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Dietary Arginine Supplementation Increases mTOR Signaling Activity in Skeletal Muscle of Neonatal Pigs[1,2]

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Abstract

Dietary arginine supplementation increases growth of neonatal pigs, but the underlying mechanisms are unknown. This study was conducted to test the hypothesis that the arginine treatment activates translation initiation factors and protein synthesis in skeletal muscle. Piglets were fed milk-based diets supplemented with 0 or 0.6% L-arginine between 7 and 14 d of age. Following a 7-d period of arginine supplementation, at 1 h after the last meal, jugular venous blood samples were obtained for metabolite analysis, whereas longissimus muscle and liver were collected to determine the abundance and phosphorylation state of the mammalian target of the rapamycin (mTOR), ribosomal protein S6 kinase 1 (S6K1), eukaryotic initiation factor (eIF) 4E-binding protein-1 (4E-BP1), eIF4E, and eIF4G. Fractional rates of protein synthesis were measured in muscle and liver using the [^{3}H]phenylalanine flooding-dose technique. Arginine supplementation increased ($P < 0.05$) daily gain, the plasma insulin concentration, and protein synthesis in skeletal muscle but not in liver. The arginine treatment enhanced the formation of the active eIF4E·eIF4G complex but reduced the amount of the inactive 4E-BP1·eIF4E complex in muscle. These changes were associated with elevated levels of phosphorylated mTOR and 4E-BP1 in muscle of arginine-supplemented piglets ($P < 0.05$). Neither the total amounts nor phosphorylation levels of the translation initiation factors in the liver differed between control and arginine-supplemented piglets. Collectively, these results suggest that dietary arginine supplementation increases mTOR signaling activity in skeletal muscle, but not in liver, of milk-fed neonatal pigs. The findings provide a molecular mechanism for explaining the previous observation that increased circulating arginine stimulated muscle protein synthesis and promoted weight gain in neonatal pigs. J. Nutr. 138: 867–872, 2008.

Introduction

Arginine is an essential amino acid for maximal growth of neonatal pigs (1). There is growing evidence that arginine displays remarkable metabolic and regulatory versatility in cells (2). Arginine takes part in multiple pathways of enormous nutritional and physiological importance, including the synthesis of protein, nitric oxide, creatine, proline, glutamate, polyamines, and agmatine (3). Notably, piglets have a high requirement of arginine for growth (4), but sow milk provides at most 40% of arginine requirements by the 7-d-old pig (5,6). On the basis of plasma concentrations of arginine, ammonia, and nitric oxide metabolites, Wu et al. (1) have suggested that an arginine deficiency is a major factor limiting maximal growth of sow-reared piglets. In support of this proposition, dietary arginine supplementation markedly enhances protein accretion and the efficiency of nutrient utilization in milk-fed piglets (7). Additionally, an increase in circulating arginine was associated with enhanced protein synthesis in skeletal muscle of neonatal pigs (8).

Many studies have demonstrated that increasing the overall availability of amino acids can enhance protein synthesis in skeletal muscle and liver by enhancing the activities of positive regulators of translation initiation factors (9–13). Interestingly, an acute infusion of leucine to piglets also stimulates the phosphorylation of the mammalian target of the rapamycin (mTOR),[5] ribosomal protein S6 kinase (S6K1), and eukaryotic initiation factor (eIF)-4E-binding protein-1 (4E-BP1) (9,11), which in turn

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[5] Abbreviations used: BW, body weight; 4E-BP1, eukaryotic initiation factor 4E-binding protein-1; eIF, eukaryotic initiation factor; mTOR, mammalian target of the rapamycin; S6K1, ribosomal protein S6 kinase 1.

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releases eIF4E from the inactive 4E-BP1·eIF4E complex (14–19). Free eIF4E binds to eIF4G and eIF4A to form the active eIF4F complex, which mediates the binding of mRNA to the 43S ribosomal complex (20–23). However, a role for arginine in the activation of these regulatory initiation factors has not been studied in neonatal pigs, whose growth is highly sensitive to arginine availability (8). We hypothesized that arginine can enhance the activation of translation initiation factors and protein synthesis in skeletal muscle and liver of milk-fed piglets.

Materials and Methods

Dietary treatment. Milk replacer powder for piglets was prepared according to a previous study (7), which consisted of whey protein concentrate, milk fat powder, α- casein, lactose, glucose, calcium lactate, vitamin mixture, and mineral mixture purchased from Tianke Company. L-Arginine and L-alanine were provided by Ajinomoto. The basal milk replacer powder (87.5% dry matter) was supplemented with 0.6% L-arginine or 1.23% L-alanine (isonitrogenous control) (dietary composition is summarized in **Table 1**). The liquid diet was prepared by mixing 1 kg of milk replacer powder with 4 L of water to obtain a milk solution (dry matter 17.5%). The level of arginine in the basal milk replacer powder was 0.58%, as analyzed by HPLC (7). The dosage of supplemental arginine (0.6%) was chosen because it was shown in our previous studies to further increase the weight gain of milk-fed piglets compared with piglets supplemented with 0.4% L-arginine (7).

Animals and tissue collection. We conducted the experiment in accordance with the Chinese guidelines for animal welfare and it was approved by the animal welfare committee of the Institute of Subtropical Agriculture, Chinese Academy of Sciences. Twenty-four, 7-d-old healthy piglets (Landrace × Yorkshire) with a mean body weight (BW) of 2.76 ± 0.41 kg were removed from 5 sows and housed individually in plastic-floored pens (1.5 m × 0.5 m) at an ambient temperature of 33°C in an environmentally controlled room. Piglets were fed every 4 h at 73 mL/kg BW per meal. At 14 d of age, blood samples were collected into EDTA-coated tubes from the jugular vein at 1 h after the last meal in the morning. Ten piglets were then killed after anesthesia with an intraperioneal injection of sodium pentobarbital (50 mg/kg BW) followed by quick dissection of liver and longissimus muscle. Blood samples were immediately centrifuged at 3000 × *g*; 5 min at 4°C and the supernatant (plasma) was stored at −80°C for analysis of amino acids and hormones. Liver and muscle samples were rapidly frozen in liquid nitrogen and stored at −80°C for Western blotting analysis.

TABLE 1 Composition and nutrient content of diets[1]

	Control	Arginine
Ingredients, %		
Whey protein concentrate (34% CP)	60.0	60.0
Milk fat powder (11% CP)	26.0	26.0
α-Casein	6.2	6.2
Lactose	3.6	3.6
Glucose	0.42	1.05
Calcium lactate (13% Ca)	1.0	1.0
Vitamin mixture[2]	0.1	0.1
CaH_2PO_4 (22% P)	1.0	1.0
Mineral mixture[3]	0.2	0.2
L-Alanine	1.23	0.0
L-Arginine	0.0	0.6
L-Methionine	0.1	0.1
L-Lysine-HCl	0.1	0.1
Terramycin-HCl	0.05	0.05
Nutrient content		
Crude protein, %	26.01	26.01
Digestible energy, *MJ/kg*	14.39	14.39
Calcium, %	0.90	0.90
Total phosphorus, %	0.7	0.7
L-Lysine, %	1.58	1.58
L-Methionine, %	0.53	0.53
L-Arginine, %	0.58	1.18

[1] The dry matter content of the powder diet was 87.5%.
[2] Provided the following (mg/kg powder diet): retinyl acetate, 0.76; cholecalciferol, 0.055; all-rac-α-tocopheryl acetate, 16; menadione sodium bisulfate, 0.50; vitamin B-12, 0.02; riboflavin, 4.0; niacin, 20; pantothenic acid, 12; choline chloride, 600; folic acid, 0.30; thiamin, 1.5; pyridoxine, 2.0; biotin, 0.08.
[3] Provided the following (mg/kg power diet): Zn (as $[C_5H_{11}NO_2S]_2Zn$), 100; Mn (as $[C_2H4N_2O_2]_2Mn$), 5.0; Fe (as $[C_2H_4O_2N]_2Fe$), 100; Cu (as $[C_2H_5NO_2]_2Cu$), 10; I (as KI), 0.2; Se (as Na_2SeO_3), 0.30.

Measurement of tissue protein synthesis. Seven-d-old piglets received dietary supplementation with alanine (control) or arginine for 7 d, as described above. At d 14 of age, 1 h after feeding in the morning, we determined rates of protein synthesis in longissimus muscle and liver using i.p. administration of a flooding dose of phenylalanine as described (13), except that [^{3}H]phenylalanine was used as a tracer (8). Blood samples were obtained from the jugular vein at 10, 20, and 30 min after the injection of [^{3}H]phenylalanine to determine its specific activity in plasma (10). Specific activities of [^{3}H]phenylalanine in the tissue free pool and protein of muscle and liver were measured to calculated fractional rates of protein synthesis, as described by Watford and Wu (10).

Chemical analysis. Amino acids in plasma were analyzed by HPLC, as previously described (5). Concentrations of insulin in plasma were determined using porcine RIA kits (Shanghai Biological Manufacturing). The intra- and inter-assay CV were 8.2 and 12.4%, respectively, for insulin.

Protein immunoblot analysis. Frozen liver or muscle samples were powdered under liquid nitrogen using a mortar and pestle. The powdered tissue was homogenized in 7 volumes of buffer (20 mmol/L HEPES, pH 7.4, 100 mmol/L KCl, 0.2 mmol/L EDTA, 2 mmol/L EGTA, 1 mmol/L dithiothreitol, 50 mmol/L NaF, 50 mmol/L β-glycerolphosphate, 0.1 mmol/L phenylmethanesulphonylfluoride, 1 mmol/L benzamidine, 0.5 mmol/L sodium vanadate, and 1 μmol/L microcystin, leucine and arginine). The homogenate was centrifuged at 10,000 × *g*; 10 min at 4°C. This solution was mixed with an equal volume of a 2× sample buffer (2 mL of 0.5 mol/L Tris, pH 6.8, 2 mL glycerol, 2 mL of 10% SDS, 0.2 mL of β-mercaptoethanol, 0.4 mL of a 4% solution of bromphenol blue, and 1.4 mL of water). The samples were boiled for 5 min and cooled on ice before being used for Western blot analysis. The samples were subjected to electrophoresis on a 7.5% polyacrylamide gel for detection of S6K1, a 15% polyacrylamide gel for detection of 4E-BP1, or a 6% polyacrylamide gel for detection of mTOR. Proteins were electrophoretically transferred to polyvinylidene difluoride membranes. The blots were incubated with primary antibodies for total eIF4E, phosphospecific eIF4E (Ser^{209}), total S6K1, phosphospecific S6K1 (Thr^{389}), and total mTOR (all were rabbit polyclonal antibodies from Santa Cruz Biotechnology); a goat anti-eIF4G antibody (Santa Cruz Biotechnology); and phosphorylated (Ser^{2448}) mTOR (Cell Signaling). The membrane was then washed with 1×Tris-buffered saline containing 0.1% Tween 20 and incubated with a secondary antibody (horseradish peroxidase-conjugated goat anti-mouse; Zhongsan Gold Bridge) at 4°C for 3 h. The blots were exposed to X-ray film in a cassette equipped with a DuPont Lightning Plus intensifying screen. After development, films were scanned using a Microtek ScanMaker V scanner connected to a Lenovo Y300 computer. Images were obtained for Adobe Photoshop and quantitated using Scanimage software.

Quantification of the 4E-BP1·eIF4E and eIF4G·eIF4E complexes. The 4E-BP1·eIF4E and eIF4G·eIF4E complexes were quantified as described previously (14–18). Briefly, eIF4E was immunoprecipitated with an anti-eIF4E polyclonal antibody (Santa Cruz biotechnology) from aliquots of the supernatants obtained from centrifugation of the liver and muscle

homogenates at 10,000 × *g* for 10 min. Antibody-antigen complexes were collected using magnetic beads and subjected to electrophoresis using a 15% polyacrylamide gel. Proteins were then electrophoretically transferred to a polyvinylidene difluoride membrane. The blots were incubated with a rabbit polyclonal anti-eIF4E antibody, a rabbit polyclonal anti-4EBP1 antibody, or a goat anti-eIF4G antibody for 12 h at 4°C. The phosphorylated forms of 4E-BP1 were measured after immunoprecipitation of 4E-BP1 from the tissue homogenates after centrifugation at 10,000 × *g*. The various phosphorylated forms of 4E-BP1 were separated by SDS-PAGE and analyzed by protein immunoblotting. The blots were developed with enhanced chemiluminescence and the autoradiographs were scanned for analysis, as described above.

Statistical analysis. Experimental data for each group are expressed as means with pooled SEM. Statistical evaluation of the data were carried out using the General Linear Model procedure of the Analysis Systems Institute (SAS, 2000) followed by the Student-Newman-Keuls multiple comparison test. Differences between the groups were considered significant at $P < 0.05$.

Results

Milk intake and growth performance. Daily food intake did not differ between control and arginine-supplemented piglets during a 7-d experimental period (**Table 2**). The initial BW at d 7 of age did not differ between the 2 groups (Table 2). However, dietary supplementation with arginine increased ($P < 0.05$) BW and daily weight gain of piglets by 10% and 38%, respectively. The ratio of dry matter intake: BW gain (g/g) was 1.93 and 1.52, respectively, for control and arginine-supplemented piglets (SEM = 0.13; $n = 12$; $P < 0.05$).

Plasma amino acids and insulin. Plasma concentrations of arginine, citrulline, and ornithine were 25, 20, and 19% greater ($P < 0.05$) in arginine-supplemented piglets than in control piglets, respectively (**Table 3**). Dietary supplementation with L-arginine did not affect plasma concentrations of lysine, histidine, methionine (Table 3), isoleucine, glutamate, leucine, phenylalanine, threonine, tyrosine, or valine (data not shown). Compared with control piglets, plasma insulin concentration was enhanced ($P < 0.05$) in arginine-supplemented piglets (Table 3).

Tissue protein synthesis. Fractional rates of protein synthesis were higher ($P < 0.01$) in liver than in skeletal muscle of piglets (Table 3). Dietary supplementation with arginine enhanced ($P < 0.05$) the fractional rate of protein synthesis in longissimus muscle by 24%. However, hepatic protein synthesis did not differ between control and arginine-supplemented piglets (Table 3).

Amounts of eIF4G·eIF4E and 4E-BP1·eIF4E complexes. Compared with piglets fed the control diet, dietary arginine supplementation reduced ($P < 0.05$) the association of eIF4E with 4E-BP1 (**Fig. 1**) but increased ($P < 0.05$) the formation of the active eIF4G·eIF4E complex (**Fig. 2**) in longissimus muscle. In liver, the abundance of eIF4E·eIF4G or 4E-BP1·eIF4E complex did not differ between control piglets and arginine-supplemented piglets. The arginine treatment did not affect the percentage levels (%) of phosphorylated eIF4E in skeletal muscle (2.24 and 2.30 in control and arginine-treated piglets, respectively; SEM = 0.46, $n = 10$) or liver (3.62 and 4.05 in control and arginine-treated piglets, respectively; SEM = 0.29, $n = 10$).

TABLE 2 Effect of dietary L-arginine supplementation on food intake and growth performance of piglets[1]

	Control	Arginine	SEM
Initial BW, *kg*	2.72	2.75	0.03
Final BW, *kg*	3.38	3.67*	0.09
Average daily gain, *g/d*	93.7	129.3*	8.63
Daily dry matter intake, *g/d*	180.0	196.0	15.6

[1] Values are means with pooled SEM, $n = 12$. The diets of piglets were supplemented with 0.6% L-arginine or 1.23% L-alanine (isonitrogenous control) between d 7 and 14 after birth. *Different from the control, $P < 0.05$.

TABLE 3 Effects of dietary arginine supplementation on plasma concentrations of insulin and amino acids and fractional rates of protein synthesis in skeletal muscle and liver in 14-d-old piglets[1]

	Control	Arginine	SEM
Plasma concentrations			
Insulin, *pmol/L*	36.8	52.8*	3.1
Arginine, *μmol/L*	142.0	174.0*	6.7
Histidine, *μmol/L*	76.0	77.0	3.6
Lysine, *μmol/L*	218.0	217.0	19.0
Methionine, *μmol/L*	66.0	59.0	5.3
Citrulline, *μmol/L*	73.0	86.0*	1.7
Ornithine, *μmol/L*	71.0	85.0*	3.7
Fractional rates of protein synthesis, *%/d*			
Longissimus muscle	15.4	19.2*	0.67
Liver	75.9	78.6	3.2

[1] Values are means with pooled SEM, $n = 12$ (treatment for plasma metabolites) or 9 (treatment for fractional rates of protein synthesis). The diets of piglets were supplemented with 0.6% L-arginine or 1.23% L-alanine (isonitrogenous control) between d 7 and 14 after birth. *Different from the control, $P < 0.05$.

Phosphorylation levels for 4E-BP1, S6K1, and mTOR. Phosphorylation at Thr^{46} and the γ-isoform of 4E-BP1, the repressor protein of eIF4E, is illustrated in **Figure 3**. Three forms of phosphorylated 4E-BP1 (α, β, and γ) were detected in both muscle and liver. The γ-form is most phosphorylated (Fig. 3). Dietary arginine supplementation markedly stimulated ($P < 0.05$) the phosphorylation of 4E-BP1 (Fig. 3) and mTOR (**Fig. 4**) in longissimus muscle. However, in liver, the levels of phosphorylated 4E-BP1 (Fig. 3) and mTOR (Fig. 4) did not differ between control and arginine-supplemented piglets. The arginine treatment did not affect S6K1 phosphorylation in skeletal muscle (0.86 and 0.98 in control and arginine-treated piglets, respec-

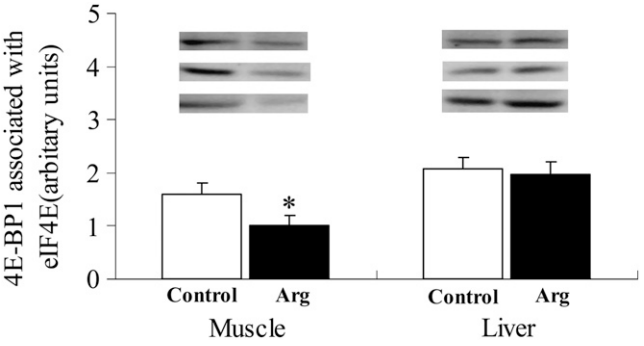

FIGURE 1 Effect of high dietary arginine on the association of 4E-BP1 with eIF4E in skeletal muscle and liver of pigs. The diets of piglets were supplemented with 0.6% L-arginine or 1.23% L-alanine (isonitrogenous control) between d 7 and 14 after birth. At d 14, 1 h after feeding, muscle and liver were obtained from piglets. Extracts of samples were immunoprecipitated with an anti-eIF4E antibody. Data are means ± SEM, $n = 10$. *Different from the control, $P < 0.05$.

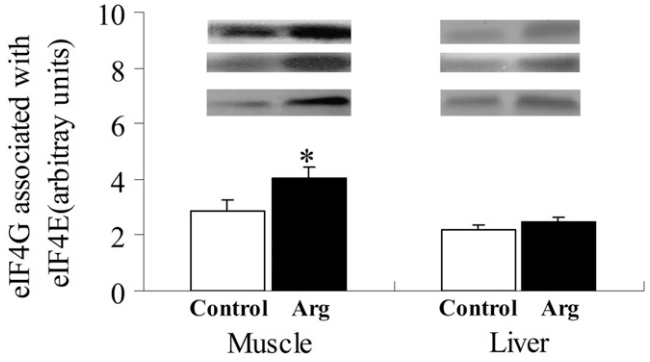

FIGURE 2 Effect of high dietary arginine on the association of eIF4G with eIF4E in skeletal muscle and liver of pigs. The diets of piglets were supplemented with 0.6% L-arginine or 1.23% L-alanine (isonitrogenous control) between d 7 and 14 after birth. At d 14, 1 h after feeding, muscle and liver were obtained from piglets. Extracts of samples were immunoprecipitated with an anti-eIF4E antibody. Data are means ± SEM, $n = 10$. *Different from the control, $P < 0.05$.

tively; SEM = 0.06, $n = 10$) or liver (0.89 and 1.04 in control and arginine-treated piglets, respectively; SEM = 0.14, $n = 10$).

In either skeletal muscle or liver, the relative abundance of total mTOR, eIF4E, S6K1, and 4E-BP1 proteins did not change between the 2 groups of piglets.

Discussion

To our knowledge, this is the first study to determine the effect of dietary arginine supplementation on the activation of translation initiation factors in muscle and liver of neonatal pigs after a meal. We confirmed the previous report that supplementing piglets with L-arginine increased plasma concentrations of insulin, as well as the growth of milk-fed piglets (7). Notably, novel and important findings from the present study are that the growth-promoting effect of arginine supplementation was associated with increased formation of the active eIF4G·eIF4E complex, activation of positive regulators of protein synthesis, and fractional rate of protein synthesis in skeletal muscle (Figs. 1–4; Table 3).

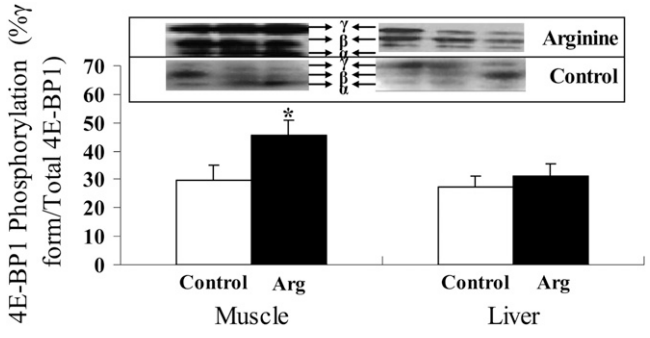

FIGURE 3 4E-BP1 phosphorylation in skeletal muscle and liver of piglets supplemented with 0.6% L-arginine or 1.23% L-alanine (isonitrogenous control) between d 7 and 14 after birth. At d 14, 1 h after feeding, muscle and liver were obtained from piglets. The phosphorylated forms of 4E-BP1 were measured after immunoprecipitation of 4E-BP1 from the tissue homogenates after centrifugation at 10,000 × g for 10 min at 4°C. Position of α-, β-, and γ-forms are denoted. Data are means ± SEM, $n = 10$. *Different from the control, $P < 0.05$.

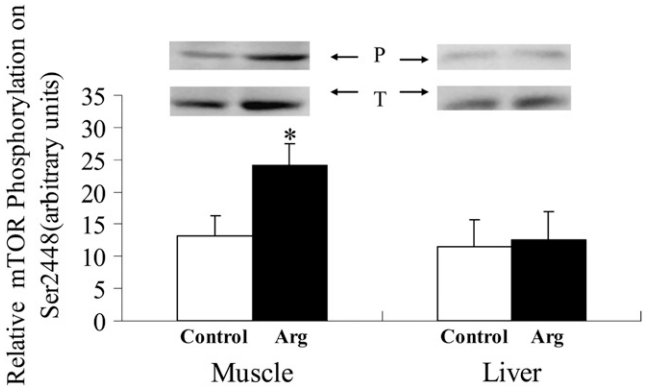

FIGURE 4 Phosphorylation state of mTOR in skeletal muscle and liver of piglets supplemented with 0.6% L-arginine or 1.23% L-alanine (isonitrogenous control) between d 7 and 14 after birth. At d 14, 1 h after feeding, muscle and liver were obtained from piglets. Tissue extracts were separated by 2 6% SDS-polyacrylamide gel electrophoresis and 1 was for determination of phosphorylation of mTOR at Ser^{2448} and another was for total mTOR. Values for phosphorylated mTOR were normalized for total mTOR content. Data are means ± SEM, $n = 10$. *Different from the control, $P < 0.05$.

Arginine is an essential amino acid for young pigs (24) but is severely deficient in milk-fed piglets (25) due to relative low concentrations in milk protein and reduced intestinal release of citrulline, an effective precursor of arginine (5). Recent studies have demonstrated that enhanced protein accretion in skeletal muscle and the whole body of milk-fed piglets can be achieved by elevating plasma levels of arginine through either dietary arginine supplementation (7,26) or enhancing endogenous arginine synthesis (8). This can now be explained by an arginine-induced increase in muscle protein synthesis (Table 3). It is noteworthy that recent studies have shown that arginine can increase protein synthesis in the small intestine of neonatal pigs (27) and that it also activates mTOR and other kinase-mediated signaling pathways in intestinal epithelial cells (28), where arginase activity is limited. Consistent with these reports, our results indicate that dietary supplementation with L-arginine can activate the mTOR signaling pathways in skeletal muscle of neonatal pigs (Figs. 1–4). Because muscle growth depends on the balance between protein synthesis and degradation, future studies are necessary to determine the effect of arginine on protein degradation in cells and the whole body.

In contrast to skeletal muscle, addition of arginine to culture medium did not affect mTOR expression or phosphorylation in mammalian hepatocytes (29), which rapidly degrade arginine by an exceedingly high activity of arginase (2). This is consistent with our finding that dietary supplementation with L-arginine did not affect the levels of total and phosphorylated mTOR or the fractional synthesis of proteins in piglet liver. However, because the flooding-dose method was used to measure global protein synthesis in this study (9), we cannot exclude the possibility that dietary arginine supplementation may increase the synthesis of specific protein(s) in the liver.

Another novel and important finding from this work is that dietary arginine supplementation affected the phosphorylation of 4E-BP1 in piglet muscle. When 4E-BP1 is phosphorylated, it is dissociated from the inactive eIF4E·4E-BP1 complex, thereby releasing eIF4E for binding with eIF4G to form the active eIF4G·eIF4E complex (19–21). Accordingly, dietary supplementation with L-arginine reduced concentrations of the eIF4E·4E-

BP1 complex and increased the formation of the active eIF4G·eIF4E complex in longissimus muscle of milk-fed neonatal pigs (Fig. 2). This would be expected to stimulate the formation of the 43S complex for initiating muscle protein synthesis (30). The changes in the abundance of 4E-BP1·eIF4E or eIF4G·eIF4E complex in muscle were not the result of altered expression of eIF4E protein, because the 2 groups did not differ in the content of phosphorylated or total eIF4E in skeletal muscle. Because piglet tissues were obtained at a single time point for the determination of the phosphorylation state of various protein factors, the finding that there was no detectable alteration in S6K1 phosphorylation at 60 min after the last meal does not exclude the possibility that a transient change in tissues might have been missed. In addition, it should be noted that the results of the current study indicate only associations between dietary arginine supplementation and components of the mTOR signaling pathway but do not unequivocally demonstrate causality.

Arginine is a potent stimulator of insulin and somatotropin secretion (30). Considerable work has demonstrated that both insulin (31–33) and amino acids (34–36) play critical roles in the regulation of protein synthesis in piglets. Except for arginine, ornithine, and citrulline, plasma concentrations of other measured amino acids did not differ between control and arginine-supplemented piglets (Table 3). It is noteworthy that published studies show that an increase in arginine availability enhanced the growth of neonatal pigs in the absence of a change in plasma levels of insulin or growth hormone (7,8). Therefore, the effect of arginine could be brought about independent of alterations in these hormones. In support of this notion, there is emerging evidence from studies with cultured muscle cells that arginine can directly stimulate the phosphorylation of proteins in the mTOR signaling pathway (37; G. Wu, unpublished data). However, it is possible that the maximum effect of dietary arginine supplementation on muscle protein synthesis and growth may be mediated in part by elevated levels of the circulating anabolic hormones via the insulin signaling pathway. This possibility can be examined by using the in vivo techniques of insulin and amino acid clamps that were developed by Davis et al. (34–36) for studies with neonatal pigs.

In conclusion, dietary supplementation with L-arginine to milk-fed piglets increased the phosphorylation of 4E-BP1, thereby enhancing the assembly of the active eIF4G·eIF4E complex and protein synthesis in skeletal muscle. These novel findings provide a molecular mechanism for explaining the previous observation that arginine supplementation enhanced piglet growth.

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