Feeding Colostrum Increases Circulating Insulin-Like Growth Factor I in Newborn Pigs Independent of Endogenous Growth Hormone Secretion

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ABSTRACT: Our objective was to examine the influence of feeding and endogenous GH secretion on circulating IGF-I in colostrum-deprived newborn pigs fed colostrum (n = 4), formula (control, n = 4), or water (n = 4). In another four formula-fed pigs, GH was ablated (GRF-A) with two intravenous injections of a GH releasing-factor antagonist (N-Ac-Tyr¹,D-Arg²)-GRF(1-29)-NH₂. Blood was serially sampled in all pigs to measure plasma IGF-I and GH profiles. Feeding increased plasma IGF-I concentration two- to fourfold and decreased GH secretion. Despite a more than 80% decrease in the plasma GH in GRF-A pigs, the circulating IGF-I concentration was similar to that in control pigs. In colostrum-fed pigs, plasma IGF-I was higher than that in control pigs, despite equal nutrient intake and lower circulating GH. There were no differences in plasma IGF binding protein (IGFBP)-3 levels among the treatment groups. However, the relative abundance of plasma IGFBP-4 was lower, and that of IGFBP-1 higher, in unfed pigs than in any of the three fed groups. The plasma insulin concentration was not different among fed pigs, but it was lower in unfed pigs. Our results indicate that the circulating IGF-I concentration is more dependent on nutrient intake than on GH in newborn pigs, despite relatively high GH concentrations. However, because the nutrient content in the formula was designed to match that of colostrum, a factor other than nutrient intake and GH was responsible for the maximal increase in circulating IGF-I concentration observed in colostrum-fed pigs.

Key Words: Somatotrophin, GRF-Antagonists, IGF Binding Proteins, Pigs

Introduction

Postnatal growth is largely dependent on somatotropic function, which is mediated via the endocrine/paracrine anabolic actions of GH and IGF-I (Florini et al., 1996). It is generally held that neonatal growth is GH-independent and that the somatotropic axis is not functional until well beyond the neonatal period. However, recent studies of our own (Wester et al., 1998) and others (Matteri et al., 1997) have shown that the somatotropic axis is functional and responsive in neonatal pigs, although it is much less sensitive than in mature pigs. It is well-established that level of nutrition also directly affects the tissue expression and circulating concentration of IGF-I, especially in neonates (Thissen et al., 1994). Studies in newborn pigs indicate that the circulating IGF-I concentration in colostrum-fed pigs is higher than in pigs fed either mature milk or a formula with a macronutrient content similar to that of colostrum, which suggests a possible nutrient-independent induction of IGF-I by colostrum ingestion (Dauncey et al., 1994; Burrin et al., 1995). The endocrine regulation of the tissue expression and circulating concentration of IGFBPs

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IGF-I in neonates is poorly understood. Although the neonate seems to be relatively insensitive to GH (Harrell et al., 1997), there is a well-documented surge in circulating GH in newborn pigs associated with parturition, after which it declines precipitously (Klindt, 1986). It is unknown whether the surge in the circulating GH concentration in newborn pigs increases circulating IGF-I or whether it is affected by colostrum ingestion. In the present study, we used a GH-releasing factor (GRF) antagonist approach to establish the relative effects of nutrient intake and GH on circulating IGF-I in colostrum-fed newborn pigs. We also examined whether the apparent nutrient-independent, maximal increase in circulating IGF-I observed in colostrum-fed pigs is mediated via increased circulating GH concentrations.

Materials and Methods

Animals and Design. From two litters of conventional crossbred pigs (Texas A&M University, College Station, TX), 16 newborn, unsuckled pigs were obtained immediately after birth and weighed. Before the start of the experiment, the umbilical artery of each pig was catheterized with an 18-gauge polyvinyl chloride catheter under general anesthesia. Pigs were allowed to recover for at least 1 h before the start of the experiment. Animals were housed separately, with a dry towel for bedding, at an ambient temperature maintained at approximately 29°C. Pigs were assigned randomly to one of four treatments (n = 4 per treatment): unfed, formula-fed (control), formula-fed with administration of a GH-releasing factor receptor antagonist (GRF-A), and colostrum-fed. Pigs were gavage-fed every 2 h either formula or colostrum at a rate of 30 mL/kg BW for a total of 18 h. Unfed pigs were given a similar volume of water every 2 h for a total of 8 h. The formula was prepared to match the macronutrient composition of colostrum (Burrin et al., 1995). The colostrum was derived from pooled samples taken from conventional sows within 24 h after parturition. With the initial litter, an 8-h serial blood collection was used to characterize pulsatile secretion of GH. From those data, we determined that a 4-h collection was sufficient to accurately quantify GH secretion. The GRF-A peptide [(N-Ac-Tyr1,D-Arg2)-GRF(1-29)-NH2] (Bachem Bioscience, King of Prussia, PA) was dissolved in 10 mM acetic acid and administered via the umbilical artery in two equal bolus doses (200 μg/kg BW) at 0 and 9 h after the first feeding. Animals not receiving GRF-A were dosed with an equal volume of saline. The experimental protocol was approved by the Animal Care and Use Committee of Baylor College of Medicine and in accordance with the National Research Council’s (1996) Guide for the Care and Use of Laboratory Animals.

Blood Sampling and Analysis. Serial blood samples were collected from the umbilical artery (1.5 mL) into heparinized syringes every 15 min for 4 h. Samples were centrifuged at 3,000 × g for 20 min at 4°C, and the plasma was collected. In order to maintain hematocrit and colloidal osmotic pressure, packed red blood cells were resuspended in an equal volume of 5% human serum albumin and returned to each respective pig every 30 min. Additional blood was obtained from the last collection to measure plasma IGF-I, IGF binding proteins (IGFBP), and insulin.

Plasma GH concentration was measured with a double-antibody homologous RIA (Klindt et al., 1983). Intra- and interassay CV from assays using procedures identical to this assay routinely were <10 and 15%, respectively. The secretory profile of GH was analyzed using the Pulsar peak-fitting program to estimate the mean GH concentration, baseline, peak amplitude, and peak frequency. Plasma concentration of IGF-I was measured with a RIA after acidification and chromatography to remove binding proteins (Lee et al., 1991). Recombinant human IGF-I (Bachem) was used for standards and iodination. The Iodogen® method (Pierce Chemical, Rockford, IL), with a 5-min reaction and an .8- × 15-cm Sephadex G-50 medium column, was used to iodinate and purify the hormone. Intra- and interassay CV were 8.3 and 6.5%, respectively. Plasma insulin was measured with a RIA (Linco Research, St. Louis, MO). The human specific antibody used in this assay exhibited 100% cross-reactivity with porcine insulin. All samples were analyzed in one assay in which the intraassay CV was 7.0%.

Analysis of IGFBP was performed using a western ligand blot described by Wester et al. (1998). Briefly, a 1:10 dilution of serum was separated using SDS-PAGE and transferred to a nitrocellulose membrane. After blocking, the membrane was incubated in the presence of radioactivity-labeled IGF-I. Nonspecific bound tracer was washed away, and the membrane was then exposed to x-ray film in the presence of intensifying screens at −70°C for 7 d. Autoradiographs were analyzed using laser densitometry (LKB Ultra Scan, LKB, Stockholm, Sweden). Relative molecular weights of IGFBP were estimated by comparison to Coomassie blue-stained protein standards electrophoresed under identical conditions.

Statistical Analysis. Observations were analyzed by one-way ANOVA with treatment as the main effect. Differences among treatments were compared using Fisher’s protected least significant difference test. Results are presented as means with pooled SEM from the one-way ANOVA. Differences with P < .05 were considered significant.

Results

The birth weights among the four treatment groups were similar, with a mean value of 1.42 ± .2 kg. Pigs
treated with GRF-A had significantly reduced GH secretion (Figure 1). Compared with formula-fed controls, GRF-A decreased the frequency and amplitude of GH secretion, which resulted in a 83 and 80% decrease in the baseline and mean plasma GH concentration, respectively (Figures 2 and 3). Despite large differences in circulating GH concentrations in formula-fed control and GRF-A groups, the circulating IGF-I concentrations were similar (Figure 3). Feeding decreased the GH secretion and increased the circulating IGF-I concentration. In colostrum-fed pigs, the circulating IGF-I concentration was twofold greater, and the mean GH concentration was threefold greater than in those given GRF-A. Although the plasma IGF-I concentration in colostrum-fed pigs was greater than that of formula-fed controls, the mean plasma GH concentration was about 50% less. Serum insulin concentrations were increased by feeding but were similar among the groups (unfed, 1.95 ± 0.16 μU/mL; colostrum, 135 ± 53.2 μU/mL; control, 102.8 ± 27.5 μU/mL; GRF-A, 89.7 ± 32.5 μU/mL).

Western ligand blotting and autoradiography of plasma indicated five bands of IGF binding that corresponded to apparent molecular weights of 43, 39, 34, 28, and 24 kDa. The bands detected can be putatively identified by comparing with published reports using immunological methods (Lee et al., 1991; McCusker et al., 1991; Shimasaki and Ling, 1991: Wester et al., 1998) as differentially glycosylated forms of IGFBP-3 for the 43- and 39-kDa bands, IGFBP-2 for the 34-kDa band, IGFBP-1 for the 29-kDa band, and IGFBP-4 for the 24 kDa band. When the ligand blot was compared with results in our laboratory using immunoblotting to measure IGFBP in neonatal pig plasma, an anti-human IGFBP-3 antiserum recognized only the 43- and 39-kDa bands, and an anti-human IGFBP-2 antiserum recognized only the 34-kDa band (Wester et al., 1998). In our previous report, neither the 29- nor the 24-kDa band was detected by immunoblotting with IGFBP-2 or -3 antibodies, indicating that those bands were not proteolytic products of either IGFBP-2 or -3. Furthermore, the 29- and 24-kDa bands correspond to proteins previously identified as IGFBP-1 in neonatal pig serum (McCusker et al., 1991) and IGFBP-4 (Shimasaki and Ling, 1991). Based on densitometric

![Figure 1](image_url)

Figure 1. Growth hormone secretory profiles in newborn pigs given only water (unfed) or those fed formula (control), formula and administered a GH-releasing factor antagonist (GRF-A), or colostrum.
scanning analysis, the abundance of IGFBP-3 was not affected by treatment; however, other binding proteins differed among treatments (Table 1). When expressed as a percentage of total plasma IGFBP abundance, the proportion of IGFBP-4 was highest and that of IGFBP-1 was lowest \((P < .05)\) in unfed pigs, but these were not different among fed groups.

**Discussion**

The importance of GH and the somatotropic axis to postnatal growth is well-established. However, despite the dogma that neonatal growth is GH-independent, few studies have examined the function of the somatotropic axis during the neonatal period. Our primary objective in this study was to establish the relative effects of nutrient intake and GH on circulating IGF-I in colostrum-fed newborn pigs. In order to determine the relationship between circulating GH and IGF-I, we chose to ablate GH secretion using a GRF receptor antagonist approach used previously (Lumpkin et al., 1989; Wheeler et al., 1991; Jaffe et al., 1993) to eliminate pulsatile GH secretion. This approach has advantages compared with hypophysectomy, because it avoids surgical stress and does not affect either the secretion of other pituitary hormones or feed intake.

As evidence of the efficacy of the GRF antagonist approach, we observed significant reductions in all aspects of GH secretion by administration of the GRF antagonist, such that mean plasma GH concentration...
Table 1. Plasma insulin-like growth factor binding proteins (BP) in newborn pigs unfed, fed formula (control), fed formula and administered a growth hormone-releasing factor antagonist (GRF-A), or fed colostrum

<table>
<thead>
<tr>
<th></th>
<th>Unfed</th>
<th>Control</th>
<th>GRF-A</th>
<th>Colostrum</th>
<th>Pooled SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP-1 Area of intensity, absorbance unit (AU)·mm</td>
<td>2.57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.30&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.54&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>.69</td>
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<td>BP-2</td>
<td>1.86&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.76&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.39&lt;sup&gt;b&lt;/sup&gt;</td>
<td>.34</td>
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<td>BP-3</td>
<td>1.64&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.97&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.72&lt;sup&gt;b&lt;/sup&gt;</td>
<td>.49</td>
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<td>BP-4</td>
<td>.62&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.54&lt;sup&gt;b&lt;/sup&gt;</td>
<td>.94&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>.42</td>
</tr>
<tr>
<td>Relative intensity, %</td>
<td>37.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>22.6&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.2</td>
</tr>
<tr>
<td>BP-2</td>
<td>28.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>31.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>26&lt;sup&gt;b&lt;/sup&gt;</td>
<td>27.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.9</td>
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<td>30.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>22.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.1</td>
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<td>BP-4</td>
<td>9.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>22.2&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>17.6&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>4.8</td>
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<sup>a,b</sup>Values in the same row with different superscripts are different (P < .05) as determined by Fisher’s (protected) least significant difference test (n = 4 per treatment group).

in GRF-A treated pigs was less than 20% of that in controls. In the report by Lumpkin et al. (1989) using immature rats, GRF antagonist administration eliminated pulsatile GH secretion but did not affect the basal plasma GH concentration. A similar response was observed when human subjects were administered the same antagonist; injection of the antagonist reduced mean and pulsatile secretion of GH by 40 and 75%, respectively, without reducing basal levels (Jaffe et al., 1993). In contrast to the previous reports, we observed greater reductions in amount and frequency of GH secreted (50%) as well as a decrease in basal concentration of GH (83%). We should note that even though we observed ablation of GH secretion after acute treatment of newborn pigs with the GRF antagonist, neither GH secretion nor circulating IGF-I concentration was affected in 8-d-old neonatal pigs treated with a similar daily dose of the GRF antagonist in the previous 7 d (our unpublished observations). Decreased responsiveness of GH secretion to GRF-receptor blockade with advancing age also has been reported in monkeys and may be related to maturation of the ultra-short loop feedback system for GRF (Wheeler et al., 1991).

Despite the significantly lower plasma GH concentration in GRF-A-treated pigs than that in control pigs, the circulating IGF-I and IGFBP-3 concentrations were similar. Because plasma IGF-I and IGFBP-3 levels are usually increased by GH, the lack of change in these measures suggests that somatotropic function is unresponsive to physiological changes in the circulating GH concentration in newborn pigs. The unresponsiveness of newborn pigs to GH may be largely explained by the relatively low abundance of hepatic GH receptors and IGF mRNA (Breier et al., 1989; Peng et al., 1996). The observed unresponsiveness or uncoupling of somatotropic function in newborn pigs is in contrast to the recent evidence that pharmacological doses of exogenous GH markedly increased circulating IGF-I concentrations in neonatal pigs and calves (Hammon and Blum, 1997; Mattierri and Carroll, 1997; Wester et al., 1998). Taken together, the results suggest that even though the somatotropic axis is functional and responsive to pharmacological doses of GH in neonatal pigs, it is considerably less sensitive than in adult pigs and does not play a significant physiological role in neonatal growth.

As an alternative explanation, the unresponsiveness of the plasma IGF-I concentration to changes in circulating GH could have been caused by the relatively short duration (i.e., 18 h) of exposure to the GRF antagonist. This is unlikely, given our observation of a significant increase in circulating IGF-I within 24 h after GH treatment in 3-d-old pigs, and the fact that more rapid (<12 h) increases in plasma IGF-I and tissue IGF-I mRNA expression with GH treatment have been reported in more mature pigs (Evock-Clover et al., 1992; Ramsay et al., 1995). Furthermore, a recent report in neonatal calves indicated that the plasma IGF-I concentration was significantly increased within 24 h after exogenous GH administration (Hammon and Blum, 1997). The circulating IGF-I concentration can not only increase rapidly with GH treatment, but also, as our study with 7 d-old pigs showed, plasma IGF-I concentration was significantly decreased after only 12 h of fasting (Davis et al., 1996).

The second objective of this study was to determine the relative effect of nutrient intake and its contribution to the elevated levels of circulating IGF-I in colostrum-fed newborn pigs. As predicted from previous studies (Buonomo and Klindt, 1993; Burrin et al., 1995), we found that feeding alone markedly increased circulating IGF-I more than threefold. However, despite the fact that macronutrient intake was similar to that of the formula-fed groups, the circulating IGF-I concentration was significantly higher (~60%) in pigs fed colostrum. Even though this observation suggests that some colostral factor other
than macronutrient intake increases circulating IGF-I, our results suggest that it is probably not GH, because the circulating GH level among the four treatment groups was lowest in colostrum-fed pigs. Moreover, there is evidence that the intestinal absorption of colostrum-borne IGF-I is limited and thus would not contribute to the increased circulating IGF-I in colostrum-fed newborn pigs and calves (Burrin et al., 1996; Donovan et al., 1996; Xu and Wang, 1996; Hammon and Blum, 1997). It is also unlikely that any GH or GRF ingested in colostrum is absorbed from the intestine given that the circulating GH concentration was higher in control than in the colostrum group. The observation that colostrum increases plasma IGF-I maximally and independent of macronutrient intake may explain how colostrum ingestion also elicits a maximal response in brain, cardiac, and skeletal muscle protein synthesis (Burrin et al., 1995, 1997).

The increased circulating concentration of IGF-I in each of the three feeding groups was closely paralleled by a decreased mean plasma GH concentration, possibly indicative of a negative feedback effect of circulating IGF-I on GH secretion. The elevated levels of GH in late porcine fetal development, and the subsequent, precipitous ontogenic decline in circulating GH concentration after birth, may be attributed to an increase in the sensitivity of the pituitary hormones to the inhibitory feedback actions of somatostatin and IGF-I that develop soon after birth (Elsaesser et al., 1995; Torronteras et al., 1997). Indeed, our findings with newborn pigs (<24 h after birth) are consistent with a rapid maturation of this inhibitory feedback action between circulating IGF-I and GH secretion. Furthermore, any negative feedback effect of IGF-I on GH secretion was altered by GRF-A.

Implications

Findings from our study suggest that feeding colostrum elicits a maximal stimulation of circulating insulin-like growth factor (IGF-I) in newborn pigs. The colostrum-induced stimulation of circulating IGF-I can be partially attributed to nutrient intake, but not endogenous growth hormone secretion. The maximal stimulation of circulating IGF-I in colostrum-fed pigs is independent of macronutrient intake and is associated with increased protein synthesis and tissue growth. Although IGF-I has well-known anabolic effects, a direct link between circulating IGF-I and tissue growth is only speculative. Nevertheless, the results suggest that ingestion of colostrum may confer distinct advantages for growth and development on the newborn pig.

Literature Cited


