Regulation of neonatal liver protein synthesis by insulin and amino acids in pigs


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O’Connor, Pamela M. J., Scot R. Kimball, Agus Suryawan, Jill A. Bush, Hanh V. Nguyen, Leonard S. Jefferson, and Teresa A. Davis. Regulation of neonatal liver protein synthesis by insulin and amino acids in pigs. Am J Physiol Endocrinol Metab 286: E994–E1003, 2004. First published February 3, 2004; 10.1152/ajpendo.00391.2003.—The high efficiency of protein deposition during the neonatal period is driven by high rates of protein synthesis, which are maximally stimulated after feeding. Infusion of amino acids, but not insulin, reproduces the feeding-induced stimulation of liver protein synthesis. To determine whether amino acid-stimulated liver protein synthesis is independent of insulin in neonates, and to examine the role of amino acids and insulin in the regulation of translation initiation in neonatal liver, we performed pancreatic glucose-amino acid clamps in overnight-fasted 7-day-old pigs. Pigs (n = 9–12/group) were infused with insulin at 0, 10, 22, and 110 ng·kg⁻¹·min⁻¹ to achieve 0, 2, 6, and 30 μU/ml insulin, respectively. At each insulin dose, amino acids were maintained at fasting or fed levels or, in conjunction with the highest insulin dose, allowed to fall to below fasting levels. Insulin had no effect on the fractional rate of protein synthesis in liver. Amino acids increased fractional protein synthesis rates in liver at each dose of insulin, including the 0 μU/ml dose. There was a dose-response effect of amino acids on liver protein synthesis. Amino acids and insulin increased protein synthesis rates by 2.20±0.33- and 4.17±0.66-fold, respectively, at the highest dose of amino acids. In the presence of insulin, amino acid-induced stimulation of liver protein synthesis was maximal at an amino acid infusion rate of 30 μU/ml. Amino acids increased the phosphorylation of the 70-kDa ribosomal protein ribosomal subunit (11, 24, 28). Thus, in the neonate, feeding increases the phosphorylation of the 70-kDa ribosomal protein S6 kinase (S6K1) and the eukaryotic initiation factor-4E (eIF4E)-binding protein 4E-BP1, which enhances the availability of eIF4E for 48S ribosomal complex formation and that this response does not require insulin.

neonate; growth; translation initiation; nutrition; eukaryotic initiation factor-4E

GROWTH AND PROTEIN DEPOSITION RATES ARE MORE RAPID DURING THE NEONATAL PERIOD THAN AT ANY OTHER STAGE OF POSTNATAL LIFE (8, 12, 18). Dietary protein is utilized with high efficiency in neonates (10), and this is most likely due to the enhanced rates of protein synthesis that occur in response to feeding (4, 9, 13). Studies in suckling pigs have shown that protein synthesis is high in the neonate and declines with development (4, 5). Feeding stimulates protein synthesis in the liver, as it does in other tissues of the neonate (4, 5).

Amino acids play a key role in the regulation of liver protein synthesis in the neonate (7). Studies using our novel hormone-substrate clamps have shown that the infusion of amino acids at a dose that reproduces fed-state plasma amino acid concentrations increases protein synthesis in liver of the neonatal pig, and the magnitude of the amino acid-stimulated increase in protein synthesis is similar to that which occurs in response to feeding (4, 7). Although insulin stimulates whole body amino acid disposal and skeletal muscle protein synthesis in the neonate, in neonatal liver, protein synthesis is insensitive to insulin infusion, and this effect persists with development (6, 7, 56).

Acute modulation of tissue protein synthesis rates, including the rate due to amino acid and hormonal stimulation, is regulated by changes in the rate of translation of mRNA via alterations in the rate of peptide chain initiation (15, 27, 33). Previous studies in our laboratory have shown that the postprandial increases in protein synthesis in liver and in skeletal muscle of the neonate are associated with an increase in the efficiency of the translation process, i.e., the amount of protein synthesized per unit RNA (4). This increase in translational efficiency is due primarily to increases in the activation of translation initiation factors involved in the binding of mRNA, and not initiation methionyl-tRNA (met-tRNA), to the 40S ribosomal subunit (11, 24, 28). Thus, in the neonate, feeding increases the phosphorylation of the 70-kDa ribosomal protein S6 kinase (S6K1) and the eukaryotic initiation factor-4E (eIF4E)-binding protein 4E-BP1, thus enhancing eIF4E availability by releasing it from the inactive 4E-BP1-eIF4E complex and increasing the association of eIF4E with eIF4G. These responses to feeding are more pronounced in skeletal muscle than in liver. The postprandial changes in translation initiation in the neonate are dependent on the activation of the protein kinase termed mammalian target of rapamycin (mTOR) and are associated with activation of upstream signaling proteins, including PKB and phosphatidylinositol 3-kinase (28, 49). However, in vivo studies utilizing neonatal pigs suggest that feeding does not alter eIF2B activity, which regulates the binding of met-tRNA, to the 40S ribosomal subunit (11).

Recently, we demonstrated that insulin and amino acids independently stimulate muscle protein synthesis in the neonate and that this effect involves modulation of translation factors that regulate mRNA binding to the ribosomal complex (40, 41). In the current study, we wished to determine whether 1) amino acid-induced stimulation of liver protein synthesis is independent of insulin, 2) amino acids exert a dose-response effect on liver protein synthesis, and 3) amino acid-induced liver protein synthesis is regulated by changes in the activation
of specific translation initiation factors that modulate availability of eIF4E for 48S ribosomal complex formation.

METHODS

Animals. Multiparous sows (n = 11; crossbred Yorkshire × Landrace × Hampshire × Duroc; Agriculture Headquarters, Texas Department of Criminal Justice, Huntsville, TX) were housed in lactation crates in individual, environmentally controlled rooms, maintained on a commercial diet (5084, PMI Feeds, Richmond, IN), and provided water ad libitum throughout the lactation period. After farrowing, 30 piglets remained with the sow but were not given supplemental creep feed. Piglets were studied at 5–8 days of age (2.1 ± 0.4 kg). Three to five days before the infusion study, pigs were anesthetized, and catheters were surgically inserted into a jugular vein and a carotid artery with sterile techniques, as described previously (55). Piglets were returned to the sow until studied. The previously described protocol (40) was approved by the Animal Care and Use Committee of Baylor College of Medicine. The study was conducted in accordance with the National Research Council’s Guide for the Care and Use of Laboratory Animals.

Pancreatic glucose-amino acid clamps. After an overnight fast, pigs were placed unanesthetized in a sling restraint system, as previously described by O’Connor et al. (40). The average basal concentration of blood glucose (YSI 2300 STAT Plus, Yellow Springs Instruments, Yellow Springs, OH) and plasma branched-chain amino acid (BCAA) concentrations (3) to be used in the subsequent pancreatic glucose-amino acids clamp procedure were established during a 30-min basal period. The clamp was initiated with a primed (20 ng/kg, continuous (100 μg/kg·h−1) somatostatin (BACHEM, Torrance, CA) infusion. After a 10-min infusion of somatostatin, a continuous infusion of replacement glucagon (150 ng·kg−1·h−1; Eli Lilly, Indianapolis, IN) was initiated and continued to the end of the clamp period. Insulin was infused at 0, 10, 22, or 30 μU/ml to simulate below fasting, fasting, intermediate, or fed level (7, 55). At the highest insulin dose only, amino acids were also added to maintain its concentration within 10% of the desired level (7). The glucose clamp was then inactivated by incubating the blot in 15% hydrogen peroxide for 30 min at room temperature, and then the membranes were reprobed with the monoclonal anti-eIF4E antibody (29). Values obtained using the anti-4E-BP1 antibody were normalized for the amount of eIF4E in the immunoprecipitates.

Measurement of S6K1 phosphorylation. Liver homogenates were combined with an equal volume of SDS sample buffer, and the diluted samples were subjected to electrophoresis on a 7.5% polyacrylamide gel (34). The samples were then analyzed by protein immunoblot analysis using rabbit anti-rat S6K1 polyclonal antibodies, as previously described (11).

Measurement of eIF2B activity. eIF2B activity in fresh liver supernatants was measured as the exchange of [3H]GDP bound to eIF2 for unlabeled GDP or GTP, as previously described (11, 23). Briefly, an eIF2α-[3H]GDP binary complex was formed in the absence of magnesium chloride. The eIF2α-[3H]GDP complex was then stabilized by the addition of magnesium to a final concentration of 2 mM. The eIF2α-[3H]GDP complex was incubated with samples containing eIF2B in the presence of a 100-fold molar excess of unlabeled, HPLC-purified GTP at 30°C for various times. The reaction mixture was filtered through a nitrocellulose filter, the filters were washed, and radioactivity bound to the filter was quantitated using a liquid scintillation counter.

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

\[ K_s = \frac{\text{specific radioactivity of the protein-bound phenylalanine}}{\text{specific radioactivity of the tissue-free phenylalanine}} \times \frac{1}{\text{time of labeling in minutes}} \]

where Sb (dpm/min) is the specific radioactivity of the protein-bound phenylalanine and Sa (dpm/min) 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.

Analysis of variance (ANOVA; general linear modeling) was used to assess the effect of insulin, amino acids, and their interaction, and to determine whether there was a linear or quadratic relationship between BCAA and translation initiation factor activity, and between Ks and translation initiation factor activity. Student’s t-test was used to test for specific differences between groups. To determine the effectiveness of the clamp procedure, amino acid and insulin concentrations in each treatment group were compared with their basal concentrations by use of t-tests. Probability values of P < 0.05 were considered statistically significant.
RESULTS

Infusions, hormones, and substrates. Pancreatic glucose-amino acid clamps were performed in fasted 7-day-old pigs by infusion of somatostatin (to block insulin secretion), glucagon (at replacement levels), and glucose (as needed to maintain fasting levels). Insulin was infused at four doses to achieve levels that simulated 1) less than fasting, 2) fasting, 3) intermediate between fasting and fed, or 4) fed conditions. Amino acids were clamped by monitoring plasma BCAA concentrations and adjusting the infusion rate of a balanced amino acid mixture to maintain these concentrations at fasting or fed levels. This resulted in BCAA of ~500 or 1,000 nmol/ml, respectively; at the highest insulin dose, amino acids were also allowed to fall to less than the fasting levels of ~250 nmol/ml. As shown in Table 1, targeted plasma insulin levels, i.e., ~0, 2, 6, and 30 μU/ml, and BCAA levels were largely achieved in all treatment groups. Concentrations of total amino acids from previously reported data are also shown for comparison (40). Circulating glucose and glucagon concentrations were largely maintained at basal fasting levels during the infusion of somatostatin, glucagon, insulin, and/or amino acids (data not shown).

Liver protein synthesis. Previous studies have shown that amino acid infusion in the neonatal pig stimulates protein synthesis in liver when insulin is clamped at the fasting level (2 μU/ml) (7). To examine the interaction of insulin and amino acids in the stimulation of liver protein synthesis, we used the pancreatic-substrate clamp technique to achieve four different insulin doses of ~0, 2, 6, or 30 μU/ml while amino acids were either clamped at the fasting level (500 BCAA nmol/ml) or increased to the fed level (1,000 BCAA nmol/ml). Raising amino acids from the fasting to the fed level increased the fractional rate of protein synthesis in liver (P < 0.001; Fig. 1). The magnitude of the effect of amino acids on liver protein synthesis was similar at each dose of insulin (10%–20%), including when insulin was reduced to nearly zero by somatostatin infusion (P < 0.05; Fig. 1). Insulin had no effect on the fractional rate of protein synthesis in the liver, and there was no interaction of insulin and amino acids on liver protein synthesis, as determined by ANOVA. However, examination of the individual treatment effects by Student’s t-test revealed that protein synthesis was increased only in response to the highest insulin dose in the presence of hyperaminoacidemia (P < 0.005).

To determine whether there is a dose-response effect of amino acids on liver protein synthesis, insulin was infused to simulate the fed level while amino acids were clamped at either the fasting level (500 BCAA nmol/ml) or the fed level (1,000 BCAA nmol/ml) or allowed to fall to below fasting levels (250 BCAA nmol/ml). A progressive increase in liver protein synthesis occurred as amino acids were raised from below fasting to fasting levels (P < 0.05) and from fasting to fed amino acid levels (P < 0.001; Fig. 2).

Effect of amino acids and insulin on translation initiation factors. To determine whether amino acids and insulin interact in the translational control of liver protein synthesis, we determined the effect of raising amino acid levels from the fasting to the fed level, in the presence of insulin doses ranging from...

Table 1. Plasma insulin and amino acid levels in response to insulin and amino acid infusion during pancreatic glucose-amino acid clamps in 7-day-old pigs

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>AA Groups</th>
<th>Baseline</th>
<th>Insulin Groups</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>&lt;Fasting</td>
<td>Fasting</td>
</tr>
<tr>
<td>Insulin</td>
<td>Fasting</td>
<td>1.5±0.2</td>
<td>0.6±0.1*</td>
</tr>
<tr>
<td></td>
<td>Fed</td>
<td>1.7±0.2</td>
<td>0.7±0.2*</td>
</tr>
<tr>
<td></td>
<td>&lt;Fasting</td>
<td>1.8±0.2</td>
<td>ND</td>
</tr>
<tr>
<td>TAA</td>
<td>Fasting</td>
<td>3.245±53</td>
<td>3.014±156</td>
</tr>
<tr>
<td></td>
<td>&lt;Fasting</td>
<td>3.354±134</td>
<td>ND</td>
</tr>
<tr>
<td>BCAA</td>
<td>Fasting</td>
<td>544±19</td>
<td>507±47</td>
</tr>
<tr>
<td></td>
<td>Fed</td>
<td>560±25</td>
<td>888±55</td>
</tr>
<tr>
<td></td>
<td>&lt;Fasting</td>
<td>601±22</td>
<td>ND</td>
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</tbody>
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Values are means ± SE; n = 8–12 per group. Plasma concentrations of insulin are in μU/ml, and amino acids [AA; total (TAA), branched chain (BCAA), essential, and nonessential] are in nmol/ml. ND, not determined. *Significantly different from baseline (time 0) values (P < 0.05).
below fasting to fed levels, on translation initiation factor content and/or phosphorylation. Both 4E-BP1 and S6K1 are downstream of mTOR and thus dependent on the activity of this kinase for activation. Through protein immunoblot analysis, we determined the phosphorylation state of S6K1. Hyperphosphorylation of the protein results in decreased mobility during SDS-polyacrylamide gel electrophoresis (54). Amino acids \((P < 0.001)\) increased S6K1 phosphorylation (Fig. 3A).

The stimulation of S6K1 phosphorylation by amino acids occurred at the basal insulin dose \((P < 0.001)\) and tended to occur at the zero, intermediate, and highest insulin doses \((P < 0.10)\). Insulin also increased S6K1 phosphorylation \((P < 0.001)\); specifically, an effect of insulin occurred in the presence of hyperaminoacidemia at the basal and high insulin doses \((P < 0.05)\).

Studies in cell culture have shown that eIF4E plays a critical role in the binding of mRNA to the 43S preinitiation complex (45, 48) and that the function of eIF4E may be influenced by either its phosphorylation state or its availability for formation of the active eIF4E-eIF4G complex (38, 42). To determine whether insulin and/or amino acids affected eIF4E phosphorylation status, the amount of eIF4E present in the phosphorylated form as a percentage of the total eIF4E was determined. There was no significant effect of insulin or amino acids on eIF4E phosphorylation (data not shown).

The availability of eIF4E can be regulated through changes in the amount of eIF4E bound to 4E-BP1 such that phosphor-
ylation of 4E-BP1 causes disassembly of the eIF4E-eIF4E-BP1 complex (29). Phosphorylation of 4E-BP1 at the Thr70 site has been shown to be important in regulating its association with eIF4E when phosphorylated (28, 29). Amino acids (P < 0.001) stimulated 4E-BP1 phosphorylation at the Thr70 site (Fig. 3B). Examination of specific differences among individual groups revealed that the effect of amino acids on 4E-BP1 phosphorylation occurred at the low, basal (P < 0.05), and highest doses (P < 0.001) and tended to occur at the intermediate insulin dose (P < 0.10). Insulin increased 4E-BP1 phosphorylation (P < 0.05), with an effect of insulin occurring in the presence of euaminoacidemia at the intermediate dose of insulin (P < 0.01), and in the presence of hyperaminoacidemia, at the intermediate and high doses of insulin (P < 0.005) compared with the zero insulin dose.

Phosphorylation of 4E-BP1 in cell culture and in situ decreases the association of 4E-BP1 with eIF4E, thereby allowing eIF4E to bind to eIF4G (11, 29, 35). To determine the combined effect of amino acids and insulin on 4E-BP1 association with eIF4E, eIF4E was immunoprecipitated with an anti-eIF4E antibody, followed by immunoblot analysis with an anti-4E-BP1 antibody. Amino acids (P < 0.001) decreased the amount of 4E-BP1 present in the eIF4E immunoprecipitate (Fig. 3C). Examination of specific differences among individual groups revealed that the effect of amino acids on 4E-BP1-eIF4E content occurred at the basal (P < 0.001), intermediate (P < 0.005), and high doses (P < 0.01) of insulin and tended to occur at the zero insulin dose (P < 0.10). There was no effect of insulin on the association of 4E-BP1 with eIF4E.

The binding of met-tRNAi to the 40S ribosomal subunit is primarily regulated by changes in eIF2B activity (31). Stimulation of liver protein synthesis or translation initiation factor activation with BCAA. We have previously demonstrated that amino acid infusion to the fed level, in the presence of fasted insulin levels, can reproduce the feeding-induced stimulation of liver protein synthesis in the neonate (7). Amino acid-stimulated liver protein synthesis has also been demonstrated in growing animals (15, 46) but not in adults (1, 39). A developmental decline in amino acid-stimulated liver protein synthesis has also been previously demonstrated in our clamp studies performed in growing pigs (7). However, whether there was a potentially permissive effect of fasting insulin levels on amino acid-stimulated liver protein synthesis was not ruled out in our previous study (7). In the current study, by using pancreatic glucose-amino acid clamps, we show the stimulatory effect of amino acids to be independent of insulin in that the effect of amino acids occurred at each dose of insulin, including the zero insulin dose. In the presence of fed insulin levels, we also delineated a dose-response effect of amino acids on neonatal liver protein synthesis. Furthermore, we demonstrate that neonatal liver is so sensitive to amino acids that even increasing plasma amino acid concentrations from below fasting to fasting levels stimulated protein synthesis. These findings highlight the importance of protein in the diet of the young animal.

Liver protein synthesis in response to amino acids. There are conflicting reports on the role of insulin in the regulation of liver protein synthesis. Some cell culture studies and studies in vivo in diabetic rats suggest a role of insulin in the regulation of liver protein synthesis (19, 21). However, most animal studies have shown an inability of insulin to stimulate liver protein synthesis (39, 47, 50). Our previous studies (6, 7) and this current study find no effect of insulin on the fractional rate of protein synthesis in liver of the neonate. This suggests that the postprandial rise in amino acids, independent of the postprandial rise in insulin, mediates the feeding-induced stimulation of liver protein synthesis in the neonate. It could be argued that the small amount of insulin (0.6 μU/ml) present in the pigs infused with somatostatin without insulin replacement was sufficient to play a permissive role in the stimulation of liver protein synthesis by insulin. However, this seems unlikely,
given that the lowest insulin level was less than the fasting
level (~2 μU/ml) and was at the lowest level of detection of
the insulin assay (0.5 μU/ml). Furthermore, the possibility that
fed levels of insulin may enhance the stimulation of liver
protein synthesis by amino acids should not be overlooked.

Effects of amino acids and insulin on translation initiation
factors. Our previous feeding studies, with rapamycin as a
blocker of mTOR, revealed that the feeding-induced stimu-
lation of liver protein synthesis in neonates is mTOR dependent
(28). The phosphorylation of S6K1 is primarily mTOR depen-
dent (25, 51), and previous studies in neonatal pigs have shown
that feeding, through its activation of mTOR, causes hyper-
phosphorylation of S6K1 (11, 28). Increases in the phosphor-
ylation of S6K1 result in hyperphosphorylation of ribosomal
protein S6 and thus facilitate the translation of mRNAs con-
taining terminal oligopyrimidine tracts at the 5′ end, which
encode elements of the translation apparatus, including ro-
bosomal proteins and elongation factors (32, 36). Therefore,
activation of S6K1 appears to increase the synthesis of proteins
involved in mRNA translation. In the present study, amino
acids increased S6K1 phosphorylation. Previous in vitro and in
vivo studies have shown similar findings (2, 20). Amino acid
deprivation studies (52) have shown a decrease in S6K1
phosphorylation; however, conclusions drawn as to the regu-
laratory role of amino acids from these substrate deprivation
studies are not equivalent to those conclusions derived from

Fig. 4. Correlations of protein synthesis rates (A)
and translation initiation factor activity (B-F) in
liver with BCAA during hyperinsulinemia.
substrate supply studies. Although an effect of insulin on S6K1 phosphorylation was unexpected, the change occurred only in the presence of hyperaminoacidemia and did not result in an increase in the global rate of liver protein synthesis. Stimulation of S6K1 phosphorylation in liver by insulin has been reported recently in the fetus (47).

A key step in translation initiation is the binding of mRNA to the 43S preinitiation complex, which is mediated by eIF4F, a complex of proteins that includes eIF4E. Assembly of the eIF4F complex can be regulated by the phosphorylation (38) or availability (44) of eIF4E. The eIF4E phosphorylation status, as a percentage of the total eIF4E, was examined in the current study, as it has been shown in cell culture studies to influence mRNA binding to the 43S preinitiation complex, with ultimate increases in protein synthesis (37, 38, 48). However, no effect of insulin and/or amino acids on eIF4E phosphorylation was observed in this study. These results extend the findings of our previous study that show that feeding has no effect on eIF4E phosphorylation in the neonate. This implies that, in vivo, modulation of eIF4E phosphorylation status is unlikely to be a key regulatory step in the feeding-induced stimulation of liver protein synthesis by amino acids in neonatal pigs. Alternatively, an effect of insulin and/or amino acids on eIF4E phosphorylation could have occurred at an earlier point in time.

An important regulatory step in the mRNA binding process is the reversible association of eIF4E with eIF4G, following the dissociation of 4E-BP1 from eIF4E. This results in active eIF4F complex assembly, thus enabling mRNA binding to the 43S preinitiation complex (17). Previous studies in mature rats and neonatal pigs have shown a decrease in the inactive 4E-BP1-eIF4E complex in response to refeeding (11, 28, 57). In the current study, amino acids, but not insulin, decreased the 4E-BP1-eIF4E complex assembly. The effect of amino acids occurred in the presence of the basal, intermediate, and higher doses of insulin. No effect of insulin on the 4E-BP1-eIF4E complex formation in liver of the ovine fetus has been reported (47).

A major site in the regulation of translation initiation is the binding of met-tRNAi to the 40S ribosomal subunit, an event mediated by eIF2 (30, 44). This step is regulated by eIF2B, which exchanges GTP for GDP on eIF2 (31). Studies in cell
culture suggest that insulin and amino acids increase eIF2B activity (14, 26, 53). In situ and in vivo studies suggest that amino acid imbalance induces a decrease in eIF2B activity and protein synthesis in rat liver (1, 46). Studies in diabetic animals suggest that insulin plays an important role in increasing eIF2B activity (22). In the current study, neither insulin nor amino acids had any effect on eIF2B activity. However, this was not unexpected, as previous in vivo studies in both mature rats and neonatal pigs suggest that eIF2B activity is not regulated by changes in nutritional status (11, 28, 57).

Correlations. The linear relationships of plasma BCAA concentrations with S6K1 phosphorylation, 4E-BP1 phosphorylation, 4E-BP1-eIF4E complex content, and protein synthesis in liver of the neonate in the current study suggest that a maximal response of protein synthesis and translation initiation factor activation to BCAA was not achieved. This raises the possibility that higher concentrations of amino acids may further increase translation initiation factor activity and global rates of protein synthesis. This conclusion is supported by the linear relationship between the activity of these translation initiation factors and protein synthesis. Because we infused amino acids to raise circulating amino acids to levels similar to those of pigs fed sow’s milk, this suggests that the feeding of a higher protein diet could further increase protein synthesis. Furthermore, the positive linear correlation between global protein synthesis rates and translation initiation factor modulation (S6K1 and 4E-BP1 phosphorylation) and a negative linear relationship between global protein synthesis rates and 4E-BP1-eIF4E content imply a direct response of this mTOR-dependent translation initiation pathway and global protein synthesis rates in the liver. In contrast, eIF2B activity showed no relationship with amino acid levels or with protein synthesis.

Fig. 6. Correlations of protein synthesis rates (A) and translation initiation factor activity (B-E) with plasma insulin. Continuous line, correlation during hyperaminoacidemia; dotted line, correlation during euaminoacidemia.
rates, consistent with our previous findings that feeding does not alter eIF2B activity in neonatal pigs. Thus the results suggest that the postprandial rise in amino acids, by modulation of mRNA binding and not met-tRNA binding, to the 40S ribosomal subunit plays an important role in the feeding-induced stimulation of liver protein synthesis in the neonate. Insulin’s lack of effect on and correlation with liver protein synthesis is not surprising. However, the quadratic correlation between S6K1 and 4E-BP1 phosphorylation and plasma insulin suggests an mTOR-mediated effect of insulin on these initiation factors that does not appear to modulate global rates of liver protein synthesis.

Perspectives. Our previous studies have demonstrated that feeding stimulates protein synthesis in virtually all tissues of the body of the neonate and that the magnitude of the feeding response can be reproduced in skeletal muscle by the infusion of insulin or amino acids and in liver by the infusion of amino acids but not insulin. Recent studies using pancreatic glucose-amino acid clamps demonstrated the independent stimulatory roles of insulin and amino acids on global rates of protein synthesis in skeletal muscle by mechanisms that are associated with enhanced 4E-BP1 and S6K1 phosphorylation and eIF4F complex formation, and by mechanisms independent of this pathway. The results of the current study, also using pancreatic glucose-amino acid clamps, suggest that both amino acids and insulin stimulate S6K1 and 4E-BP1 phosphorylation and eIF4F complex formation, and from this response is independent of insulin.

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