ABSTRACT: The postprandial increases in AA and insulin independently stimulate protein synthesis in skeletal muscle of piglets. Leucine is an important mediator of the response to AA. We have shown that the postprandial increase in leucine, but not isoleucine or valine, acutely stimulates muscle protein synthesis in piglets. Leucine increases muscle protein synthesis by modulating the activation of mammalian target of rapamycin (mTOR) complex 1 and signaling components of translation initiation. Leucine increases the phosphorylation of mTOR, 70-kDa ribosomal protein S6 kinase-1, eukaryotic initiation factor (eIF) 4E-binding protein-1, and eIF4G; decreases eIF2α phosphorylation; and increases the association of eIF4E with eIF4G. However, leucine does not affect the upstream activators of mTOR, that is, protein kinase B, adenosine monophosphate-activated protein kinase, and tuberous sclerosis complex 1/2, or the activation of translation elongation regulator, eukaryotic elongation factor 2. The action of leucine can be replicated by α-ketoisocaproate but not by norleucine. Interference by rapamycin with the raptor-mTOR interaction blocks leucine-induced muscle protein synthesis. The acute leucine-induced stimulation of muscle protein synthesis is not maintained for prolonged periods, despite continued activation of mTOR signaling, because circulating AA fall as they are utilized for protein synthesis. However, when circulating AA concentrations are maintained, the leucine-induced stimulation of muscle protein synthesis is maintained for prolonged periods. Thus, leucine acts as a nutrient signal to stimulate translation initiation, but whether this translates into a prolonged increase in protein synthesis depends on the sustained availability of all AA.

Key words: amino acid, leucine, muscle, newborn, pig, translation initiation

INTRODUCTION

Leucine, a branched-chain AA (BCAA), is an essential AA (EAA) that cannot be synthesized de novo by humans and other mammals (Hutson, 2006). It is now well recognized that leucine not only has a role as a substrate for protein synthesis, but also acts as a nutrient signal that regulates protein synthesis in various tissues of the body, including skeletal muscle (Anthony et al., 2001). Several studies have shown that leucine administration increases skeletal muscle protein synthesis in rodents (Crozier et al., 2005) and humans (Drummond and Rasmussen, 2008). Although the ability of leucine to stimulate protein synthesis is apparent, its mode of action is less clear (Proud, 2007). It has been established in studies originating with yeast and mammalian cell culture that leucine activates the mRNA translation machinery through the mammalian target of rapamycin (mTOR), a master kinase that is crucial for cell growth (Kimball et al., 1999; Du et al., 2007). One of the main goals of domestic animal production is to improve animal growth by means of increasing skeletal muscle mass. Thus, consideration of leucine in the nutritional management of farm animals is particularly important because of its role in the regulation of muscle protein synthesis. In this review, we present an overview of our studies on the effect of leucine ad-
ministration on protein synthesis conducted in vivo in neonatal pigs.

**REGULATION OF NEONATAL GROWTH**

During the neonatal period, the growth rate is greater than at any other state of postnatal life, and this is crucial for the long-term well-being of the organism (Reeds et al., 2000). This period is characterized by rapid protein deposition and greater nutritional efficiency, as well as marked differences in the growth rates of individual tissues and a series of maturational changes (Goldspink and Kelly, 1984; Denne and Kalhan, 1987; Davis et al., 1989; Reeds et al., 2000). More specifically, our early work and that of others show that neonates are very efficient in utilizing dietary AA for protein deposition, and this ability decreases markedly with development (McCranken et al., 1980; Burrin et al., 1991, 1992; Davis et al., 1991, 1993, 1996; Fiorotto et al., 1991; Wray-Cahen et al., 1998). Furthermore, the gain in protein mass is more rapid in skeletal muscle than in other tissues in the body (Davis and Fiorotto, 2009).

Early postnatal morbidity and mortality have posed significant challenges to the swine industry and increased the cost of animal production (Odle et al., 1996). Promotion of growth, muscling, and feed efficiency are primary goals of swine production. Thus, optimization of the diet to increase growth, sustain health, and minimize the consequences of disease during the early postnatal period is critically important for animal agriculture.

**ROLE OF AA IN THE REGULATION OF PROTEIN SYNTHESIS**

Protein synthesis is a large energy-consuming, complex process that is governed by mediators, such as hormones and nutrients (Ma and Blenis, 2009). More than 3 decades ago, investigators demonstrated that AA alone can stimulate protein synthesis in skeletal muscle in vitro (Buse and Reid, 1975; Fulks et al., 1975). Fulks et al. (1975) used diaphragms isolated from young rats to study the effect of insulin, glucose, and AA on protein turnover. In this study, the diaphragms from young rats were desirable because the muscle is thin and permits the rapid diffusion of nutrients or hormones. Fulks et al. (1975) found that addition of AA mixtures to the medium stimulated protein synthesis. Although the results of AA studies in vitro are important, Garlick and Grant (1988) questioned the physiological significance of those findings. One of the obvious concerns was that the AA concentrations used were 5 to 10 times greater than normal physiological concentrations (Lundholm and Schersten, 1977; Li and Jefferson, 1978). Thus, Preedy and Garlick (1986) conducted an in vivo study in which a normal physiological concentration of an AA mixture was infused into young rats and found that AA did not stimulate protein synthesis unless glucose was also infused. Preedy and Garlick (1986) concluded that AA increased the sensitivity of muscle to insulin, allowing it to stimulate protein synthesis at normal physiological concentrations (Preedy and Garlick, 1986; Garlick and Grant, 1988).

In our early studies, we developed the pancreatic-substrate clamp technique to identify the individual roles of AA and insulin in the regulation of protein synthesis in neonatal pigs (Wray-Cahen et al., 1997; Davis et al., 2002); we used an AA mixture containing EAA and nonessential AA that was similar to the AA composition of body proteins (Davis et al., 2002). When we infused the AA mixture to raise circulating AA to fed amounts, we demonstrated that AA alone could stimulate muscle protein synthesis (Davis et al., 2002). This response of protein synthesis to AA decreases with age in parallel with the age-related reduction in the postprandial rate of muscle protein synthesis. Furthermore, we determined that the stimulatory effect of AA occurs in the presence of fasting concentrations of insulin, at concentrations of insulin that are intermediate between the fasting and the fully fed levels, and even at below fasting insulin concentrations that are undetectable by RIA (O’Connor et al., 2003).

Now that several studies in different species ranging from rodents to humans (Preedy and Garlick, 1986; Garlick and Grant, 1988; Zanetti et al., 1999) have shown that AA can stimulate muscle protein synthesis, the obvious question would be: is the ability of each AA to induce protein synthesis equal? Surprisingly, this question was addressed by 2 independent groups approximately 35 yr ago (Buse and Reid, 1975; Fulks et al., 1975). In their studies, Fulks et al. (1975) demonstrated that of the AA in their mixture, only the BCAA enhanced protein synthesis. Further experiments showed that leucine alone or isoleucine and valine together stimulated protein synthesis. Using a hemidiaphragm preparation, Buse and Reid (1975) found that leucine acts as regulator that can stimulate protein synthesis and inhibit protein degradation in vitro. Since these remarkable findings, investigators have been seeking the answer as to whether leucine has similar effect in vivo in several species, including humans (Matthews, 2005).

The uniqueness and the ability of leucine to regulate protein synthesis are well-established (Garlick, 2005). However, the notion that leucine acts as a signaling molecule that activates cellular protein synthetic machineries is still unsettled (Avruch et al., 2009). Biochemical and genetic approaches have been used to dissect the molecular mechanisms by which leucine stimulates protein synthesis (Proud, 2007). There is a general consensus that in order for leucine to stimulate protein synthesis, it must first activate mTOR complex 1 [mTORC1; Figure 1 (Avruch et al., 2009; Kim, 2009)]. The master protein kinase, mTOR, is the central component of 2 independently regulated complexes: mTORC1 and mTORC2 (Sabatini, 2006; Lian et al., 2008). The mTORC1 consists of mTOR,
raptor, and the G-protein β-like protein, GβL/LST8, whereas mTORC2 is composed of mTOR, rictor, GβL, and mammalian stress-activated protein kinase-interaction protein 1. Whereas mTORC1 participates in major metabolic processes, including protein synthesis, mTORC2 has a relatively minor role (Huang and Manning, 2009). The most notable function of mTORC2 is to partly activate protein kinase B (PKB) by phosphorylation of the S473 site (Liao and Hung, 2010).

Whereas the AA signaling pathway is critical to the cellular regulation of growth, the insulin signaling pathway senses and coordinates general nutrient status (Hietakangas and Cohen, 2009). Both the insulin and AA signaling pathways converge at mTOR to regulate protein synthesis and, thus, growth. The insulin signaling pathway is well-characterized (Bevan, 2001). Briefly, upon insulin binding to its receptor, early steps in the insulin signaling cascade (i.e., insulin receptor, insulin receptor substrate 1, and phosphoinositide 3-kinase) are activated, resulting in the activation of PKB (Figure 1). By several proposed mechanisms, the activated PKB stimulates the activation of mTORC1. Activated PKB can phosphorylate the tuberous sclerosis complex (TSC)2, resulting in the disruption of the TSC1-TSC2 complex, a potent inhibitor of mTORC1 activation. In contrast, adenosine monophosphate-activated protein kinase (AMPK), an energy sensor that is activated by energy deficiency in the cell, can activate TSC1-TSC2, resulting in the inhibition of mTORC1. In other mechanisms, PKB can also phosphorylate proline-rich Akt substrate 40 kDa (PRAS40) and alleviate the suppression effect of PRAS40 on mTORC1.

Unlike insulin, the mechanism by which leucine stimulation leads to the activation of mTORC1 is not well understood (Avruch et al., 2009). Several possible signaling components have been identified that respond to changes in intracellular leucine with subsequent activation of mTORC1 (Figure 1). These include Ras homolog enriched in brain, mitogen-activated protein kinase kinase kinase kinase 3, vacuolar sorting protein 34, and Ras-related GTP-binding protein A-D (Avruch et al., 2006; Findlay et al., 2007; Backer, 2008; Shaw, 2008). Reviews of postulated mechanisms by which leucine activates these signaling components are presented elsewhere (Avruch et al., 2006; Findlay et al., 2007; Backer, 2008; Shaw, 2008).

Figure 1. A simple model of the molecular mechanisms by which insulin and AA/leucine regulate mRNA translation in the cytosol of eukaryotic cells. IRS-1 = insulin receptor substrate 1; PI-3K = phosphoinositide 3-kinase; PDK = phosphoinositide-dependent kinase 1; PKB = protein kinase B; MAP4K3 = mitogen-activated protein kinase kinase kinase kinase kinase 3; Vps34 = vacuolar sorting protein 34; Rag A-D = Ras-related GTP-binding protein A-D; eIF = eukaryotic initiation factor; mTOR = mammalian target of rapamycin; eEF2 = eukaryotic elongation factor 2; PRAS40 = proline-rich Akt substrate 40 kDa; Rheb = Ras homolog enriched in brain; TSC1/2 = tuberous sclerosis complex 1/2; S6K1 = ribosomal protein S6 kinase 1; AMPK = adenosine monophosphate-activated protein kinase.
A common consensus is that both insulin and leucine utilize and activate similar signaling components downstream of mTORC1, leading to the stimulation of mRNA translation and translation elongation (Proud, 2002; Figure 1). The mTORC1 regulates mRNA translation by phosphorylating 2 of its effectors, ribosomal protein S6 kinase 1 (S6K1) and eukaryotic initiation factor (eIF) 4E-binding protein-1 (4EBP1; Kimball, 2001; Proud, 2002). Phosphorylated 4E-BP1 releases eIF4E from the inactive eIF4E-4EBP1 complex, allowing the formation of the active eIF4E-eIF4G complex (eIF4F complex). The increased formation of eIF4F preferentially induces translation of mRNA containing long, highly-structured 5′-untranslated region (Kimball, 2001). Through the mTORC1-dependent pathway, leucine can also stimulate translation elongation by inhibiting the phosphorylation of eukaryotic elongation factor 2 (eEF2; Kimball, 2001; Proud, 2002). Amino acids also can influence protein synthesis in an mTORC1-independent fashion by preserving eIF2B activity (Kimball, 2001; Figure 1). Leucine inhibits phosphorylation of the α subunit of eIF2, which permits the activation of eIF2B, and promotes the translation of all mRNA (i.e., global protein synthesis; see review by Kimball, 2001).

**ACUTE EFFECTS OF LEUCINE ON PROTEIN SYNTHESIS AND ACTIVATION OF SIGNALING COMPONENTS THAT LEAD TO mRNA TRANSLATION IN NEONATAL PIGS**

Our early work demonstrated that AA were very effective in promoting the activation of components of mRNA translation and protein synthesis in muscle of neonatal pigs (Davis et al., 2002; O’Connor et al., 2003). Because leucine is considered a unique regulator of protein synthesis (Buse and Reid, 1975), we first determined the acute effect of different physiological amounts of leucine on protein synthesis in skeletal muscle of neonatal pigs (Escobar et al., 2005). We found that an acute (i.e., 1 h) infusion of leucine stimulated muscle protein synthesis in a dose-dependent manner (Figure 2A). To determine whether the ability to promote muscle protein synthesis is specific to leucine, we compared the response to leucine with the response to the other BCAA, isoleucine and valine, administered in equimolar concentrations. The results clearly demonstrated that leucine, but not isoleucine or valine, stimulated muscle protein synthesis (Figure 2B). In addition, leucine induced-stimulation occurred in the LM, which contains primarily fast-switch glycolytic fibers, and the masseter muscle, which contains more oxidative fibers (Figure 2B).

Although these studies demonstrated that leucine could stimulate muscle protein synthesis for up to 1 h, the response was not maintained after 2 h of infusion (Figure 3A and 3B). To further examine these responses, we evaluated the signaling components downstream of mTORC1. The increase in muscle protein synthesis after 1 h of leucine infusion was associated with an increase in the phosphorylation of S6K1 and 4EBP1 (Figure 3C and 3E; Escobar et al., 2005), and this increase in phosphorylation was sustained even after 2 h (Figure 3D and 3F) despite the absence of a protein synthesis response at 2 h. These data indicated that other factors are responsible for the lack of any effect of leucine on protein synthesis after 2 h. Importantly, we found that the 2- to 3-fold increase in circulating leucine concentrations was associated with a 50% reduction in the circulating concentrations of the other BCAA, valine and isoleucine (Figure 4A and 4B). Likewise, the concentrations of the other EAA had also decreased by the same magnitude (Figure 4C and 4D; Escobar et al., 2005). The results led us to speculate that the decline in the other EAA, as they are used for protein synthesis, may have limited the ability of leucine to continue to stimulate protein synthesis. Thus, the ability of leucine to
stimulate protein synthesis may be dependent upon the availability of substrate AA for protein synthesis.

To determine whether the leucine-induced stimulation of protein synthesis in skeletal muscle of neonatal pigs is dependent upon the availability of EAA, we infused neonatal pigs for 2 h with saline, leucine alone, or leucine in the presence of an AA clamp where a balanced AA mixture (without leucine) was infused to maintain circulating concentrations of all AA at fasting levels during the increase of leucine (Escobar et al., 2007). The amount of leucine achieved was about 2- to 3-fold greater than fasting levels and was similar to fed leucine levels regardless of whether we infused leucine alone or leucine in the presence of an AA clamp. This AA clamp was successful in preventing the reduction in the circulating concentration of other EAA during the infusion of leucine (Figure 5). With this protocol, the 2-h leucine infusion increased the phosphorylation of S6K1 and 4EBP1, as well as the formation of the active complex of eIF4E bound to eIF4G, similar to that which occurred at 1 h (Figure 6A). Importantly, when hypoaminoacidemia was prevented and euaminoacidemia was maintained with the AA clamp, the leucine-induced stimulation of muscle protein synthesis was sustained for 2 h (Figure 6B). The results indicate that the stimulation of protein synthesis by parenteral leucine infusion is dependent on the availability of AA.

**LEUCINE-INDUCED STIMULATION OF MUSCLE PROTEIN SYNTHESIS IS MTORC1 DEPENDENT**

The majority of information obtained on the molecular mechanism by which AA or leucine enhance protein synthesis has been based on in vitro or cell
culture studies (Du et al., 2007; Atherton et al., 2010). Because little was known of the mechanism of action of leucine in vivo, we examined the effect of rapamycin, a drug that acutely inhibits only mTORC1 activation, on the leucine-induced stimulation of protein synthesis and the activation of signaling components in skeletal muscle of 7-d-old pigs (Suryawan et al., 2008). We found that leucine and rapamycin had no effect on the phosphorylation of AMPK, PKB, and TSC2, but it severely reduced raptor-mTOR interaction. The inhibitory effect of rapamycin was absent in both the rictor-mTOR interaction and the GβL-mTOR interaction, verifying cell culture work showing that acute treatment with rapamycin has no effect on mTORC2 activation (Toschi et al., 2009). Consistent with these observations, rapamycin completely blocked leucine-induced phosphorylation of mTOR (Figure 7A), S6K1, and 4EBP1, as well as the leucine-induced formation of an active eIF4E-eIF4G complex (Figure 7B), whereas the formation of the inactive eIF4E-4EBP1 complex was increased significantly (Figure 7C; Suryawan et al., 2008). We did not detect any effect of rapamycin on the phosphorylation of eEF2, although studies in cell cultures indicate that eEF2 phosphorylation is mTOR-dependent (Proud, 2004). Importantly, we found that disruption of leucine-induced activation of mTORC1 has a detrimental effect on protein synthesis in vivo because rapamycin completely blocked leucine-induced muscle protein synthesis (Figure 7D; Suryawan et al., 2008), consistent with findings in cell culture (Talvas et al., 2006). In contrast, other studies from our laboratory demonstrated that rapamycin only partially blocked the feeding-induced stimulation of muscle protein synthesis in neonatal pigs (Kimball et al., 2000). Together the results indicate that, whereas leucine-stimulated protein synthesis is mTOR-dependent, the portion of the feeding-induced stimulation of muscle protein synthesis that is mediated by insulin or nonleucine AA or both is, at least in part, mTOR-independent.

**LEUCINE OR KETOISOCAPROATE?**

Recognition of leucine as a nutrient signal is challenged by the fact that leucine can be easily metabolized by most tissues, including skeletal muscle (Suryawan et al., 1998). Thus, at this point it is not
clear whether the anabolic effects of leucine are due to leucine itself or one of its metabolites. There are 2 primary steps of the BCAA catabolic pathways in tissues (Hutson et al., 2005; Figure 8). The first step, which is readily reversible, is the transamination of the BCAA, leucine, isoleucine, and valine, by branched-chain aminotransferase (BCAT). There are 2 mammalian BCAT: a mitochondrial (BCATm) and a cy-
tosolic (BCATc) isozyme (Hutson et al., 2005). The transamination products are α-ketoisocaproate (KIC), α-keto-β-methylvalerate, and α-ketoisovalerate, respectively. The second step, which is irreversible, is oxidative decarboxylation catalyzed by the branched-chain keto acid dehydrogenase complex. The products of this process can enter the tricarboxylic acid cycle to produce energy or other metabolic components.

The notion of whether or not the molecular structure of leucine is crucial for its ability to stimulate protein synthesis is unclear. Several studies in cell culture (Yagasaki et al., 2003) and rodent models (Lynch et al., 2002a) have examined the effects of a metabolite of leucine, KIC, and norleucine, an isomer of leucine, on protein synthesis, with conflicting results. In our recent study, we evaluated the effects of leucine, KIC, and norleucine infusion for 60 min on muscle protein synthesis and the activation of translation initiation factors in overnight fasted neonatal pigs (Escobar et al., 2010). After infusion, as expected, the concentration of each infused individual compound was increased significantly when compared with the control treatment, and the infusion of KIC and leucine increased the concentrations of each other. When we examined the activation of translation initiation, we found that both leucine and KIC, but not norleucine, enhanced the phosphorylation of 4EBP1 and the formation of the eIF4E·eIF4G complex (Figures 9A and C), and reduced the formation of the inactive eIF4E·4EBP1 complex (Figure 9B). As a consequence, only leucine and KIC increased muscle protein synthesis in neonatal pigs (Figure 9D).

Our findings regarding the effect of KIC are consistent with other studies performed in cell culture (Yagasaki et al., 2003) and in rodents (Lynch et al., 2002b). Unfortunately, the true value of this observation is still highly debatable. This is because KIC easily can be converted into leucine by BCAT in most tissues (see Figure 8). Thus, the use of BCATm (–/–) transgenic mice (She et al., 2007) would be appropriate to study the actual effect of KIC on muscle protein synthesis. Unlike the KIC data, our data on norleucine are not consistent with studies in rats (Lynch et al., 2002b). Lynch et al. (2002b) found that acutely, norleucine is as effective as leucine in stimulating protein synthesis and the phosphorylation of S6K1 and 4EBP1 in skeletal muscle of mature rats. Furthermore, these investigators showed that chronic norleucine and leucine supplementation (12 d) produced similar results (Lynch et al., 2002a). The reason for the discrepancy between our study and theirs is unknown; thus, further study of the effect of norleucine in domestic animals is warranted.

**PROLONGED EFFECTS OF LEUCINE ON THE REGULATION OF TRANSLATION INITIATION**

Although there is ample evidence that leucine acutely stimulates muscle protein synthesis in neona-
chronic increases in leucine had no effect on the phosphorylation of PKB, indicating that leucine infusion did not activate the insulin signaling pathway (Wilson et al., 2010a). The phosphorylation of AMPK was not increased, indicating that there was no significant change in energy status in the cell. Furthermore, TSC2 phosphorylation was unchanged. This is important because studies in cell culture had provided controversial evidence that TSC2 has a role in AA signaling (Smith et al., 2005), although our recent in vivo studies demonstrated that insulin, but not AA, increase TSC2 phosphorylation and, thus, decrease the activation of the mTORC1 inhibitor (Suryawan et al., 2007). The interactions among the components of mTORC1 and mTORC2 are necessary for their activation (Toschi et al., 2009). Leucine, with or without an AA clamp, did not affect the interaction between mTOR and raptor, in mTORC1 (Wilson et al., 2010a), and had no effect on the interaction of mTOR with G3L, in both mTORC1 and mTORC2, and the interaction of mTOR with rictor, in mTORC2 in muscle. As predicted, prolonged leucine infusion, with or without an AA clamp, induced the phosphorylation of mTOR, but had no effect of the phosphorylation of PRAS40 and raptor. In contrast, we found that prolonged leucine infusion, with or without an AA clamp, enhanced the phosphorylation of S6K1 and 4EBP1, increased the formation of the active eIF4E·eIF4G complex (Figure 10A), and reduced the formation of the inhibitory eIF4E·4EBP1 complex. The elongation process, which is partly regulated by eEF2, is essential for mRNA translation (Proud, 2004). Previous studies examining the effect of feeding on the regulation of translation in neonatal muscle were unable to detect any change in eEF2 phosphorylation, suggesting that the elongation process is not a limiting step in this condition (Proud, 2004). Thus, it is not surprising that in this study, leucine did not alter the phosphorylation of eEF2 (Figure 10B; Wilson et al., 2010a). We also determined the phosphorylation of eIF2α, an inhibitor of eIF2B (see Figure 1). The results show that long-term treatment with leucine, with or without the AA clamp, decreased the phosphorylation of eIF2α (Figure 10C), which would release the inhibitory effect of this factor, and thus promote the binding of methionyl-tRNA to the 40S ribosomal complex and allow mRNA translation to proceed. Most importantly, we showed that long-term leucine infusion increased muscle protein synthesis, but only in the presence of an AA clamp when euaminoacidemia was maintained (Figure 10D). These results stress the importance of AA availability for supporting the leucine-induced stimulation of protein synthesis.

The aforementioned data presented were obtained from the LM, which is composed of primarily fast-twitch glycolytic fibers. Because of the potential for use of leucine clinically and in animal production, it was also important to examine the effect in muscles of different fiber types (Wilson et al., 2010b). Therefore, we examined the gastrocnemius muscle, which has a more

![Branched-chain acetyl CoA](image)

**Figure 8.** A schematic of the branched-chain AA (BCAA) metabolic pathway. The first step of BCAA metabolism is the reversible transamination of Leu, Ile, or Val, which is catalyzed by the branched-chain aminotransferase (BCAT) isozymes (BCATm and BCATc). In the second step, the products, α-ketoisocaproate (KIC), α-keto-β-methylvalerate (KMV), or α-ketoisovalerate (KIV), undergo oxidative decarboxylation, which is catalyzed by the branched-chain keto acid dehydrogenase (BCKD) enzyme complex. This irreversible process produces branched-chain acetyl CoA, which can enter the tricarboxylic acid (TCA) cycle.
Figure 9. Effect of saline (Sal), Leu, α-ketoisocaproic acid (KIC), and norleucine (Nleu) infusion for 1 h on eukaryotic initiation factor (eIF) 4E-eIF4E-binding protein-1 (4EBP1) phosphorylation (panel A), the abundance of eIF4E-4EBP1 complex (panel B), the abundance of the eIF4E-eIF4G complex (panel C), and fractional protein synthesis rates (panel D) in skeletal muscle of neonatal pigs. Results are presented as means ± SEM. *Differs from saline group ($P < 0.05$). AU = arbitrary units. Data are from Escobar et al. (2010).

Figure 10. Effect of 24-h Leu infusion, with or without AA clamp, on the eukaryotic initiation factor (eIF) 4E-eIF4G (eIF4E-eIF4G) complex abundance (panel A), the phosphorylation of eukaryotic elongation factor 2 (eEF2; panel B) and eIF2α (panel C), and fractional protein synthesis rates (panel D) in skeletal muscle of neonatal pigs. Results are presented as means ± SEM. *Differs from saline (Sal) group ($P < 0.05$). AU = arbitrary units. Data are from Wilson et al. (2010a).
mixed fiber type, and the masseter muscle, which is composed of primarily oxidative fibers. We also examined the right and left ventricles of the heart because the left heart undergoes hypertrophy during the first few days after birth (Escobar et al., 2006). We found that leucine increased protein synthesis in the gastrocnemius and masseter muscles but this only occurred in the presence of an AA clamp where hypoaaminoacidemia was prevented (Wilson et al., 2010b). However, there was no significant effect of leucine on protein synthesis in the heart. Consistent with protein synthesis data, we found that leucine, with or without an AA clamp, stimulated the phosphorylation of S6K1 and 4EBP1 in the gastrocnemius and masseter muscles but not in the heart.

In this study, we also determined protein synthesis rates and the phosphorylation of S6K1 and 4EBP1 in visceral tissues (Wilson et al., 2010b). We found that leucine alone, and in the presence of an AA clamp, increased the phosphorylation of S6K1 and 4EBP1 in liver and pancreas, but not in the kidney and jejunum. However, maintenance of euaminoacidemia, using an AA clamp, was required for the stimulation of protein synthesis in the liver and pancreas by leucine. Taken together, these data indicate that prolonged parenteral infusion of leucine enhances the activation of the mTOR signaling pathway in skeletal muscle, liver, and pancreas of neonatal pigs. However, the leucine-induced activation of translation initiation factors is not affected by changes in the circulating concentrations of other AA. Most important, prolonged parenteral infusion of leucine stimulates protein synthesis in most peripheral and visceral tissues but this effect is dependent on the availability of all other AA.

SUMMARY AND CONCLUSIONS

There is a growing body of evidence indicating that leucine acts as an anabolic agent that stimulates protein synthesis in various tissues in the body. But until now, the beneficial effect of physiological leucine administration on protein synthesis in newborn pigs had not been elucidated. Our data from several studies show that acute (i.e., 1 h) administration of leucine promotes muscle protein synthesis by activating translation initiation factors downstream of mTORC1. Our finding that KIC, but not norleucine, can replace leucine action is interesting, but more studies are necessary to evaluate the chemical structures required for the leucine-induced stimulation of protein synthesis. Using rapamycin, we also demonstrated that mTORC1 plays a crucial role in mediating the action of leucine on muscle protein synthesis in vivo. Although the activation of translation initiation factors was preserved after a 2-h leucine infusion, muscle protein synthesis stimulation was not, unless the leucine-induced reduction in EAA concentrations was rescued by an AA clamp. This novel and important finding also applied to chronic (24 h) leucine infusion studies. Moreover, our observations that chronic leucine administration promotes protein synthesis in skeletal muscles of different fiber types and several visceral tissues are encouraging. However, the ultimate challenge relevant to animal production would be the use of leucine supplementation in newborn feeding practices to promote growth, the challenge that we are currently pursuing.

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Leucine stimulates protein synthesis

2015


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