an estimation of the protein synthetic capacity (Cp) of the tissue. Protein synthetic efficiency (Ksyn) was estimated by multiplying the fractional synthesis rate (K, %/d) by the ratio of protein to RNA to yield grams of protein synthesized per gram of total RNA per day.

Vitamins and minerals: The mineral and vitamin premixes provided ingredients at the following mass concentrations (mg/L) in the formula: calcium carbonate, 1600; monocalcium phosphate, 5910; cobalt carbonate, 19.71; magnesium sulfate, 1005; potassium chloride, 3154; potassium iodide, 0.33; sodium bicarbonate, 2759; zinc sulfate, 99; retinyl palmitate, 9; ergocalciferol, 0.625; dl-α-tocopherol acetate, 110; ascorbic acid, 225; inositol, 25; choline chloride, 375, menadione, 11.25; p-aminobenzoic acid, 25; niacin, 21.25; riboflavin, 5.0; pyridoxine HCl, 5.0; thiamine HCl, 5.0; calcium pantothenate, 15.0; biotin, 0.10; folic acid, 0.45; vitamin B-12, 0.00675.
## TABLE 1

Vital organ protein and RNA contents relative to body weight (BW) in unfed newborn pigs and those fed mature milk, colostrum or formula for 24 h.\(^1,2\)

<table>
<thead>
<tr>
<th>Protein, mg/kg BW</th>
<th>Newborn</th>
<th>Mature milk</th>
<th>Colostrum</th>
<th>Formula</th>
<th>Pooled SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>1949(^a)</td>
<td>1548(^b)</td>
<td>1351(^b)</td>
<td>1263(^b)</td>
<td>292</td>
</tr>
<tr>
<td>Heart</td>
<td>555</td>
<td>619</td>
<td>649</td>
<td>592</td>
<td>84</td>
</tr>
<tr>
<td>Lung</td>
<td>1431</td>
<td>1395</td>
<td>1655</td>
<td>1603</td>
<td>230</td>
</tr>
<tr>
<td>Kidney</td>
<td>793(^a)</td>
<td>960(^ab)</td>
<td>1098(^b)</td>
<td>1166(^b)</td>
<td>201</td>
</tr>
<tr>
<td>Spleen</td>
<td>97(^a)</td>
<td>114(^a)</td>
<td>154(^b)</td>
<td>130(^ab)</td>
<td>32</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>RNA, mg/kg BW</th>
<th>Newborn</th>
<th>Mature milk</th>
<th>Colostrum</th>
<th>Formula</th>
<th>Pooled SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>26.36</td>
<td>21.24</td>
<td>19.54</td>
<td>17.73</td>
<td>5.38</td>
</tr>
<tr>
<td>Heart</td>
<td>11.67(^a)</td>
<td>13.08(^a)</td>
<td>18.80(^b)</td>
<td>15.37(^ab)</td>
<td>3.40</td>
</tr>
<tr>
<td>Lung</td>
<td>30.04(^a)</td>
<td>28.29(^a)</td>
<td>50.98(^b)</td>
<td>41.09(^a)</td>
<td>10.52</td>
</tr>
<tr>
<td>Kidney</td>
<td>22.69</td>
<td>22.27</td>
<td>26.13</td>
<td>24.36</td>
<td>6.3</td>
</tr>
<tr>
<td>Spleen</td>
<td>2.80(^a)</td>
<td>3.74(^a)</td>
<td>6.12(^b)</td>
<td>5.25(^b)</td>
<td>1.47</td>
</tr>
</tbody>
</table>

\(^1\) Values are means with pooled SD, \(n = 6\) for all fed groups and \(n = 5\) unfed newborns.

\(^2\) Means in a row with the same superscript are not significantly different (\(P \geq 0.05\)) as determined by Fisher’s LSD test.

were higher than that of mature milk (48 ± 6 g/L and 4.1 ± 5 MJ/L), but not different from that of the formula (122 ± 15 g/L and 5.7 ± 7 MJ/L). Protein (g/kg BW) and gross energy (kJ/kg BW) intakes, respectively, of colostrum-fed (54 ± 4 and 2711 ± 151) and formula-fed (53 ± 3 and 2548 ± 172) pigs were not significantly different from each other, but were higher than in mature milk-fed pigs (23 ± 1 and 1924 ± 100). The amino acid composition of a pooled sample of colostrum similar to that used in this study has been reported previously (Davis et al. 1994). In that report, the total amino acid concentration of porcine colostrum was 99 g/L, a value which is lower than the protein concentration estimated here, based on the colostrum nitrogen concentration. An explanation for our overestimate of colostrum protein, based on nitrogen concentration, is likely due to the non-protein nitrogen components, such as urea, ammonia, taurine, and polyamines, that have been reported in porcine colostrum (Kelly et al. 1991, Wu et al. 1994). The predicted amino acid concentration of the formula was calculated to be 128 g/L based on the reported primary amino acid sequence of bovine casein, bovine lactalbumin and bovine albumin and their relative proportions in the diet (Brunner 1977). This predicted total amino con-

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**FIGURE 1** Fractional protein synthesis rates (\(K_s, \%\)/d) of brain and heart in unfed newborn pigs and those fed mature milk, colostrum or formula for 24 h. Values are means ± SD, \(n = 6\) for all fed groups and \(n = 5\) newborn. * \(P < 0.05\) versus newborn, *** \(P < 0.05\) colostrum versus milk and formula.

**FIGURE 2** Fractional protein synthesis rates (\(K_s, \%\)/d) of kidney, spleen and lung in unfed newborn pigs and those fed mature milk, colostrum or formula for 24 h. Values are means ± SD, \(n = 6\) for all fed groups and \(n = 5\) newborn. * \(P < 0.05\) versus newborn, *** \(P < 0.05\) colostrum and formula versus milk.
The predicted essential amino acid composition of the formula is similar to the estimated concentration content based on our measurement of nitrogen concentration. The predicted essential amino acid composition of the formula tended to be greater than that reported for colostrum. The predicted essential amino acid composition of the formula expressed as a percentage of that in colostrum was as follows: 180% lysine, 134% leucine, 182% isoleucine, 113% valine, 145% phenylalanine, 102% threonine, 161% histidine, 205% tryptophan, 118% methionine and 167% cystine. Based on the estimated amino acid composition of formula and colostrum described above, the calculated amino acid intake of formula-fed pigs was approximately 30% greater than colostrum-fed pigs. Body weight gains (g/kg BW) during the 24-h feeding period in colostrum- and formula-fed pigs (173 ± 17 and 154 ± 22) were greater than that of mature milk-fed pigs (84 ± 14); however, body weight gains in colostrum- and formula-fed pigs were not significantly different.

Brain protein content (relative to BW) was lower in the three feeding groups than in the unfed newborn group (Table 1). However, the kidney protein content in colostrum and formula-fed pigs was higher than in either the unfed newborns or those fed milk. Spleen protein content was also higher in colostrum-fed pigs than in unfed newborn pigs. The heart and lung RNA content (relative to BW) in pigs fed colostrum were higher than in either unfed newborns or milk-fed pigs (Table 1). The spleen RNA content in pigs fed colostrum and formula were higher than in either unfed newborns or milk-fed pigs.

The fractional protein synthesis rate in all vital organs was significantly increased by feeding in all groups (Figs. 1 and 2). Among the three feeding groups, the brain and heart protein synthesis rates of colostrum-fed pigs were greater than those of either formula- or milk-fed pigs (Fig. 1). Kidney and spleen protein synthesis rates in colostrum- and formula-fed pigs were not significantly different, but both were higher than in milk-fed pigs (Fig. 2). Lung protein synthesis rates did not differ among the three feeding groups (Fig. 2).

The protein synthetic capacity of the heart and spleen in pigs fed either colostrum or formula also did not differ, but was greater in pigs fed colostrum than either the unfed newborn or milk-fed groups. In contrast, the protein synthetic capacity of the kidney in pigs fed colostrum and milk did not differ from unfed newborn pigs, while that of formula-fed pigs was lower than unfed newborn pigs. Similarly, the protein synthetic efficiency of the heart, kidney, and spleen were significantly greater in milk- and colostrum-fed pigs than in unfed newborn pigs (Table 2). The protein synthetic efficiency in the kidney of pigs fed formula was higher than either unfed newborn or milk-fed pigs.

**DISCUSSION**

The mitogenic or trophic potential of colostrum-borne growth factors has been implicated in the stimulation of tissue growth and development of the suckling neonate. In this study we extended our previous findings to determine whether the components of colostrum, in addition to the major macronutrients, are partially responsible for the marked stimulation of vital organ protein synthesis observed in newborn pigs (Burrin et al. 1992, 1995). As in our previous studies, the current findings suggest that feeding milk, colostrum or formula, markedly stimulated protein synthesis in all vital organs. However, there were tissue-specific differences in the response to each of the three diets. Protein synthesis rates in brain and heart were significantly higher in colostrum-fed pigs than in either milk or formula-fed pigs. This enhanced stimulation of brain and heart protein synthesis in response to colostrum feeding occurred despite similar macronutrient intakes and circulating glucose and amino acid concentrations in the formula- and colostrum-fed pigs (Burrin et al. 1995). This suggests that the maximal stimulation of brain and heart protein synthesis in colostrum-fed pigs was a result of one or more components in colostrum, not directly associated with macronutrients, that are absent in formula. The increase in brain protein synthesis, but not protein content, suggests that feeding enhanced protein degradation, which may be indicative of tissue remodeling associated with development.

The maximal stimulation of brain protein synthesis by colostrum was particularly interesting given the recent evidence...
that the quality of the diet fed to preterm infants can have beneficial effects on developmental outcomes (Lucas et al. 1990, 1992 and 1994). In particular, these studies have shown that preterm infants fed human milk rather than formula during the neonatal period scored significantly higher on intelligence quotient, psychomotor and mental development tests when measured at 18 mo and 8 y of age. It has been hypothesized that this apparent neurodevelopmental advantage of human milk for the preterm infant may be related to the presence of long-chain lipids, such as docosahexanoic acid (DHA), hormones and growth factors that are not found in infant formulas (Lucas et al. 1992). Other recent findings demonstrate that dietary cholesterol supplementation improves the abnormally low exploratory behavior observed in neonatal pigs genetically selected for low serum cholesterol (Schoknecht et al. 1994). Based on the composition of ingredients and limited analysis (Burrin et al. 1995), the formula we used was devoid of DHA and growth factors. Therefore, this advantage of colostrum feeding on brain protein synthesis cannot be attributed to macronutrient intake, particularly of protein and energy. Furthermore, it has been argued that some of the results of the human studies are confounded by inherent socio-behavioral differences between breast- and formula-feeding mothers. However, unlike these human studies, this controlled study with neonatal pigs is not confounded by any inherent maternal effects among feeding groups.

The kidney and spleen protein synthesis rates of colostrum- and formula-fed pigs did not differ from each other and were greater than those of milk-fed pigs, presumably reflecting the greater nutrient concentration of colostrum and formula. Thus, the dominant stimulus for kidney and spleen protein synthesis was macronutrient intake and is consistent with our previous findings for other visceral organs, particularly the liver (Burrin et al. 1995). The response to feeding milk, colostrum, or formula was associated with metabolic and endocrine changes reported previously (Burrin et al. 1995), which may explain the stimulation of vital organ protein synthesis. Insulin and amino acids are critical factors that mediate the acute stimulation of protein synthesis in response to feeding (Garlick et al. 1983; Garlick and Grant, 1988). In this study, circulating insulin and amino acid concentrations were increased with feeding in all three groups, but after 4 h of feeding, tended to be higher in colostrum- and formula-fed pigs than in milk-fed pigs (Burrin et al. 1995). This pattern of circulating insulin and amino acid concentrations likely reflects the higher protein intake of colostrum- and formula-fed versus milk-fed pigs. Therefore, the maximal rates of kidney and spleen protein synthesis in both colostrum- and formula-fed pigs compared to those of pigs fed milk may be largely attributed to higher protein intake and perhaps the resultant increase in circulating insulin and amino acid concentrations in these groups.

Our results suggest that the cellular mechanisms by which feeding stimulated protein synthesis varied among organs. Our estimates of translational efficiency and protein synthetic capacity are based on the assumption that our measurements represent largely ribosomal RNA, but does not preclude the possibility that specific mRNAs’s are either up- or down-regulated in response to diet. The increases in heart, lung and spleen protein synthesis resulted from increases in both translational efficiency and protein synthetic capacity, whereas kidney protein synthesis was mediated by increased translational efficiency. The increase in protein synthetic capacity that was evident in colostrum- and formula-fed pigs was paralleled by increased total RNA content, suggesting either enhanced ribosomal RNA synthesis or reduced degradation. This is supported by evidence from studies in rats suggesting that both amino acids and insulin increase ribosomal protein synthesis (Ashford and Pain 1986) and inhibit RNA degradation (Lardeux and Mortimore 1987).

These results, in conjunction with our earlier observations from this study, suggest that the response of protein metabolism to early feeding varies among organs. The organs associated primarily with the digestion and absorption (gastrointestinal tissues), metabolism (liver) and excretion (kidney) of macronutrients, appear to respond only to macronutrient intake itself. However, skeletal and cardiac muscle, and critically, the brain, apparently require some unidentified component of colostrum to achieve a maximum rate of protein synthesis and growth. Whether this factor is a trace nutrient (such as a specific fatty acid), a growth factor or reflects the activation of a specific growth-regulatory pathway remains unknown. Nevertheless, these findings support the critical role of colostrum ingestion in developmental regulation during the immediate neonatal period.

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LITERATURE CITED


