Response of skeletal muscle protein synthesis to insulin in suckling pigs decreases with development

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WRAY-CAHEN, DIANE, HANH V. NGUYEN, DOUGLAS G. BURRIN, PHILIP R. BECKETT, MARTA L. FIOROTTO, PETER J. REEDS, TIMOTHY J. WESTER, AND TERESA A. DAVIS. Response of skeletal muscle protein synthesis to insulin in suckling pigs decreases with development. Am. J. Physiol. 275 (Endocrinol. Metab. 38): E602–E609, 1998.—The elevated rate of muscle protein deposition in the neonate is largely due to an enhanced stimulation of skeletal muscle protein synthesis by feeding. To examine the role of insulin in this response, hyperinsulinemic-euglycemic-amino acid clamps were performed in 7- and 26-day-old pigs. Pigs were infused with 0, 30, 100, or 1,000 ng·kg⁻¹·min⁻¹ of insulin to mimic the plasma insulin levels observed under fasted, fed, refed, and supraphysiological conditions, respectively. Whole body amino acid disposal was determined from the rate of infusion of an amino acid mixture necessary to maintain plasma essential amino acid concentrations near their basal fasting levels. A flooding dose of L-[4-³H]phenylalanine was used to measure skeletal muscle protein synthesis. Whole body amino acid disposal increased progressively as the insulin infusion rate increased, and this response was greater in 7- than in 26-day-old pigs. Skeletal muscle protein synthesis was stimulated by insulin, and this response was maximal at a low insulin infusion rate (30 ng·kg⁻¹·min⁻¹). The stimulation of muscle protein synthesis by insulin was also greater in 7- than in 26-day-old pigs. These data suggest that muscle protein synthesis is more sensitive to insulin than whole body amino acid disposal. The results further suggest that insulin is a central regulatory factor in the elevated rate of muscle protein deposition and the increased response of skeletal muscle protein synthesis to feeding in the neonate.

The neonatal period is characterized by rapid growth rates, with high rates of protein synthesis to support this growth (8, 13, 19). Both the fractional rate of growth and that of protein synthesis decline with age from birth to adulthood. We have demonstrated previously that the fractional rate of protein synthesis in skeletal muscle is particularly high in the fed neonate and declines rapidly during the suckling period in both pigs and rats (6, 8). Much of this decline reflects a marked fall in the stimulation of skeletal muscle protein synthesis by feeding (6, 7, 9). This elevated stimulation of protein synthesis by feeding likely contributes to the high efficiency with which dietary amino acids are used for protein deposition in the neonate (10).

Studies in weaned, growing rats suggest that insulin is an important factor in the stimulation of skeletal muscle protein synthesis by nutrient intake (15, 16, 28). Studies performed in the ovine fetus (24) have also demonstrated that insulin can stimulate whole body protein synthesis without affecting whole body proteolysis. However, most amino acid kinetic studies conducted in adult humans suggest that the protein anabolic effect of insulin results from a decrease in the rate of proteolysis with no stimulation of protein synthesis (18, 20, 25). Taken together, these studies suggest that insulin stimulates muscle protein synthesis in growing but not in nongrowing animals. However, these studies do not indicate whether the enhanced stimulation of muscle protein synthesis by feeding in the neonate and its decrease with development are attributable to developmental differences in the response of muscle to the insulin that is secreted after a meal.

Because insulin stimulates protein accretion and cellular uptake of amino acids, experimentally induced hyperinsulinemia, in the absence of amino acid administration, is associated with a fall in both blood and intracellular amino acid concentrations (23). This fall in circulating amino acid concentrations with increasing insulin concentration is more profound and more rapid the younger the individual (33). Because a fall in amino acid concentrations could limit the ability of insulin to stimulate protein synthesis, the maintenance of basal levels of circulating amino acids during hyperinsulinemic-euglycemic clamps may be necessary for the observation of developmental changes in insulin-stimulated protein synthesis. Therefore, an amino acid clamp technique was developed (33) for use in conjunction with the hyperinsulinemic-euglycemic clamp technique (12) to determine the role of insulin, independent of changes in circulating amino acids, in the regulation of protein metabolism during early postnatal life. In our initial studies, we demonstrated that both the sensitivity and the responsiveness of whole body amino acid disposal to insulin decline over the suckling period (33). However, that study did not examine the extent to which the stimulation of amino acid disposal by insulin was due to an increase in protein synthesis or to a reduction in protein degradation.

The present study tested the hypothesis that the stimulation of skeletal muscle protein synthesis that occurs with feeding in the neonatal pig is mediated by insulin and that this response to insulin declines with early postnatal development in parallel with the previously observed (6) developmental decline in the stimulation of muscle protein synthesis by feeding. We further hypothesized that the response of skeletal muscle protein synthesis to insulin parallels the response of whole body amino acid disposal to insulin.
The specific aims of this study were 1) to determine the effect of stage of development on the response of skeletal muscle protein synthesis to the infusion of insulin, at rates which reproduce the circulating insulin concentrations observed under fasted, fed, refed, and supraphysiological conditions while circulating amino acids and glucose levels are maintained at fasting levels, and 2) to compare the insulin response for skeletal muscle protein synthesis and whole body amino acid disposal.

METHODS

Animals and surgery. Eleven multiparous sows of predominantly Yorkshire and Landrace stock were mated to boars of predominantly Hampshire and Duroc stock. One week before farrowing, sows were housed in lactation crates in individual, environmentally controlled rooms. Sows were maintained on a commercial diet (5084, PMI Feeds, Richmond, IN) throughout a 28-day lactation. After farrowing, piglets remained with the sow and were not given supplemental creep feed. Twenty-six piglets from five litters were studied at 6.5 ± 0.6 days of age (age range 6–7 days; 2.2 ± 0.3 kg) and 37 piglets from six litters were studied at 26.4 ± 1.0 days of age (age range 25–28 days; 7.3 ± 0.8 kg). 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.

Three to five days before the insulin infusion study, pigs were anesthetized and catheters were surgically inserted into a jugular vein and a carotid artery, as described previously [49]. The hyperinsulinemic-euglycemic-amino acid clamp procedure was initiated, five blood samples were acquired and immediately analyzed for blood glucose and BCAA concentrations. Immediately after blood glucose determination, an aliquot of blood was centrifuged at 14,000 rpm for 30 s (Eppendorf model 5415) and the plasma was analyzed for BCAA concentration. A 2.5-min enzymatic kinetic assay was used to determine the total BCAA concentration (2). The assay used the enzyme branched-chain dehydrogenase (EC 1.4.1.19) to catalyze the conversion of BCAA to their respective ketoacids and NADH in the presence of NAD+.

Blood samples were collected into heparinized syringes from the venous catheter. Between sampling times, the venous catheter was filled with physiological saline containing 30 IU/ml sodium heparin.

Over the 30-min basal period before the clamp procedure was initiated, five blood samples were acquired and immediately analyzed for blood glucose (YSI 2300 STAT Plus, Yellow Springs Instruments, Yellow Springs, OH) and for total branched-chain amino acids (BCAA) to establish the average basal concentration of blood glucose and plasma BCAA to be used in the subsequent euglycemic-amino acid clamp procedure. A basal sample also was obtained at this time for the later determination of plasma insulin and amino acid concentrations.

The hyperinsulinemic-euglycemic-amino acid clamp procedure used in this study was similar to the technique that we have previously described [33], except that BCAA were monitored instead of lysine. The BCAA assay (2) is more rapid than the lysine assay (3) and, in preceding trials, the amino acid clamps used in the BCAA assay gave similar results to those used in the lysine assay. During the clamp procedure, insulin was infused at 0, 30, 100, or 1,000 ng·kg⁻¹·min⁻¹.

In preliminary studies, we found that normalizing insulin infusion rates to body weight raised the 0.66 power resulted in the same insulin infusion rate (ng·kg⁻¹·0.66·min⁻¹), giving similar plasma insulin concentrations at both 7 and 26 days of age. Insulin stocks (100 and 1,000 mg/ml) were prepared beforehand by dissolving lyophilized insulin (Eli Lilly, Indianapolis, IN) in 0.01 N HCl and then mixing it with the diluent (sterile physiological saline containing 40 ml of filter-sterilized pig serum/1 ml saline). The insulin infusates were prepared by diluting the appropriate stock with the diluent just before infusion. Pigs that received no exogenous insulin received an infusion of the diluent. The concentration of insulin in the infusates was adjusted on an individual basis so that a fixed-volume infusion rate delivered the desired dose per kilogram raised to the 0.66 power. Depending on the size of the pig and dose of insulin infused, 5, 10, 20, 30, or 50% dextrose solutions were infused during the insulin infusion. Trophamine 10% (McGaw, Irvine, CA) was used as the mixed amino acid infusate during the clamp procedure. It was infused either undiluted or diluted with sterile physiological saline as appropriate for the insulin dose and weight of each pig. Undiluted, Trophamine 10% contains (g/100 ml): isoleucine, 0.82; leucine, 1.4; lysine, 0.82; methionine, 0.34; phenylalanine, 0.48; threonine, 0.42; tryptophan, 0.2; valine, 0.78; cysteine, <0.016; histidine, 0.48; tyrosine, 0.24; alanine, 0.54; arginine, 1.2; proline, 0.68; serine, 0.38; glycine, 0.36; aspartic acid, 0.32; glutamic acid, 0.5; and taurine, 0.025. Tyrosine was primarily in the N-acetyl-tyrosine form.

After a 10-min priming infusion, adapted from DeFronzo et al. (12), insulin was infused at a constant rate (12 ml/h). Venous blood samples (0.2 ml) were acquired every 5 min and immediately analyzed for glucose and BCAA concentrations. Immediately after blood glucose determination, an aliquot of blood was centrifuged at 14,000 rpm for 30 s (Eppendorf model 5415) and the plasma was analyzed for BCAA concentration. A 2.5-min enzymatic kinetic assay was used to determine the total BCAA concentration (2). The assay used the enzyme branched-chain dehydrogenase (EC 1.4.1.19) to catalyze the conversion of BCAA to their respective ketoacids and NADH in the presence of NAD+.

Larger blood volumes were acquired at 60, 90, 100, 110, 120, 180, 190, 200, 210, 215, 225, and 240 min; plasma was harvested and frozen for later metabolite analysis. Dextrose and amino acid infusions were initiated 3–6 min after the start of the insulin infusion and thereafter adjusted as necessary to maintain blood glucose and BCAA concentrations within 10% of the average basal concentration for each pig.

Muscle protein synthesis in vivo. The fractional rate of protein synthesis was measured with a flooding dose of L-[4-14C]phenylalanine (17) injected at 3.5 h after the initiation of the clamp procedure. Pigs were killed at 4 h, and samples of longissimus dorsi were collected and rapidly frozen. The specific radioactivities of the protein hydrolysate, homogenate supernatant, and blood supernatant were determined as previously described (8).

Plasma amino acids and insulin. The concentrations of individual plasma BCAA and phenylalanine from frozen plasma samples obtained after 0 and 210 min of the insulin infusions were measured with an HPLC method (9). Plasma containing methionine sulfone (internal standard) was filtered through a 10,000-molecular weight filter. Amino acids were precolumn derivitized with phenylisothiocyanate and separated on a PICO-TAG reverse-phase column (Waters, Milford, MA). Derivatized amino acids were detected on-line spectrophotometrically, and quantities were calculated using an amino acid standard (Pierce, Rockford, IL).

Plasma radioimmunoreactive insulin concentrations were measured using a porcine insulin radioimmunoassay kit (Linco, St. Louis, MO) that used porcine insulin antibody and insulin standards.
human insulin standards. The inter- and intra-assay coefficients of variation for insulin were 5.0 and 4.7%, respectively.

Calculations and statistics. The fractional rate of protein synthesis ($K_s$; percentage of protein mass synthesized in a day) was calculated as

$$K_s (\% / \text{day}) = \frac{[S_b / S_a] \times (1.440/t]}{100}$$

where $S_b$ is the specific radioactivity of the protein-bound phenylalanine, $S_a$ is the specific radioactivity of the tissue-free phenylalanine at the time of tissue collection and the linear regression of the blood specific radioactivity of the animal at 5, 15, and 30 min against time, and t is the time of labeling in minutes.

Individual amino acid concentrations at each insulin dose and age were compared with basal amino acid concentrations using Scheffe's Multiple-Comparison Test in general linear models analysis of variance (GLM ANOVA; NCSS 97, Number Cruncher Statistical Systems, Kaysville, UT). Statistical comparisons of plasma insulin concentrations, amino acid and glucose disposal rates (calculated from data obtained during the last hour of each infusion), and protein synthesis rates were made with GLM ANOVA to assess dose and age effects and their interaction. A nested design with pigs blocked by litter within age was used. When there were significant interactions between dose and age, pairwise comparisons of dose were made within age groups using Fisher's least significant difference test (29). A set of orthogonal contrasts designed to determine the lowest dose at which a maximal response was achieved was also used. Linear and quadratic regression analyses were performed to evaluate the relationship between glucose and amino acid disposal rates. Results are presented as means ± SE. Probability values of $P < 0.05$ were considered statistically significant and are not reported in the text.

**RESULTS**

Hyperinsulinemic-euglycemic-amino acid clamps. Figure 1 shows the time course of the mean blood glucose concentrations and the mean dextrose infusion rates necessary to maintain the fasting levels of blood glucose during the hyperinsulinemic-euglycemic-amino acid clamps in 7- and 26-day-old pigs in which insulin was infused at rates of 0, 30, 100, or 1,000 ng·kg$^{-2}$·min$^{-1}$ in 7- and 26-day-old pigs. Figure 1 also shows the time course of the mean plasma BCAA concentrations and the mean TrophAmine infusion rates (expressed in mmol BCAA·kg$^{-1}$·h$^{-1}$) necessary to maintain the fasting levels of plasma BCAA during the same clamps in 7- and 26-day-old pigs. The data show that circulating levels of both glucose and BCAA were generally well maintained within 10% of basal fasting values during the course of insulin infusions in both 7- and 26-day-old pigs. When no insulin was administered (0 ng insulin·kg$^{-2}$·min$^{-1}$), TrophAmine was not infused, because plasma BCAA did not fall over the infusion period at either age. The infusion rates of dextrose and TrophAmine increased with an increase in the insulin infusion rate. The rates of infusion of dextrose and TrophAmine were greater in 7- than in 26-day-old pigs, but these differences were more apparent for TrophAmine than for dextrose.

Plasma insulin. The younger pigs had slightly, but significantly, lower basal fasting plasma insulin concentrations than the 26-day-old pigs (Table 1). Similar concentrations of insulin were observed for both age groups at the 30 and 1,000 ng·kg$^{-2}$·min$^{-1}$ insulin infusion rates, but 7-day-old pigs had lower concentrations of insulin with the 100 ng·kg$^{-2}$·min$^{-1}$ insulin infusion rate. The insulin infusion rates of 30 and 100
1,000 ng·kg\(^{-0.66}\)·min\(^{-1}\) achieved the target insulin concentrations previously observed during the fed steady state and the refeed state when pigs had been fasted and then given a meal (6, 33). The highest insulin infusion rate of 1,000 ng·kg\(^{-0.66}\)·min\(^{-1}\) achieved supraphysiological insulin levels.

Plasma amino acids. Table 2 shows the circulating amino acid concentrations in the basal fasting condition and during the infusion of insulin, TrophAmine, and dextrose. Basal fasting concentrations of most

Table 2. Plasma concentrations of individual amino acids during euglycemic-amino acid clamps with increasing levels of insulin infusion in 7- and 26-day-old suckling pigs

<table>
<thead>
<tr>
<th>Age</th>
<th>Basal</th>
<th>Insulin Infusion Rate, ng·kg(^{-0.66})·min(^{-1})</th>
<th>0</th>
<th>30</th>
<th>100</th>
<th>1000</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Essential amino acids, nmol/ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arginine</td>
<td>7</td>
<td>128 ± 8</td>
<td>110 ± 20</td>
<td>198 ± 15</td>
<td>172 ± 15</td>
<td>171 ± 15*</td>
</tr>
<tr>
<td>Hidistidine</td>
<td>7</td>
<td>38 ± 3</td>
<td>32 ± 6</td>
<td>54 ± 5*</td>
<td>56 ± 5*</td>
<td>85 ± 5*</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>7</td>
<td>116 ± 6</td>
<td>86 ± 15*</td>
<td>118 ± 12</td>
<td>99 ± 12</td>
<td>132 ± 12</td>
</tr>
<tr>
<td>Leucine</td>
<td>7</td>
<td>146 ± 8</td>
<td>115 ± 20</td>
<td>173 ± 15</td>
<td>171 ± 15</td>
<td>174 ± 15</td>
</tr>
<tr>
<td>Lysine</td>
<td>7</td>
<td>124 ± 8</td>
<td>110 ± 19</td>
<td>147 ± 15</td>
<td>132 ± 15</td>
<td>172 ± 15*</td>
</tr>
<tr>
<td>Methionine</td>
<td>7</td>
<td>134 ± 6</td>
<td>147 ± 11</td>
<td>131 ± 12</td>
<td>149 ± 11</td>
<td>113 ± 12</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>7</td>
<td>94 ± 4</td>
<td>90 ± 10</td>
<td>105 ± 8</td>
<td>107 ± 8</td>
<td>132 ± 8*</td>
</tr>
<tr>
<td>Threonine</td>
<td>7</td>
<td>181 ± 11</td>
<td>148 ± 27</td>
<td>174 ± 21</td>
<td>128 ± 21</td>
<td>168 ± 21</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>7</td>
<td>46 ± 4</td>
<td>44 ± 10</td>
<td>59 ± 8</td>
<td>63 ± 8</td>
<td>89 ± 8</td>
</tr>
<tr>
<td>Valine</td>
<td>7</td>
<td>26 ± 3</td>
<td>74 ± 6</td>
<td>88 ± 6*</td>
<td>94 ± 6*</td>
<td>105 ± 6*</td>
</tr>
<tr>
<td>Alanine</td>
<td>7</td>
<td>237 ± 56</td>
<td>248 ± 44</td>
<td>231 ± 44*</td>
<td>244 ± 44</td>
<td></td>
</tr>
<tr>
<td>Asparagine</td>
<td>7</td>
<td>78 ± 9</td>
<td>35 ± 7*</td>
<td>47 ± 7*</td>
<td>13 ± 7*</td>
<td></td>
</tr>
<tr>
<td>Aspartate</td>
<td>7</td>
<td>8 ± 1</td>
<td>8 ± 2</td>
<td>8 ± 2</td>
<td>11 ± 1*</td>
<td>8 ± 1</td>
</tr>
<tr>
<td>Citrulline</td>
<td>7</td>
<td>67 ± 5</td>
<td>72 ± 19</td>
<td>67 ± 14</td>
<td>54 ± 14</td>
<td>67 ± 11</td>
</tr>
<tr>
<td>Glutamate</td>
<td>7</td>
<td>92 ± 8</td>
<td>86 ± 13</td>
<td>63 ± 10*</td>
<td>67 ± 10*</td>
<td>65 ± 10*</td>
</tr>
<tr>
<td>Glutamine</td>
<td>7</td>
<td>189 ± 28</td>
<td>175 ± 22</td>
<td>167 ± 22</td>
<td>97 ± 22*</td>
<td>127 ± 22*</td>
</tr>
<tr>
<td>Glycine</td>
<td>7</td>
<td>250 ± 16*</td>
<td>256 ± 34</td>
<td>262 ± 31*</td>
<td>256 ± 34*</td>
<td>256 ± 34*</td>
</tr>
<tr>
<td>Ornithine</td>
<td>7</td>
<td>212 ± 28</td>
<td>774 ± 55</td>
<td>709 ± 59*</td>
<td>581 ± 52*</td>
<td>481 ± 50*</td>
</tr>
<tr>
<td>Proline</td>
<td>7</td>
<td>41 ± 12</td>
<td>67 ± 10</td>
<td>61 ± 10</td>
<td>118 ± 10*</td>
<td></td>
</tr>
<tr>
<td>Serine</td>
<td>7</td>
<td>39 ± 4</td>
<td>48 ± 7</td>
<td>37 ± 8</td>
<td>46 ± 7*</td>
<td>43 ± 8</td>
</tr>
<tr>
<td>Taurine</td>
<td>7</td>
<td>164 ± 7</td>
<td>146 ± 17</td>
<td>157 ± 13</td>
<td>140 ± 13</td>
<td>140 ± 13</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>7</td>
<td>190 ± 5</td>
<td>157 ± 10</td>
<td>159 ± 10*</td>
<td>154 ± 9*</td>
<td>139 ± 10*</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>7</td>
<td>118 ± 4</td>
<td>125 ± 10</td>
<td>102 ± 8*</td>
<td>87 ± 8*</td>
<td>90 ± 8*</td>
</tr>
<tr>
<td>Valine</td>
<td>7</td>
<td>134 ± 3</td>
<td>158 ± 16</td>
<td>95 ± 6</td>
<td>81 ± 6</td>
<td>81 ± 6</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>7</td>
<td>81 ± 5</td>
<td>79 ± 12</td>
<td>49 ± 10*</td>
<td>56 ± 10*</td>
<td>19 ± 10*</td>
</tr>
</tbody>
</table>
| Dextrose infusion rate during the last hour of the hyperinsulinemic-euglycemic-amino acid clamps was not likely partitioned toward oxidation (33), the TrophAmine infusion rate was used as an indication of insulin-stimulated whole body net amino acid disposal. Figure 2 shows the average whole body amino acid disposal (expressed in nmol total amino acids·kg\(^{-1}\)·h\(^{-1}\)) during the last hour of the hyperinsulinemic-euglycemic-amino acid clamps. TrophAmine was not infused at the 0 ng·kg\(^{-0.66}\)·min\(^{-1}\) insulin infusion rate because amino acid disposal was greater in 7- than in 26-day-old pigs (P < 0.05).

Amino acid and glucose disposal. Because our previous studies suggested that the amino acids infused during hyperinsulinemic-euglycemic-amino acid clamp were not likely partitioned toward oxidation (33), the TrophAmine infusion rate was used as an indication of insulin-stimulated whole body net amino acid disposal.

- Amino acid disposal increased progressively with an increase in insulin infusion rate (P < 0.05) and decreased with age (P < 0.05).
- There was a significant interaction of insulin infusion rate and age (P < 0.05).
- The increase in amino acid disposal with increasing insulin infusion rate was greater in 7- than in 26-day-old pigs (P < 0.05).

Values are means ± SE; nos. of animals are shown in Table 1. *Amino acid values statistically significantly different (P < 0.05) from basal values.
acid disposal rates within any insulin infusion rate or age group. However, when all insulin infusion rates were considered, glucose disposal and amino acid disposal were linearly related in both 7- and 26-day-old pigs, with correlation (R²) values of 83 and 68%, respectively.

Muscle protein synthesis. The fractional rate of skeletal muscle protein synthesis in the basal fasting condition (0 ng·kg⁻⁰.⁶⁶·min⁻¹ insulin infusion rate) was nearly threefold higher in 7- than in 26-day-old pigs (Fig. 3). The infusion of insulin increased muscle protein synthesis in both 7- and 26-day-old pigs. The increase in muscle protein synthesis with the infusion of physiological levels of insulin in pigs was greater in 7- than in 26-day-old pigs, both in absolute terms (6.9 vs. 1.3 %/day) and as a percentage of basal fasting levels (58 vs. 30%). The maximum response of muscle protein synthesis to insulin was achieved by the lowest infusion rate of insulin (30 ng·kg⁻⁰.⁶⁶·min⁻¹) for both age groups. Although there was a response to supraphysiological levels of insulin in 7-day-old pigs, this response was less than that for the 100 ng·kg⁻⁰.⁶⁶·min⁻¹ insulin infusion rate. In 26-day-old pigs, supraphysiological levels of insulin did not stimulate muscle protein synthesis above basal levels.

DISCUSSION

We have demonstrated in previous studies that the stimulation of skeletal muscle protein synthesis by feeding is greater in young than in older suckling rats and pigs (6, 7, 9). Insulin levels, as well as those of circulating amino acids and glucose, increase rapidly after a meal. Therefore, in the current study, we wished to test the hypothesis that the developmental change in the response of muscle protein synthesis to feeding is due to a developmental change in the response to insulin. Because we had previously shown that circulating amino acid and glucose concentrations markedly and rapidly decrease during the infusion of insulin in young animals (33), it was necessary to use an amino acid clamp technique (33), in conjunction with the hyperinsulinemic-euglycemic clamp technique (12), to maintain substrate concentrations during the infusion of insulin. We selected insulin infusion rates that would result in circulating insulin concentrations which we had observed previously (6, 33) in suckling pigs in the fed state, in the fed steady state, and in the refed state shortly after a meal. We also examined the effects of a supraphysiological insulin level to determine whether maximum stimulation was achieved. The results of the present study demonstrated that low, physiological concentrations of insulin stimulated skeletal muscle protein synthesis in young pigs, and that this stimulatory effect of insulin was greater, in both absolute and proportional terms, at 7 than at 26 days of age. These results, together with those of our previous feeding study in suckling pigs (6), suggest that insulin is an important regulatory factor in the elevated response of protein synthesis to feeding in the neonate. Furthermore, comparison of the response of skeletal muscle protein synthesis and whole body amino acid disposal to insulin indicates that skeletal muscle protein synthesis is more sensitive than whole body amino acid disposal to insulin.

Hyperinsulinemic-euglycemic-amino acid clamp. In our previous study in which we investigated the developmental changes in the response of amino acid disposal to insulin (33), lysine was used as the representative amino acid to monitor circulating amino acid concentrations during the infusion of insulin, with the aim of maintaining circulating amino acid concentrations at the basal fasting level. In the current study, the circulating concentrations of the BCAA were monitored during the clamp procedure because the assay is more rapid (2) than the one for lysine (3). Rapid adjustment of the amino acid infusion rate is particularly important in young animals, which have high amino acid turnover rates (13, 33). Comparison of the results from the two hyperinsulinemic-euglycemic-amino acid clamp studies that used either the BCAA or lysine assays to monitor circulating amino acid concentrations indicates that the circulating levels of individual amino acids achieved at the different plasma insulin concentrations were similar in most cases. Use of the amino acid clamp technique prevented the fall in circulating essential amino acid concentrations that occurs (33) when amino acids are not infused concurrently with insulin. This drop in circulating amino acid concentrations when an amino acid mixture is not infused (33) is quite marked (decreasing to ~40% of basal) and occurs at insulin concentrations that are within the physiological range (20 μU/ml). Although there was a modest increase in the circulating concentrations of some of the essential amino acids during the hyperinsulinemic-euglycemic-amino acid clamp, the increase was not of the magnitude that occurs with feeding (6, 9). Circulat-
Studies by Garlick and co-workers (15, 28) suggest that to a lesser extent, if at all, in adult mammals (1, 27, 31). The stimulation of muscle protein synthesis by feeding is present in newborn humans (13). However, the stimulation of muscle protein synthesis in weaned, growing rats (14, 7, 9). Feeding has also been demonstrated to stimulate muscle protein synthesis at the lowest insulin infusion rate was nearly fourfold greater in 7- than in 26-day-old pigs, which is consistent with a higher insulin sensitivity during the early postnatal period. By contrast, glucose disposal rate at the highest insulin infusion rate was only 1.2-fold higher in 7- than in 26-day-old pigs and at the lowest insulin infusion rate was only 2-fold higher in 7- than in 26-day-old pigs. This suggests that the developmental change in insulin responsiveness and sensitivity of amino acid disposal may be greater than that for glucose disposal; however, full insulin dose-response curves are required to confirm this supposition. Differences in the insulin sensitivity of amino acid and glucose metabolism have been reported previously (11, 25) and are consistent with the multiple intracellular signaling pathways for insulin's action (34).

Skeletal muscle protein synthesis. Our previous studies in suckling pigs and rats demonstrated that skeletal muscle protein synthesis is stimulated by feeding and that this response is greater the younger the animal (6, 7, 9). Feeding has also been demonstrated to stimulate muscle protein synthesis in weaned, growing rats (14) and lambs (27) and to elevate whole body protein synthesis in newborn humans (13). However, the stimulation of muscle protein synthesis by feeding is present to a lesser extent, if at all, in adult mammals (1, 27, 31). Studies by Garlick and co-workers (15, 28) suggest that insulin mediates the stimulation of skeletal muscle protein synthesis by feeding in weaned, growing rats. They showed that the feeding response could be blocked by coadministration of anti-insulin serum (28) and that the infusion of physiological levels of insulin into fasted, weaned rats could restore muscle protein synthesis to rates similar to those found in the fed state (15). However, studies in adult rats and humans show little, if any, response of muscle protein synthesis to the infusion of insulin (1, 17, 20, 25).

The results of the present study demonstrate that increasing circulating insulin concentrations to the level present in the fed steady state (33), while maintaining amino acids and glucose near the fasting level, resulted in a maximal stimulation of muscle protein synthesis in suckling pigs. Higher physiological levels of insulin, similar to those present in the refed state shortly after the consumption of a meal (6), produced no further stimulation of skeletal muscle protein synthesis. These results are consistent with our previous results in young rats, which demonstrated that the fractional rates of skeletal muscle protein synthesis are similar in the fed steady state and in the refed state (9). Pigs receiving supraphysiological levels of insulin did not achieve the maximum protein synthesis response. The results of the current study also indicate that the stimulation of skeletal muscle protein synthesis by insulin decreases during early postnatal development. The maximum stimulation of skeletal muscle protein synthesis by insulin was 58% at 7 days but was only 30% at 26 days of age. Because the basal fasting rates of muscle protein synthesis were much higher at 7 than at 26 days of age, differences between the age groups in their maximum response to insulin were even more pronounced when expressed in absolute terms. In addition, the response to supraphysiological levels of insulin was only 23% over basal in the 7-day-old pigs, but there was no response at the highest insulin infusion rate in the 26-day-old pigs. The magnitude of the stimulation of skeletal muscle protein synthesis by physiological concentrations of insulin in the current study and the decrease in this response with age were similar to the response pattern we have previously observed in suckling pigs after refeeding (6). Because the plasma insulin concentrations achieved during the infusion of insulin were similar to those previously observed with feeding (6), and the circulating amino acid and glucose concentrations remained near the fasting level, the results suggest that the developmental change in the stimulation of skeletal muscle protein synthesis by feeding is due to a developmental decrease in the response to the elevation in circulating insulin (rather than amino acids and glucose) that occurs with eating.

The reduced effect of supraphysiological levels of insulin on skeletal muscle protein synthesis is difficult to interpret. During the insulin infusions, we chose to infuse an amino acid mixture (TroPhAmine) that was designed for use in human neonates. Nonetheless, the circulating concentrations of some of the nonessential amino acids fell, particularly at the high insulin infusion rates, but this decline was not as great as that observed when amino acids were not infused concurrently with insulin in a previous study (33). Whole body amino acid and glucose disposal. The amino acid infusion rate required to maintain circulating BCAA is equal to the net balance of BCAA disappearance from the blood into the tissues and the appearance of BCAA in the blood from the tissues, if amino acid catabolism remains constant. In our previous hyperinsulinemic-euglycemic-amino acid clamp studies (33), urea nitrogen levels in the blood (BUN), an indication of amino acid catabolism, either decreased or remained the same from baseline to the end of the insulin infusions. These results are consistent with studies in adult humans in which BUN concentrations, urea nitrogen excretion, and leucine oxidation either decreased or did not change during hyperinsulinemic-euglycemic clamps in which amino acids were either clamped or unclamped (14, 30). Hence, the amino acid infusion rate is a reasonable indicator of net whole body amino acid disposal, although it does not indicate the proportion of the amino acids that can be attributed to an increase in protein synthesis or to a decrease in protein degradation.

The results of the current study confirm those of our previous study (33) that insulin-stimulated whole body amino acid disposal decreases with development in neonatal pigs. The results demonstrated that amino acid disposal at the highest insulin infusion rate was twofold higher in 7- than in 26-day-old pigs, suggesting an increased responsiveness to insulin in the younger pigs. Amino acid disposal at the lowest insulin infusion rate was nearly fourfold greater in 7- than in 26-day-old pigs, which is consistent with a higher insulin sensitivity during the early postnatal period. By contrast, glucose disposal rate at the highest insulin infusion rate was only 1.2-fold higher in 7- than in 26-day-old pigs and at the lowest insulin infusion rate was only 2-fold higher in 7- than in 26-day-old pigs. This suggests that the developmental change in insulin responsiveness and sensitivity of amino acid disposal may be greater than that for glucose disposal; however, full insulin dose-response curves are required to confirm this supposition. Differences in the insulin sensitivity of amino acid and glucose metabolism have been reported previously (11, 25) and are consistent with the multiple intracellular signaling pathways for insulin's action (34).
amino acids, particularly glutamine and glycine, were markedly lower at the highest insulin concentrations. Therefore, we postulate that nonessential amino acids or, indeed, nitrogen, may have become limiting. We are currently investigating this possibility by using a modified amino acid mixture.

Because amino acids were clamped near the fasting level and insulin was elevated in the current study, the specific role of amino acids in the regulation of muscle protein synthesis in the neonate remains to be determined. Studies in weaned, growing rats suggest that an elevation in circulating amino acids enhances the sensitivity of muscle protein synthesis to insulin but that amino acid infusion alone does not stimulate muscle protein synthesis (16). In contrast, studies in adult humans and pigs have provided conflicting results, suggesting that amino acid infusion alone (5) or amino acids with an accompanying infusion of insulin (4, 32) stimulate protein synthesis.

The potential mechanisms that underlie the high rates of both basal and insulin-stimulated skeletal muscle protein synthesis in the neonate are intriguing. Previous studies in rats and pigs have demonstrated that the developmental decline in the fractional rate of protein synthesis is strongly associated with a progressive decline in the RNA-to-protein ratio (6–9). This suggests that the high basal rates of muscle protein synthesis in the 7- vs. 26-day-old pigs is likely due to an elevated ribosomal number. We postulate that the developmental changes in insulin-stimulated skeletal muscle protein synthesis during the neonatal period may be associated with developmental changes in the phosphorylation state or availability of proteins involved in the initiation of the translation of mRNA. Recent studies performed in L6 myoblasts and perfused rat hindlimbs suggest that insulin stimulates muscle protein synthesis through changes in the activity of the eukaryotic initiation factor (eIF) 4E and the eIF-4E-binding proteins 4E-BP1 and eIF-4G, which are involved in the binding of mRNA to the 40S ribosomal subunit (21, 22).

Comparison of the response of whole body amino acid disposal and skeletal muscle protein synthesis to insulin. A dominant factor determining the high rate of whole body protein synthesis in the early postnatal period appears to be the elevated rate of skeletal muscle protein synthesis (6, 13, 19). Therefore, in the current study, we wished to determine whether the response of skeletal muscle protein synthesis to insulin would parallel the insulin response of whole body amino acid disposal. The results showed that the maximum stimulation by insulin of muscle protein synthesis, but not whole body amino acid disposal, was achieved at the low insulin infusion rate of 30 ng·kg

\[-0.66\] min

\[-1\] in both 7- and 26-day-old pigs. Indeed, at the 30 ng·kg

\[-0.66\] min

\[-1\] insulin infusion rate, whole body amino acid disposal was only 60% of that at the 1,000 ng·kg

\[-0.66\] min

\[-1\] insulin infusion rate in 7-day-old pigs and was only 30% of that at the 1,000 ng·kg

\[-0.66\] min

\[-1\] insulin infusion rate in 26-day-old pigs. This suggests that skeletal muscle protein synthesis is more sensitive to insulin than is whole body amino acid disposal. This comparison implies that, although the uptake of amino acids by skeletal muscle for the synthesis of proteins contributes to insulin-stimulated whole body amino acid disposal, the appearance of amino acids from the blood due to proteolysis may also contribute to insulin-stimulated whole body amino acid disposal, particularly at higher insulin concentrations. In addition, the synthesis of proteins in visceral tissues may also contribute to whole body amino acid disposal, but maximal stimulation could be achieved at high plasma insulin levels, indicating a reduced sensitivity to insulin in visceral tissues compared with skeletal muscle.

Perspectives. Our results indicate that insulin stimulates skeletal muscle protein synthesis in young pigs and that this response to insulin declines over the suckling period. Because the plasma insulin concentrations achieved during the infusion of insulin were similar to those previously observed with feeding (6, 33), and circulating glucose and amino acid concentrations remained near the fasting level, the results suggest that the developmental change in the stimulation of skeletal muscle protein synthesis by feeding is due to a developmental change in the response to insulin. This developmental decrease in the stimulation of muscle protein synthesis by insulin is consistent with the developmental change in the response of whole body amino acid disposal to insulin (33). This enhanced response to insulin likely contributes to the more efficient use of dietary amino acids for growth in the neonate, because more of the dietary amino acids could be partitioned toward muscle protein synthesis than toward catabolism. Thus the enhanced response to insulin may play a crucial role in maintaining the high growth rate of skeletal muscle in the neonate. The greater insulin sensitivity of skeletal muscle protein synthesis than whole body amino acid disposal suggests that visceral tissue protein synthesis may be less sensitive to insulin and/or that a suppression of proteolysis is most pronounced at the higher insulin concentrations. Further study will be required to examine these possibilities.

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