Stimulation of protein synthesis by both insulin and amino acids is unique to skeletal muscle in neonatal pigs

TERESA A. DAVIS, MARTA L. FIOROTTO, DOUGLAS G. BURRIN, PETER J. REEDS, HANH V. NGUYEN, PHILIP R. BECKETT, RHONDA C. VANN, AND PAMELA M. J. O’CONNOR

United States Department of Agriculture/Agricultural Research Service, Children’s Nutrition Research Center, and Endocrinology and Metabolism Section, Department of Pediatrics, Baylor College of Medicine, Houston, Texas 77030

Received 16 November 2001; accepted in final form 18 December 2001

DURING THE NEONATAL PERIOD, the gain in protein mass in skeletal muscle is more rapid than in other tissues of the body during the early postnatal period (12, 18). This developmental decline in skeletal muscle protein synthesis varies with fiber type and is accompanied by a decrease in ribosome number and a specific accumulation of myofibrillar proteins (18, 25).

The efficiency with which dietary amino acids are utilized for protein deposition is also high in the neonate and declines markedly with development (20, 40). This high efficiency is likely due to the enhanced stimulation of protein synthesis in response to feeding (12, 15, 17, 19). The feeding-induced stimulation of protein synthesis occurs in virtually all tissues of the neonate; however, the postprandial rise in protein synthesis and the developmental decline in the response to feeding are most pronounced in skeletal muscle, particularly in those muscles that are primarily fast twitch, glycolytic (8–10, 12, 15, 17, 19). The developmental decline in the response to feeding in muscle parallels the marked fall in the nutrient-induced activation of translation initiation factors that regulate the binding of mRNA to the 40S ribosomal subunit (21).

Insulin is likely a central regulatory factor in the elevated rate of muscle protein deposition in the neonate. Results of studies, by use of the hyperinsulinemic-euglycemic-euaminoacidemic clamp technique originally developed in our laboratory, show that insulin stimulates whole body amino acid disposal in the neonate and that both the insulin sensitivity and the responsiveness of amino acid disposal are attenuated with the advancement of development (61). When insulin is infused at doses reproducing plasma insulin levels observed under fed conditions and essential amino acids and glucose are clamped at fasting levels, skeletal muscle protein synthesis increases to values observed in the fed state (62). The stimulation of protein synthesis by insulin, like the response to feeding, is greater in muscles that are predominantly fast twitch glycolytic and is not specific to myofibrillar proteins (14). This response to insulin decreases with age.
development (14, 62), in parallel with the developmental
decline in the stimulation of muscle protein synthesis
by feeding (12). Recent studies suggest that the
developmental decline in the response of skeletal mus-
cle protein synthesis to food consumption results from
a reduction in the effectiveness of the insulin-signaling
pathway to transduce to the translation apparatus the
stimulus provided by feeding (35, 37, 53).

A recent study from this laboratory (14) suggests
that the feeding-induced stimulation of visceral tissue
protein synthesis in the neonate cannot be attributed
to insulin. We found that the infusion of insulin in the
neonate stimulates protein synthesis in skeletal mus-
cle, cardiac muscle, and skin, but insulin did not stim-
ulate, and in fact tended to lower, protein synthesis in
liver, intestine, pancreas, and kidney (14). The ability
of amino acids to stimulate liver protein synthesis is
controversial, but limited studies suggest that amino
acids increase protein synthesis in the liver of immatu-
re (27, 51) but not mature (3, 44) animals. In con-
trast, amino acids appear to stimulate protein synthe-
sis in the skeletal muscle of young, adult, and elderly
populations (6, 41, 49, 58, 59). Therefore, we hypothe-
sized that protein synthesis in all tissues of the neo-
rate is responsive to the postprandial rise in amino
acid concentrations.

During the hyperinsulinemic-euglycemic-euamino-
acidemic clamps in previous studies, we infused Tro-
phamine, an amino acid mixture designed for use
in neonates (14, 61, 62). However, in these studies,
detailed examination of amino acid concentrations
during “euaminoacidemic”-hyperinsulinemic-euglycemic
clamps showed that the circulating concentrations of
some of the nonessential amino acids were lower at the
highest insulin concentrations, which could potentially
limit the availability of substrate for protein synthesis.
Therefore, we developed a new amino acid clamp mix-
ture designed specifically to keep the concentrations of
all amino acids constant during insulin stimulation.
We determined, in 7- and 26-day-old pigs, the response
to feeding-induced stimulation of visceral tissue
protein synthesis to 1) the infusion of insulin at a dose that reproduces insulin levels in the fed state
while circulating amino acids and glucose are main-
tained at fasting levels, 2) the infusion of amino acids
at a dose that reproduces fed-state amino acid levels
while insulin and glucose are kept at fasting levels, and
3) the infusion of insulin and amino acids at doses that
reproduce insulin and amino acid levels in the fed state
while glucose is kept at fasting level.

METHODS

Animals and surgery. Multiparous crossbred (Yorkshire ×
Landrace × Hampshire × Duroc) sows (n = 8; Agriculture
Headquarters, Texas Department of Criminal Justice,
Huntsville, TX) were housed in lactation crates in individual,
environmentally controlled rooms and maintained on a
commercial diet (5084, PMI Feeds, Richmond, IN) throughout
a 28-day lactation period. Sows delivered 8–12 piglets, and
these remained with the sows for nursing. Piglets were not
given supplemental creep feed. Piglets weighing 2.0 ± 0.1 kg
were studied at 7 days of age (n = 35), and piglets weighing
7.2 ± 0.1 kg were studied at 26 days of age (n = 39). Three
to five days before the insulin infusion study, pigs were anes-
thetized, and catheters were inserted surgically into a jugu-
lar vein and a carotid artery (61). Piglets were returned to
the sows until studied. The protocol was approved by the Animal
Care and Use Committee of Baylor College of Medicine.
The study was conducted in accordance with the National Re-
search Council’s Guide for the Care and Use of Laboratory
Animals.

Infusions. Pigs were placed unanesthetized in a sling
restraint system after a 12-h fast. Pigs were assigned to one of
two treatment groups: 1) fasting (Control), 2) hyperinsulinene-
ic-euglycemic-euaminoacidemic clamp (Insulin), 3) eun-
sulinemic-euglycemic-hyperaminoacidemic clamp (AA), and
4) hyperinsulinemic-euglycemic-hyperaminoacidemic clamp
(Insulin + AA). Body weights were similar in each treatment
group at each age studied. During a 30-min basal period,
blood samples were obtained and immediately analyzed for
blood glucose (YSI 2300 STAT Plus, Yellow Springs Instruments,
Yellow Springs, OH) to establish the average basal concen-
tration of blood glucose to be used in the subsequent eugly-
cemic clamp procedure (22). Plasma samples were analyzed
for total branched-chain amino acids (BCAA), by use of a
rapid enzymatic kinetic assay (5) to establish the average
basal concentration of BCAA to be used in the subsequent
euaminoacidemic or hyperaminoacidemic clamp procedure.

The clamps were initiated with a primed, constant (12 ml/h)
infusion of insulin (Eli Lilly, Indianapolis, IN) at either 0 or
100 ng·kg⁻¹·min⁻¹. Venous blood samples (0.2 ml) were
every 5 min and immediately analyzed for glucose
and BCAA concentrations. The infusion rate of dextrose
(Baxter Healthcare, Deerfield, IL) was adjusted as necessary
to maintain the blood glucose concentration within ±10% of
the average basal concentration. Euaminoacidemia was ob-
tained by adjusting the infusion rate of an amino acid mix-
ture to maintain the plasma BCAA concentration within 10%
of the fasting level. Hyperaminoacidemia was obtained by
infusing an amino acid mixture to raise plasma BCAA con-
centrations to twofold the fasting level to reproduce the level
of amino acids present in the fed state (12).

A new amino acid mixture was developed for use as an
infusion during the euaminoacidemic and hyperaminoac-
demic clamp procedures. The composition of the new amino
acid mixture was based on the amino acid composition of
body protein (20) and our previous observations on the
changes in plasma amino acid concentrations when 10% Trophamine (McGaw, Irvine, CA) was infused during hyper-
insulinemic-euglycemic-euaminoacidemic clamp studies (14,
61, 62). The new mixture utilized two dipeptides, glycyl-
lysine and alanyl-glutamine, to avoid the problem of poor
solubility of tyrosine and instability of glutamine and con-
tained higher concentrations of some of the nonessential amino acids than commercial amino acid mixtures. The new amino acid mixture contained (millimolar) arginine (20.1), histidine
(12.9), isoleucine (28.6), leucine (34.3), lysine (27.4), methio-
nine (10.1), phenylalanine (12.1), threonine (21.0), trypto-
phan (4.4), valine (34.1), alanine (27.3, 38% provided as
alanyl-glutamine), aspartate (12.0), cysteine (6.2), glutamate
(23.8), glutamine (17.1, 100% provided as alanyl-glutamine),
glycine (54.3, 4% provided as glycyl-tyrosine), proline (34.8),
serin (23.8), taurine (2.0), and tyrosine (7.2, 83% provided as
glycyl-tyrosine).

Tissue protein synthesis in vivo. Fractional rates of protein
synthesis were measured with a flooding dose of L-[4,6-³H]phe-
nylalanine (29) injected 3.5 h after the initiation of the clamp.
Blood samples were taken at 5, 15, and 30 min after the
injection for measurement of the specific radioactivity of the
extracellular free pool of phenylalanine. Pigs were killed at 4 h after the initiation of the clamp. Samples of longissimus dorsi, gastrocnemius, masseter, and cardiac muscles and skin, liver, jejunum, pancreas, kidney, and spleen were collected, rapidly frozen in liquid nitrogen, and stored at −70°C.

Total protein was isolated from all tissues sampled, and sarcoplasmic and myofibrillar proteins were isolated in longissimus dorsi muscle of 7-day-old pigs as described previously (18, 25). The specific radioactivities of the protein hydrolysates, homogenate supernatants, and blood supernatants were determined as previously described (18).

**Plasma insulin and amino acids.** Plasma immunoreactive insulin concentrations were measured using a porcine insulin radioimmunoassay kit (Linco, St. Louis, MO). Plasma amino acid concentrations were measured with a high-performance liquid chromatography method (PICO-TAG reverse-phase column, Waters, Milford, MA) as previously described (19).

**Calculations and statistics.** Fractional rates of protein synthesis (K_s) percent protein mass synthesized in a day) were 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 tissue-free phenylalanine, S_a is the specific radioactivity of the tissue free phenylalanine for the labeling period determined from the value of the animal at the time of the tissue collection, corrected by the linear regression of the blood specific radioactivity of the animal against time, and t is the time of labeling in minutes. We have demonstrated that, after a flooding dose of phenylalanine is administered, the specific radioactivity of tissue free phenylalanine is in equilibrium with the aminoacyl-tRNA specific radioactivity, and therefore, the tissue free phenylalanine is a valid measure of the tissue precursor pool specific radioactivity (16).

Three-way analysis of variance was used to assess the effect of insulin, amino acids, age, and their interaction on tissue protein synthesis. When significant interactions were detected, the value in each treatment group for each age was compared with the control value by use of t-tests. To determine the effect of the clamp procedure, individual amino acid, glucose, and insulin concentrations in each treatment group were compared with their basal concentrations by use of t-tests. Differences of P < 0.05 were considered statistically significant for all comparisons except those for plasma amino acid concentrations. Because there was an increased probability that one of the 22 amino acid comparisons in each of the four treatment groups would be significantly different among groups by random chance, a more conservative statistical approach for amino acid comparisons was used; therefore, probability values of < 0.01 were considered statistically different. Results are presented as means ± SE.

**RESULTS**

**Infusions.** Pigs at 7 and 26 days of age were infused with insulin, glucose, amino acids, and/or saline to reproduce 1) fasting insulin, glucose, and amino acid levels (Control group); 2) fed insulin, fasting glucose, and fasting amino acid levels (hyperinsulinemic-euglycemic-euaminoacidemic clamp; Insulin group); 3) fasting insulin, fasting glucose, and fed amino acid levels (euinsulinemic-euglycemic-hyperaminoacidemic clamp; AA group); and 4) fed insulin, fasting glucose, and fed amino acid levels (hyperinsulinemic-euglycemic-hyperaminoacidemic clamp; Insulin+AA group). Table 1 shows that the circulating insulin concentrations normally found in the neonatal pig in the fasting condition and after a meal (3 and 30 μU/ml, respectively) were largely reproduced in both 7- and 26-day-old pigs by the infusion of 0 and 100 ng·kg⁻¹·min⁻¹. Hyperaminoacidemia did not alter circulating insulin concentrations. Plasma glucose concentrations were maintained at basal fasting levels during the infusion of insulin and/or amino acids in 7- and 26-day-old pigs (Table 1).

Circulating essential and nonessential amino acid concentrations in control and in insulin- and/or amino acid-infused 7- and 26-day-old pigs are compared with baseline (0 time) values in Fig. 1. During the 4-h saline infusion period in control pigs, plasma amino acid concentrations remained stable in 7-day-old pigs, although leucine, isoleucine, and lysine increased and alanine and glutamate decreased in 26-day-old pigs (P < 0.01). Because circulating nonessential amino acid concentrations decreased in our previous hyperinsulinemic-euglycemic-euaminoacidemic clamp studies, which utilized Trophamine as the amino acid infusate (14, 62), in the present study, we developed a new amino acid mixture, which contains higher concentrations of nonessential amino acids than Trophamine. The circulating concentrations of both nonessential and essential amino acids were maintained largely at the fasting level during the hyperinsulinemic-euglycemic-euaminoacidemic clamp with the new amino acid infusate. The exceptions were elevations in histidine and tryptophan and a reduction in asparagine (P < 0.01). Hyperaminoacidemic clamps in the presence of euinsulinemia or hyperinsulinemia increased the circulating concentrations of essential amino acids about twofold and those of nonessential amino acids ~50% (P < 0.01).

Figure 2 shows the net whole body amino acid and glucose disposal rates, as indicated by the amino acid and glucose infusion rates during the last hour of the infusion period. Amino acids were not infused in the control animals, because plasma amino acid concentrations in control animals, because plasma amino acid concentrations in control and in insulin- and/or amino acid-infused 7- and 26-day-old pigs are compared with baseline (0 time) values in Fig. 1. During the 4-h saline infusion period in control pigs, plasma amino acid concentrations remained stable in 7-day-old pigs, although leucine, isoleucine, and lysine increased and alanine and glutamate decreased in 26-day-old pigs (P < 0.01). Because circulating nonessential amino acid concentrations decreased in our previous hyperinsulinemic-euglycemic-euaminoacidemic clamp studies, which utilized Trophamine as the amino acid infusate (14, 62), in the present study, we developed a new amino acid mixture, which contains higher concentrations of nonessential amino acids than Trophamine. The circulating concentrations of both nonessential and essential amino acids were maintained largely at the fasting level during the hyperinsulinemic-euglycemic-euaminoacidemic clamp with the new amino acid infusate. The exceptions were elevations in histidine and tryptophan and a reduction in asparagine (P < 0.01). Hyperaminoacidemic clamps in the presence of euinsulinemia or hyperinsulinemia increased the circulating concentrations of essential amino acids about twofold and those of nonessential amino acids ~50% (P < 0.01).

Table 1. Plasma insulin and glucose concentrations in response to insulin and/or amino acid infusion in 7- and 26-day-old pigs

<table>
<thead>
<tr>
<th>Age</th>
<th>Basal</th>
<th>Control</th>
<th>Insulin</th>
<th>AA</th>
<th>Insulin + AA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 days</td>
<td>2.6 ± 0.2</td>
<td>2.2 ± 0.2</td>
<td>27.2 ± 2.1</td>
<td>2.7 ± 0.1</td>
<td>28.3 ± 1.7</td>
</tr>
<tr>
<td>26 days</td>
<td>3.2 ± 0.3</td>
<td>2.3 ± 0.2</td>
<td>27.3 ± 1.9</td>
<td>3.4 ± 0.2</td>
<td>35.0 ± 1.2</td>
</tr>
<tr>
<td>Glucose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 days</td>
<td>76 ± 2</td>
<td>75 ± 2</td>
<td>78 ± 3</td>
<td>81 ± 3</td>
<td>78 ± 3</td>
</tr>
<tr>
<td>26 days</td>
<td>72 ± 2</td>
<td>71 ± 3</td>
<td>77 ± 3</td>
<td>70 ± 3</td>
<td>72 ± 3</td>
</tr>
</tbody>
</table>

Plasma concentrations of insulin in μU/ml and glucose in mg/dl are means ± SE; n = 7–12 per age and treatment group. *Insulin values in hyperinsulinemic-euglycemic-euaminoacidemic clamp (Insulin) and hyperinsulinemic-euglycemic-hyperaminoacidemic clamp (Insulin+AA) groups, but not Control or euinsulinemic-euglycemic-hyperaminoacidemic clamp (AA) groups, were significantly greater than basal, preinfusion values (P < 0.001). Glucose values in all treatment groups were not statistically different from basal values (P > 0.05).

AJP-Endocrinol Metab • VOL 282 • APRIL 2002 • www.aipendo.org
tions did not fall below 10% of the basal level. During the infusion of either insulin or amino acids, the amino acid disposal rates were about twofold greater in 7- than in 26-day-old pigs ($P < 0.01$), but the glucose disposal rates did not differ significantly with age. The effects of insulin and amino acids on amino acid disposal were additive.

**Tissue protein synthesis.** Fractional rates of protein synthesis in skeletal muscles of different fiber types in 7- and 26-day-old pigs are shown in Fig. 3. Under fasting conditions, the fractional rates of protein synthesis in longissimus dorsi, gastrocnemius, and masseter muscles were about threefold, twofold, and 50% higher in 7- than in 26-day-old pigs, respectively ($P < 0.001$). Hyperinsulinemia increased ($P < 0.005$) protein synthesis in longissimus dorsi (57%), gastrocnemius (64%), and masseter (36%) muscles, and these responses decreased with age ($P < 0.001$). Hyperaminoacidemia also increased ($P < 0.01$) protein synthesis in longissimus dorsi (48%), gastrocnemius (50%), and masseter (28%) muscles ($P < 0.01$). The amino acid response also decreased with age ($P < 0.05$). In contrast to whole body amino acid disposal, the combination of hyperinsulinemia and hyperaminoacidemia did not exert an additive effect on muscle protein synthesis ($P > 0.05$).

To determine whether the stimulation of skeletal muscle protein synthesis by insulin and/or amino acids is specific to myofibrillar proteins, protein synthesis rates in myofibrillar and sarcoplasmic proteins were determined in longissimus dorsi muscle of 7-day-old pigs in the four treatment groups. Hyperinsulinemia and hyperaminoacidemia, either alone or in combination, increased protein synthesis in both myofibrillar and sarcoplasmic protein fractions to a similar extent (60–80%; Fig. 4). Rates of protein synthesis did not differ between the two protein fractions in any treatment group.

Fractional rates of protein synthesis in cardiac muscle, skin, and spleen of 7- and 26-day-old pigs are shown in Fig. 5. The fractional rates of protein synthesis in cardiac muscle, skin, and spleen in the basal fasting condition were 15, 90, and 30% higher in 7- than in 26-day-old pigs, respectively ($P < 0.01$). Hyperinsulinemia increased protein synthesis in cardiac
muscle (50%; \(P < 0.001\)), skin (34%; \(P < 0.001\)), and spleen (26%; \(P < 0.05\)), and these responses to insulin decreased with age (\(P < 0.05\)). Hyperaminoacidemia had no effect on protein synthesis in these tissues. The combination of hyperinsulinemia and hyperaminoacidemia had no greater effect on protein synthesis than the effect of hyperinsulinemia alone (\(P > 0.05\)).

**DISCUSSION**

**Regulation of skeletal muscle protein synthesis by insulin and amino acids.** Current and previous studies suggest that insulin plays a key role in the elevated

The fractional rates of protein synthesis in the jejunum of 7- and 26-day-old pigs are shown in Fig. 7. The fractional rate of protein synthesis in jejunum was higher in 7- than in 26-day-old pigs (\(P < 0.001\)). Neither hyperinsulinemia nor hyperaminoacidemia significantly altered the rate of protein synthesis in the jejunum (\(P > 0.05\)).
rate of protein deposition and the increased response of skeletal muscle protein synthesis to feeding in the neonate. First, postprandial changes in protein synthesis in neonatal pigs are positively correlated with changes in circulating insulin concentrations (13). Second, when amino acids and glucose are maintained near fasting levels, insulin infusion increases amino acid disposal in the neonatal pig and ovine fetus (57, 61). The insulin sensitivity and responsiveness of amino acid disposal decrease with development. Third, raising insulin concentrations in the neonatal pig to levels typical of the fed state increases the rate of skeletal muscle protein synthesis to within the range normally present in the fed state, even when essential amino acids and glucose are maintained at fasting levels (21, 62). This response to insulin, like the response to feeding, is attenuated with development. Fourth, insulin infusion stimulates whole body and skeletal muscle protein synthesis in the fetal sheep, young lamb, and weaned rat (28, 38, 60) but has little, if any, effect in the adult human or rat (4, 30, 32, 39, 41). These findings suggest that the ability of skeletal muscle protein synthesis to respond to the postprandial rise in insulin wanes with development. Fifth, the developmental decline in the feeding-induced activation of the insulin-signaling pathway leading to translation initiation parallels the developmental decline in feeding- and insulin-stimulated protein synthesis (21, 35, 53). This suggests that the high activity of the insulin-signaling pathway contributes to the increased response of muscle protein synthesis rates to feeding in the neonate.

In the present study, we used an amino acid clamp technique to prevent the drop in circulating amino acid concentrations that occurs when amino acids are not infused concurrently with insulin. Because the circulating concentrations of many nonessential amino acids decreased at the highest insulin concentrations in our previous studies (14, 62), in the present study, we developed a new amino acid mixture that contained higher concentrations of nonessential amino acids than the Trophamine used previously. Using this new amino acid infusate, we maintained almost all nonessential amino acids, as well as essential amino acids, at the fasting level during the infusion of insulin. Critically, however, the stimulation of skeletal muscle protein synthesis by insulin was similar in the present and previous studies (14, 62) and, indeed, was similar to the response to feeding (12). This suggests that maintenance of nonessential amino acid concentrations is not required for the insulin-induced stimulation of skeletal muscle protein synthesis.

To determine the role of amino acids in the postprandial rise in protein synthesis, the new amino acid...
mixture was infused to raise circulating amino acid concentrations to near the fed level. Importantly, circulating concentrations of insulin, measured every 30 min during the 4-h infusion, did not increase in response to amino acid infusion. This suggests that the physiological rise in amino acid concentrations stimulated protein synthesis in skeletal muscle in the absence of a rise in insulin concentration. Although the combined infusion of amino acids and insulin increased protein synthesis, the increase was similar to that obtained during amino acid or insulin stimulation alone. This implies that insulin and amino acids may be interacting with the same signaling pathway within skeletal muscle. This idea is supported by the observation that the muscle protein synthesis response to insulin and amino acids decreased with development, in parallel with the developmental decline in the feeding-induced stimulation of skeletal muscle protein synthesis. In young, adult, and elderly populations, amino acid infusion, either alone or concurrent with insulin infusion, stimulates protein synthesis in skeletal muscle (6, 41, 49, 58, 59). This suggests that the ability of amino acids to stimulate skeletal muscle protein synthesis wanes, but is not lost, with age.

The results of the present study further show that the stimulation of muscle protein synthesis by amino acids and/or insulin was greater in those muscles containing predominantly fast-twitch, glycolytic muscle fibers. The response to both anabolic agents was greater in longissimus dorsi and gastrocnemius muscles, which contain fast-twitch fibers that are predominantly glycolytic, than in the masseter muscle, which is composed entirely of oxidative fibers. These findings are consistent with our previously reported results (19, 21) and those of others (4, 36), demonstrating that protein synthesis is more responsive to anabolic agents in muscles composed of predominantly fast fibers than in muscles of slow oxidative fibers. Furthermore, the greater developmental decline in fractional protein synthesis rates in fast-twitch glycolytic than in slower oxidative muscles in the present study is consistent with our previously reported findings in pig and rat muscles (18, 14). Because the skeletal muscles that we studied in the neonatal pig have predominantly fast-twitch contractile characteristics but different oxidative and glycolytic properties (1, 47, 54), the differences in protein synthesis rates between the muscles that we observed are most likely attributable to the distinct differences in the muscles' metabolic rather than twitch characteristics.

The results of the present study demonstrate that hyperinsulinemia and hyperaminoacidemia, alone or in combination, stimulate both myofibrillar and sarcoplasmic protein synthesis. In addition, the degree of stimulation by these agents was proportional. These results are consistent with our previous studies, which showed that the synthesis of both protein fractions is reduced with age. Hyperaminoacidemia increased protein synthesis in liver (P < 0.001), kidney (P < 0.005), and pancreas (P < 0.05), and the response in liver decreased with age (P < 0.05). *Protein synthesis rates statistically significantly different from control rates (P < 0.05).

Fig. 6. $K_s$ in liver (A), kidney (B), and pancreas (C) of 7- and 26-day-old pigs during Control, Insulin, AA, and Insulin+AA clamps. Values are means ± SE; n = 7–12 per age and treatment group. Protein synthesis decreased with age in liver (P < 0.001). Hyperaminoacidemia increased protein synthesis in liver (P < 0.001), kidney (P < 0.005), and pancreas (P < 0.05), and the response in liver decreased with age (P < 0.05).

Fig. 7. $K_s$ in jejunum of 7- and 26-day-old pigs during Control, Insulin, AA, and Insulin+AA clamps. Values are means ± SE; n = 7–12 per age and treatment group. Protein synthesis decreased with age in jejunum (P < 0.001).
increased similarly by milk feeding (26) and insulin infusion (14) in neonatal pigs. Furthermore, we have previously shown in the neonatal pig (14) that myofibrillar and sarcoplasmic protein synthesis rates are similar, as in the present study, and that the age-associated decline in the synthesis rates of the two protein fractions is proportional over this phase of development.

**Regulation of tissue protein synthesis by either insulin or amino acids alone.** Feeding increases protein synthesis in virtually all tissues of the neonatal pig (7, 8, 12, 15). In the present study, infusing insulin at a dose that reproduced plasma insulin levels in the fed state while maintaining glucose and essential and non-essential amino acids at the fasting level stimulated protein synthesis in cardiac muscle, skin, and spleen. However, in a previous study (14), infusing insulin at doses reproducing fed-state plasma insulin levels while keeping glucose and essential amino acids, but not nonessential amino acids, at fasting levels stimulated protein synthesis in cardiac muscle and skin but not spleen. This suggests that keeping nonessential amino acid concentrations at fasting levels is not required for the insulin-induced stimulation of protein synthesis in cardiac muscle or skin, although it may play a permissive role in spleen. Furthermore, an elevation in amino acids in the presence of either euinsulinemia or hyperinsulinemia had no effect on protein synthesis in cardiac muscle, skin, or spleen. Thus the results are consistent with the hypothesis that the postprandial rise in insulin, but not amino acids, largely mediates the stimulation of protein synthesis by feeding in the cardiac muscle, skin, and spleen of the neonate.

Insulin also appears to regulate protein synthesis in the cardiac muscle of young (48) but not adult (41) rats, consistent with the developmental decline in the insulin-induced stimulation of cardiac muscle protein synthesis observed in the present study. Amino acid infusion has no effect on cardiac muscle protein synthesis in the adult rat (41), as in the neonatal pigs of the present study, suggesting that cardiac muscle may be resistant to the anabolic effects of amino acids throughout the life cycle.

Our previous study (14) showed that insulin infusion does not stimulate, and in some cases reduces, protein synthesis in liver, intestine, pancreas, and kidney, despite maintenance of glucose and essential amino acids at fasting levels. In the present study, the infusion of physiological levels of insulin, when nonessential and essential amino acids and glucose were clamped at fasting levels, did not stimulate, but also did not reduce, protein synthesis in these tissues. This suggests that the reduction in protein synthesis in liver and intestine during the infusion of insulin in the previous study (14) was due to a reduction in substrate, i.e., nonessential amino acids or nitrogen. Moreover, the infusion of amino acids at doses reproducing fed-state amino acid levels increased protein synthesis in liver, pancreas, and kidney in the present study. These responses to amino acid infusion occurred in the presence of either euinsulinemia or hyperinsulinemia. The results are suggestive of an important role of amino acids, but not insulin, in the regulation of protein synthesis in the liver, pancreas, and kidney of the neonate.

Many (1, 44, 55), but not all (33, 34), studies report an inability of insulin to stimulate liver protein synthesis. However, none have reported a reduction in liver protein synthesis with insulin infusion. The reduction in liver protein synthesis in the previous insulin infusion study (14) may have been due to the more profound effect of insulin on circulating amino acids in neonatal animals, which have higher amino acid turnover rates than older animals (23, 61). Indeed, amino acids appear to stimulate liver protein synthesis in growing (27, 51), but not adult or elderly (3, 44), animals, consistent with the developmental decline in the amino acid-induced stimulation of liver protein synthesis in the present study.

In the present study, we found that the physiological elevation of insulin, amino acids, or both had no effect on protein synthesis in the jejunum. Insulin infusion in healthy adults (42) and in diabetics (46) reduces protein synthesis in splanchnic tissues, consisting primarily of gut and liver. However, insulin deprivation modestly reduces, and insulin treatment restores, protein synthesis rates in the small intestinal mucosa of diabetics (11), suggesting that insulin may be required for the maintenance of mucosal protein synthesis. Feeding stimulates intestinal protein synthesis in growing animals (9, 12, 15), but in the present study, amino acids were infused peripherally rather than enterally. Thus we postulate that supplying amino acids to the mucosa via the luminal rather than the arterial route is likely the most important modulator of the feeding-induced stimulation of intestinal protein synthesis. Indeed, previous studies in neonatal pigs (52) have shown that, in the fed state, dietary amino acids make a greater contribution than arterial amino acids to jejunal protein synthesis.

**Whole body amino acid and glucose disposal.** Previous studies (61) showed that insulin stimulates the utilization of amino acids for protein deposition in the whole body of the neonatal pig and that both the insulin sensitivity and responsiveness of amino acid disposal decrease with development. The present study extends our previous work to show a marked developmental decline in insulin-stimulated amino acid disposal in the presence of either fasted- or fed-state amino acid levels. Furthermore, the amount of amino acids required to raise amino acid concentrations to near the fed state in the presence of either fasting- or fed-state insulin levels also decreased with development. This developmental decrease in amino acid disposal in response to hyperinsulinemia and/or hyperaminoacidemia paralleled the developmental decline in the ability of these anabolic agents to stimulate protein synthesis in most tissues. We have recently shown a marked developmental decline in both insulin receptor abundance and the feeding-induced activation of the insulin receptor in skeletal muscle of the neonate (53). This developmental change in the activation of
the insulin receptor is propagated down the insulin-signaling pathway to many of the eukaryotic initiation factors that regulate translation (21, 35). Furthermore, the feeding-induced stimulation of muscle protein synthesis is dependent on activation of a protein kinase, mammalian target of rapamycin or mTOR (37), which has been shown in vitro to be important in mediating the signal generated by both amino acids and insulin (31, 50). The lack of developmental decline in insulin-stimulated glucose disposal in the presence of either euaminoacidemia or hyperaminoacidemia is surprising and implies differential developmental regulation of these two insulin-stimulated metabolic responses.

_Perspectives._ Feeding stimulates whole body protein synthesis in the newborn infant, but the effect is much smaller in the adult (24, 43, 56), implying that different mechanisms regulate protein synthesis in adults and neonates. Studies using the neonatal pig as an animal model of the human infant (45) have shown that feeding-induced stimulation of protein synthesis in virtually all tissues of the body of the neonate, a response that decreases with development (12). The results of the present study strongly support the hypothesis that the feeding-induced stimulation of protein synthesis in skeletal muscle is mediated by the postprandial rise in both insulin and amino acids. Protein synthesis in cardiac muscle, skin, and spleen is responsive to insulin but not amino acid stimulation, whereas liver, kidney, and pancreas are responsive to amino acid but not insulin stimulation. Because neither insulin nor amino acid infusion altered intestinal protein synthesis, we postulate that amino acid stimulation in the luminal rather than arterial route may be required for the feeding-induced stimulation of intestinal protein synthesis. Thus it appears that the stimulation of protein synthesis by feeding is mediated by either insulin or amino acids in most tissues of the neonate, but in skeletal muscle, the feeding-induced stimulation of protein synthesis is regulated by both insulin and amino acids. The ability of skeletal muscle to respond to the postprandial rise in two anabolic stimuli likely contributes to the efficient utilization of nutrients for growth in the neonate and the more rapid gain in protein mass in skeletal muscle than in other tissues of the body in the neonate.

We thank W. Liu, J. Rosenberger, J. Henry, and X. Chang for technical assistance, J. Cunningham and F. Biggs for care of animals, E. O. Smith for statistical assistance, L. Loddeke for editorial assistance, A. Gillum for graphics, and J. Croom for secretarial assistance. We acknowledge Eli Lilly for the generous donation of porcine insulin.

This work is a publication of the US Department of Agriculture, Agricultural Research Service (USDA/ARS) Children’s Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine and Texas Children’s Hospital, Houston, Texas. This project has been funded in part by National Institute of Arthritis and Musculoskeletal and Skin Diseases Institute Grant R01 AR-44474 and the USDA/ARS under Cooperative Agreement no. 58–6250–6–001. This research was also supported in part by National Institutes of Health Training Grant T32 HD-07445. The contents of this publication do not necessarily reflect the views or policies of the US Department of Agriculture, nor does mention of trade names, commercial products, or organizations imply endorsement by the US Government.

Present address of P. J. Reeds: University of Illinois, Dept. of Animal Sciences, 1207 W. Gregory, Rm. 432, Urbana, IL 61801. Present address of R. Vann: Central Mississippi Research and Extension Center, Mississippi State University, 1320 Seven Springs Rd., Raymond, MS 39154.

REFERENCES


