Stimulation of muscle protein synthesis by somatotropin in pigs is independent of the somatotropin-induced increase in circulating insulin

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Submitted 25 February 2008; accepted in final form 2 May 2008

Chronic treatment with somatotropin, also known as growth hormone, increases weight gain without raising dietary intake and thereby improves the efficiency with which dietary amino acids are utilized for growth (5, 6, 19). In addition to enhancing protein deposition, somatotropin treatment also decreases the accretion of fat (17, 37, 35). Previously, we demonstrated that 1 wk of porcine somatotropin (pST) treatment increases whole body protein accretion by minimizing protein loss during fasting and maximizing protein gained after feeding (48, 49). Further studies showed tissue-specific differences in the regulation of protein synthesis by pST treatment (2, 4). Chronic treatment with pST increased muscle protein synthesis by enhancing the activation of translation initiation factors that regulate the binding of mRNA and methionyl-tRNA to the ribosomal complex, whereas in the liver pST treatment increased protein synthesis by increasing ribosome number (2). However, the mechanism by which pST treatment is able to elicit these effects has not been identified.

Treatment with pST increases circulating levels of both insulin and growth hormone (16), and it has been suggested that this is a consequence of insulin resistance to glucose uptake (27). However, the reduced effect of insulin on glucose disposal during pST treatment appears to occur as a result of decreased glucose uptake by adipocytes, whereas the insulin-stimulated glucose uptake in skeletal muscle is not blunted by pST (16, 52). In addition to the promotion of glucose uptake, insulin also stimulates muscle protein synthesis in skeletal muscle of growing animals (10, 40). Studies in rapidly growing untreated pigs have demonstrated that, when insulin is raised to the fed level, protein synthesis in skeletal muscle is enhanced, even when amino acids and glucose are maintained at the fasting level using pancreatic glucose-amino acid clamps (10, 40, 52). The phosphorylation of both 4E-BP1 and ribosomal protein S6 (23), which had been thought to promote the translation of mRNAs that encode proteins that regulate the binding of mRNA and methionyl-tRNA to the ribosomal complex, whereas in the liver pST treatment increased protein synthesis by increasing ribosome number (2). However, the mechanism by which pST treatment is able to elicit these effects has not been identified.

The binding of insulin to its receptor on the cell surface initiates a cascade of events that lead to the activation of protein kinase B (PKB) and the master protein kinase mammalian target of rapamycin (mTOR) (26, 51). Activation of mTOR induces the phosphorylation of eukaryotic initiation factor 4E-binding protein-1 (4E-BP1), which in the unphosphorylated state binds to eIF4E, forming an inactive complex (36). Phosphorylation of 4E-BP1 leads to the dissociation of eIF4E from 4E-BP1, allowing eIF4E to associate with eIF4G to form, in conjunction with eIF4A, the active eIF4F complex (36), which mediates the binding of mRNA to the 40S ribosomal subunit (23). In addition, activated mTOR phosphorylates ribosomal protein S6 kinase-1 (S6K1), thereby activating ribosomal protein S6 (23), which had been thought to promote the translation of mRNAs that encode proteins that regulate translation (30). However, recent studies suggest that this activation is not required for regulating ribosomal protein translation (44). The phosphorylation of both 4E-BP1 and S6K1 increases the efficiency of translation initiation and, hence, promotes protein synthesis. In rapidly growing swine, insulin increases muscle protein synthesis by enhancing the activation of translation initiation factors that regulate the binding of mRNA and methionyl-tRNA to the ribosomal complex, whereas in the liver pST treatment increased protein synthesis by increasing ribosome number (2). However, the mechanism by which pST treatment is able to elicit these effects has not been identified.

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binding of mRNA to the ribosomal complex (40). In hepatoma cells in culture, somatotropin activates mTOR via phosphoinositide 3-kinase (PI3K) signaling, leading to an increase in the abundance of the active eIF4E-eIF4G complex (28).

This study aimed to determine whether the increase in skeletal muscle protein synthesis, observed in pigs chronically treated with pST, is a consequence of the pST-induced increase in circulating insulin levels. In addition, we wished to determine whether the mTOR signaling pathway modulates the observed changes. This study was performed in rapidly growing pigs (~20 kg) treated with pST for 7–10 days and subjected to pancreatic glucose-amino acid clamps, where circulating insulin levels were manipulated to achieve those of fasted and fed control and pST-treated pigs.

**MATERIALS AND METHODS**

**Animals and design.** Thirty-seven 8- to 10-wk-old crossbred (Landrace × Yorkshire × Hampshire × Duroc) female pigs (Agricultural Headquarters, Texas Department of Criminal Justice, Huntsville, TX) weighing 11 ± 0.22 kg were housed in individual cages. Since relatively high protein intakes are required to obtain the maximum promoting effects of pST (7), the animals were fed a 24% protein diet (Producers Cooperative Association, Bryan, TX) at 6% of their body weight and provided water ad libitum. Pigs were adjusted to the diet (Producers Cooperative Association, Bryan, TX) at 6% of their body weight and provided water ad libitum. Pigs were adjusted to the diet for 7 days and then randomly assigned to one of two treatment groups, either saline (control) or recombinant pST (gift of Dr. Frank Dunshea) at a rate of 150 μg·kg body wt\(^{-1}\)·day\(^{-1}\) for 7–10 days. The dose of saline or pST was divided into two daily injections (75 μg/kg body wt) and was administered in the subcutaneous region. Body weights were measured daily, and the dietary intake and treatment doses were adjusted accordingly. Pigs treated with pST were offered the diet at 6% of their body weight per day, and control pigs were pair fed to the intake level of the pST-treated pigs to minimize any confounding effect of differences in food intake. Daily feed allowance was divided into two meals to coincide with time of injection.

Three to five days before infusions, the pigs (17.35 ± 0.25 kg) were fasted overnight, and the jugular vein and carotid artery of each pig were catheterized using sterile techniques under general anesthesia (Aerrane; Anaquest, Madison, WI) as described previously (8). After surgery, pigs were returned to their cages and resumed their regular treatment regimen. 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.

**Pancreatic glucose-amino acid clamps.** Control and pST-treated pigs (19.96 ± 0.23 kg) were fasted overnight (16 h) before the infusion studies. On the day of infusion, pigs were administered their daily dose of pST (150 μg/kg body wt) or saline 60 min before infusion. Clamps were performed using techniques similar to those previously described (40, 48, 53) (Fig. 1). Over a 30-min period before the initiation of the clamp, basal blood glucose (YSI 2300 STAT Plus; Yellow Springs Instruments, Yellow Springs, OH) and branched-chain amino acid (BCAA) concentrations were determined as described previously (9). Previous studies have demonstrated that, after a flooding dose of \(^{[3]H}\)phenylalanine injection 75 min after the initiation of the clamp procedure (22), Pigs were killed at 2 h, and samples of the longissimus dorsi muscle were collected and rapidly frozen. The specific radioactivity of the protein hydrolysate, homogenate supernatant, and blood supernatant were determined as described previously (9). Previous studies have demonstrated that, after a flooding dose of \(^{[3]H}\)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 (12).

**Protein immunoblot analysis.** Proteins from longissimus dorsi muscle homogenates were separated on polyacrylamide gels (PAGE). For each assay, all samples were run at the same time on triple-wide gels (CBS Scientific C, Del Mar, CA) to eliminate interassay variation. Proteins were electrophoretically transferred to polyvinylidene difluoride transfer membranes (Pall, Pensicola, FL), which were incubated with appropriate primary antibodies, washed, and exposed to an appropriate secondary antibody, as previously described (14).

For normalization, immunoblots preformed with anti-phosphospecific antibodies were stripped in stripping buffer (Pierce Biotechnol-
ogy, Rockford, IL) and reprop with the corresponding nonphosphospecific antibodies. Blots were developed using an enhanced chemiluminescence kit (GE Health Sciences, Buckinghamshire, UK), visualized, and analyzed using a ChemiDoc-It Imaging System (UVP, Upland, CA). Primary antibodies that were used in the immunoblotting were PKB (total and Ser¹⁷³, Cell Signaling Technology, Beverly, MA), 4E-BP1 (total; Bethyl Laboratories, Montgomery, TX, and Thr70, Cell Signaling), and S6K1 (total, Ser²³⁵/²³⁶, and Ser²⁴⁰/²⁴⁴, Cell Signaling).

Quantification of eIF₄E·4E-BP1 and eIF₄E·eIF4G complexes. These complexes were immunoprecipitated using an anti-eIF₄E monoclonal antibody (gift of Dr. Leonard Jefferson, Penn State University College of Medicine, Hershey, PA) from aliquots of fresh tissue homogenates (18). Briefly, samples were homogenized in seven volumes of buffer (in mM: 20 HEPES, 2 EGTA, 50 NaF, 100 KCl, and 0.2 EDTA, pH 7.4) containing Sigma P3840 Protease Inhibitor Cocktail (Sigma Chemical, St. Louis, MO) and centrifuged at 10,000 g for 10 min at 4°C. Supernatants were incubated overnight at 4°C with constant rocking with anti-eIF₄E antibody. Immunoprecipitates were recovered with goat anti-mouse IgG magnetic beads (PolySciences, Warrenton, PA), washed, resuspended in sample buffer as described elsewhere (18), and immediately subjected to protein immunoblot analysis using rabbit anti-4EBP1 (Bethyl Laboratories) antibody or rabbit anti-eIF4G (Novus Biologicals, Littleton, CO). Amounts of 4E-BP1 and eIF4G were corrected by the eIF4E recovered from the immunoprecipitate.

Calculations and statistics. The fractional rate of protein synthesis (Kₜ, % protein mass synthesized in a day) was calculated as $Kₜ = \left(\frac{E₀}{Eₜ}\right) \times (1.440/t) \times 100$

where E₀ (in pmol/nmol) is the specific radioactivity of the protein-bound phenylalanine, Eₜ (in pmol/nmol) is the specific radioactivity of the tissue free phenylalanine at the time of tissue collection, t is the time of labeling in minutes, and 1.440 is the minutes-to-day conversion.

ANOVA was carried out using a general linear model to determine main statistical differences, including the interaction term. Between group analysis was performed using a t-test. Probability values of <0.05 were considered significant for all comparisons. Data are presented as means ± SE.

RESULTS

Plasma hormone and substrate concentrations. The plasma hormone and substrate concentrations shown in Tables 1 and 2 are mean values obtained either at baseline or at the end of the infusion. To determine the effectiveness of pST treatment, circulating PUN, IGF-I, and somatotropin concentrations were measured. Treatment of pigs with pST reduced circulating levels of PUN by ~30% (P < 0.001) compared with saline-treated controls (Table 1). Compared with saline-treated controls, pST-treated pigs demonstrated 63% (P = 0.001) and 2,600% (P < 0.001) increases in plasma IGF-I and somatotropin levels, respectively (Table 1).

The effectiveness of the clamp in attaining the desired insulin, glucose, and amino acid concentrations was shown in Table 2. Target insulin levels of 1 L) fasting control and pST pigs (5 µU/ml), 2) fed levels of control pigs (25 µU/ml), and 3) fed levels of pST-treated pigs (50 µU/ml) during the 2-h clamp were largely achieved in all groups. Plasma glucose levels were for the most part maintained within 10% of fasting, preclamp levels at each insulin level. However, glucose levels in the fasting insulin groups (5 µU/ml) were higher than baseline levels (P < 0.05), even though additional glucose was not provided to these groups. Additionally, pigs treated with pST had higher glucose concentrations than control animals (P < 0.002) in all treatment periods (although this reached significance only in the preclamp phase). BCAA, essential (EAA), nonessential (NEAA), and, hence, total (TAA) amino acid concentrations were successfully maintained over the course of the infusion period by use of the amino acid clamp method (Table 2).

Glucose and amino acid disposal rates. Net whole body glucose and amino acid disposal rates were calculated from the average infusion rate of dextrose and a balanced amino acid mixture required to maintain circulating levels within 10% of fasting baseline. Glucose disposal rates were altered by circulating insulin levels (P < 0.001); this was driven by the large increase in glucose uptake observed between 5 and 25 µU/ml (P < 0.001) of insulin (Fig. 2A). Additionally, raised plasma insulin levels increased whole body amino acid disposal rates in control and pST-treated animals at fed insulin concentrations (25 and 50 µU/ml), but there was no further increase between 25 and 50 µU/ml of insulin (Fig. 2B).

Skeletal muscle protein synthesis. The fractional rate of skeletal muscle protein synthesis was increased by both insulin and pST treatment (P < 0.005; Fig. 3). Increasing circulating insulin concentrations to either 25 or 50 µU/ml increased fractional rates of protein synthesis by 20–25% compared with fasting insulin levels (5 µU/ml, P = 0.001). However, there was no significant difference between the two fed insulin levels (P > 0.15), indicating that maximal rates of protein synthesis may be achieved at or before 25 µU/ml. Pigs treated with pST had higher rates of protein synthesis in the skeletal muscle compared with those treated with saline only, and there was no interaction of the effect of pST and insulin.

Translation initiation factors. To determine whether chronic pST treatment increased skeletal muscle protein synthesis by promoting insulin-induced translation initiation, Western blot analysis was performed. PKB phosphorylation (Fig. 4) on Ser¹⁷³ was used as an indicator of the activation of early steps in the insulin-signaling pathway. Raising insulin concentrations from fasting (5 µU/ml) to levels seen in fed animals (25 or 50 µU/ml) increased PKB phosphorylation (P < 0.001). However, doubling the insulin concentration from 25 to 50 µU/ml showed no additional effect on PKB phosphorylation. There was no significant difference on PKB phosphorylation between control and pST-treated animals, suggesting that pST does not stimulate protein synthesis via this pathway.

Phosphorylation of PKB leads to the activation of mTOR, a master kinase, which phosphorylates both S6K1 and 4E-BP1 (26, 51). S6K1 phosphorylation on its Thr³⁸⁹ residue was increased when insulin concentrations were increased from

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**Table 1. Plasma hormone and substrate concentrations in control and pST-treated pigs at the beginning of the insulin clamp**

<table>
<thead>
<tr>
<th></th>
<th>Saline</th>
<th>pST</th>
</tr>
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<tbody>
<tr>
<td>PUN, mg/dl</td>
<td>12.2 ± 0.5</td>
<td>8.0 ± 0.48</td>
</tr>
<tr>
<td>IGF-I, ng/ml</td>
<td>126.6 ± 29.2</td>
<td>290.1 ± 32.8</td>
</tr>
<tr>
<td>pST, ng/ml</td>
<td>4.6 ± 2.8</td>
<td>116.9 ± 49.1</td>
</tr>
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Values are means ± SE; n = 11–13/treatment group. PUN, plasma urea nitrogen; IGF-I, insulin-like growth factor I; pST, porcine somatotropin. §P < 0.001 vs. saline-treated pigs. Significance determined by t-test analysis.
fasting to fed levels (P < 0.001; Fig. 5A), but no additional stimulation was detected when insulin levels were doubled from 25 to 50 μU/ml. Phosphorylation of 4E-BP1 on Thr70 was also increased when the circulating insulin level was increased from fasting to fed levels (P < 0.001; Fig. 5B) with no additional stimulation measured when insulin levels were doubled from 25 to 50 μU/ml. The phosphorylation of 4E-BP1 causes the disassociation of the inactive 4E-BP1-eIF4E complex (36). Measurement of the abundance of the 4E-BP1-eIF4E complex revealed that increasing insulin concentration to fed levels was associated with a reduction in the abundance of this complex (P < 0.001; Fig. 6A). Disassociation of 4E-BP1-eIF4E allows eIF4E to be free to associate with eIF4G and form the active eIF4E-eIF4G complex (36). As predicted, fed levels of insulin showed a greater abundance of the active eIF4E-eIF4G complex compared with pigs maintained at fasting insulin concentrations (P < 0.001; Fig. 6B), with no additional stimulation detected when insulin levels were doubled from 25 to 50 μU/ml. There was no effect of pST treatment on S6K1 phosphorylation, 4E-BP1 phosphorylation, or 4E-BP1-eIF4E and eIF4E-eIF4G complex formation in skeletal muscle.

**DISCUSSION**

Treatment with pST for one wk increases the rate of skeletal muscle protein synthesis, but the mechanism by which this occurs has not yet been elucidated (2, 4–6). Chronic treatment with pST doubles postprandial circulating levels of insulin (16), a known promoter of protein synthesis in growing animals (10, 41). Insulin activates the insulin-signaling pathway...
by binding to its receptor, leading to a phosphorylation-signaling cascade that activates mTOR (26, 51). This master protein kinase promotes the formation of the active eIF4E binding protein-1 (4E-BP1; Fig. 4) phosphorylation in skeletal muscle at the end of a pancreatic glucose-amino acid clamp in control and pST-treated pigs. Pigs were infused with insulin at 18, 90, and 200 ng·kg\(^{-1}\)·min\(^{-1}\) to reproduce fasting (5 μU/ml), fed control (25 μU/ml), or fed pST-treated (50 μU/ml) levels, while glucose and amino acids were clamped at baseline fasting levels. ANOVA indicates a significant effect of insulin (P < 0.001). Results of paired t-test: †response to insulin dose different from fasting (5 μU/ml, P < 0.05). Values are means ± SE; n = 5–7 per group.

Chronic treatment with pST is known to alter circulating levels of PUN and IGF-I (21, 49). Previous studies have demonstrated that treatment with pST reduces the activity of urea cycle enzymes (3) and, hence, urea synthesis (49). IGF-I levels have been shown to increase in response to pST treatment (2, 21, 25). The increase of 63% in pST-treated animals in the current study is slightly lower than previously reported (2, 49), the reason for this is not known. Taken together, the reduced PUN and increased IGF-I concentrations confirm the effectiveness of the pST treatment regimen.

Plasma glucose levels are elevated in response to chronic treatment with pST (24, 27, 52), and this rise may be the result of reduced tissue glucose uptake in response to pST-induced insulin resistance (15, 48). In this study, pigs treated with pST did not show a significant reduction in whole body glucose disposal compared with control animals. The lack of effect observed in the current study may be a result of the large variation associated with the measurement of glucose uptake. Alternatively, as the majority of studies investigating the effects of pST treatment are conducted in mature animals near market weight, differences in the body composition between those mature animals and the current rapidly growing pigs may account for the observations. Several studies have shown that insulin resistance in response to long-term pST stimulation occurs in adipose tissue (16, 52, 53), with little or no reported effect on skeletal muscle (17, 52). In pST-treated animals, the decreased response to insulin in adipose tissue may be a consequence of the redistribution of nutrients in pST-treated animals from fat accretion to protein deposition (17, 32). Consequently, mature animals may display a greater insulin resistance to glucose disposal due to higher levels of adipose tissue compared with younger rapidly growing pigs. Importantly, we also found that pigs treated with pST did not show an altered response of whole body amino acid disposal to insulin.

**Protein synthesis rates.** In this study, treatment of pigs with pST increased muscle protein synthesis rates by ~16% compared with saline-treated control animals. It was shown previously that pST treatment increases the efficiency with which nutrients are used for muscle mass gain by redistributing nutrients from fat to muscle (5, 6, 17, 19, 35, 48). Treatment with pST has been shown to increase whole body (24, 47) and skeletal muscle (5, 33, 45) protein synthesis rates; however, the mechanism through which this occurs is unknown. Previous studies conducted in our laboratory have shown that pST increases muscle protein synthesis in the fed state but not in the fasted state (2). Although pigs were fasted overnight in the
current study, pST treatment increased the rate of muscle protein synthesis and there was no interactive effect of pST and insulin. Differences between studies may have arisen due to altered periods of pST treatment, i.e., 7–10 days in the current study vs. 7 days in the previous studies. Alternatively, the different measurement times of protein synthesis rates may have influenced the results; i.e., in the current study, pigs were killed and tissues frozen for analysis of protein synthesis rates 3 h after the last pST injection, whereas in the study by Bush et al. (2), tissue sampling occurred 8 h after the last injection. As pST levels peak 1–6 h postinjection (16), the main effect of pST on muscle protein synthesis in the fasting state may have occurred during this time window and declined by 8 h postinjection. However, even 8 h postinjection, pST treatment continued to promote skeletal muscle protein synthesis in animals maintained in the fed state in the previous study (2). Therefore, it appears that a pST-mediated increase in muscle protein synthesis in the fasting state may be transient and that this transient effect could be due to limited substrate availability to maintain increased rates of muscle protein synthesis in the fasted animal.

Postprandial insulin concentrations in pST-treated animals are double that observed in controls (16), and because insulin is a known promoter of skeletal muscle protein synthesis (40), this study investigated whether increased insulin levels alone could account for the rise in protein synthesis in pST-treated animals after feeding. While insulin did increase skeletal muscle protein synthesis in both control and pST-treated animals, pST did not further enhance protein synthesis rates. Furthermore, increasing circulating insulin levels from 25 μU/ml (as seen in fed controls) to 50 μU/ml (as seen in fed pST-treated animals) had no significant effect on protein synthesis rates in the skeletal muscle. This supports studies by Vann et al. (48), wherein raising insulin levels from 25 to 50 μU/ml, as in the current study, did not further enhance whole body protein synthesis. However, that study showed no stimulation of protein synthesis when insulin levels were raised from fasting to 25 μU/ml. Since that study was conducted at the whole body level, tissues such as the liver (2), which do not respond to insulin stimulation and have higher rates of protein synthesis than do muscle, may have obscured the stimulatory effect of insulin in skeletal muscle. Therefore, our results suggest that the pST-induced increase in protein synthesis rates is not a consequence of elevated circulating insulin levels.

Translation initiation. Translation initiation rates can be altered by several anabolic stimuli, including amino acids, insulin, and IGF-I (11, 18, 20, 31). Anabolic agents have been shown to alter the formation of the active eIF4E-eIF4G complex, which aids the binding of the m7GTP cap to the 5′ end of the mRNA (37–39, 46). Insulin promotes activation of translation initiation via activation of mTOR involved signaling through the PI 3-kinase/PKB pathway (1, 50), which in turn activates the eIF4E-signaling cascade complex. In the current study, the phosphorylation of PKB was increased by insulin but not by pST. Increasing insulin concentrations from fasting to 25 μU/ml caused PKB phosphorylation to rise; however, further raising of insulin levels to 50 μU/ml was unable to further enhance phosphorylation of PKB, indicating that there may be a limit on the ability of insulin to stimulate the insulin-signaling cascade. As PKB is upstream of mTOR, it is not surprising that a similar pattern of phosphorylation was seen for the downstream translation effectors regulated by mTOR, i.e., 4E-BP1 and S6K1 (36, 46). As 4E-BP1 and S6K1 are well known substrates of mTOR activation, the phosphorylation of these two factors were taken as a measure of mTOR activity. Insulin increased 4E-BP1 and S6K1 phosphorylation and formation of the active eIF4E-eIF4G complex between fasting and 25 μU/ml, but raising insulin from 25 to 50 μU/ml insulin did not enhance this effect. This supports our protein synthesis data, which suggests that increased insulin levels in pST-treated animals does not account for the increase in protein synthesis in these animals. We (2) previously showed that pST treatment of growing pigs did not further enhance the effect of feeding on PKB or S6K1. However, in enterally fed growing pigs, pST treatment increased 4E-BP1 phosphorylation, and consequently eIF4E-eIF4G association was increased (2). The ability of feeding, but not insulin, to enhance eIF4E-eIF4G binding in pST-treated pigs suggests that maintaining glucose and amino acids at the fasting rather than the fed level may limit translation initiation. Alternatively, translation initiation and, hence, protein synthesis may be enhanced via an mTOR-independent pathway. Insulin is also known to promote the activity of eIF2B (42), an initiation factor involved in bringing the initiation codon to the ribosome (42). The activity of eIF2B was not measured in this study; however, in
of our previous work, eIF2B activity was increased by pST treatment but was unaffected by feeding (2).

A study conducted by Hayashi and Proud (28) in cell culture suggested that increased levels of protein synthesis in response to pST treatment are signaled through the mTOR pathway in hepatocytes. In that study, treatment of rat hepatoma cells with either wortmannin (an PI 3-kinase inhibitor) or rapamycin (an inhibitor of mTOR) blocked somatotropin-induced phosphorylation of 4E-BP1 and S6K1, suggesting that somatotropin activates the PI 3-kinase/mTOR pathway in the liver (28). However, the concentration of somatotropin used in that study was not reported. Also, as the study was conducted in hepatic cells, direct comparison to the current study is not possible, because differences in protein synthesis activation have been shown to occur in skeletal muscle and liver (2). However, activation of the PI 3-kinase/PKB pathway in the liver may not indicate a role for insulin in the activation of translation initiation by pST, as somatotropin binding to its own receptor causes phosphorylation of the Janus family tyrosine kinase-2 (34), which can activate PI 3-kinase (29, 43).

Perspectives. The results of the current study demonstrate that treatment of growing pigs with pST for 7–10 days increases skeletal muscle protein synthesis independently of the pST-induced elevation in circulating insulin levels and the mTOR pathway. In the present study, increasing circulating insulin levels above those normally associated with feeding did not further increase the activation of indexes of translation initiation. Since mTOR is one conversion point for several anabolic and growth-signaling pathways and has been shown to be activated by somatotropin receptor activation in some tissues, a role of mTOR activation under differing nutritional conditions, such as in the presence of fed amino acid levels, deserves further investigation.

ACKNOWLEDGMENTS

We thank M. Fiorotto for helpful discussions, J. Fleming for technical assistance, D. Miller and J. Stubblefield for care of animals, E. O. Smith for statistical assistance, A. Gillum for graphics, and L. Weiser for secretarial assistance. Present address of J. S. Jeyapalan: Pediatric Critical Care Medicine, PO Box 100296, Gainesville, FL 32610. Present address of J. Frank: Dept. of Animal Science, University of Arkansas, Fayetteville, AR 72701.

GRANTS

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, Houston, TX. This project was supported by National Research Initiative Competitive Grant no. 2005-35206-15273 from the USDA Cooperative State Research, Education, and Extension Service. The contents of this publication do not necessarily reflect the views or policies of the US Department of Agriculture, nor does the mention of trade names, commercial products, or organizations imply endorsement by the US Government.

REFERENCES

SOMATOTROPIN REGULATION OF MUSCLE PROTEIN SYNTHESIS


