Stimulation of In Vitro Rat Muscle Protein Synthesis by Leucine Decreases with Age

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ABSTRACT Aging is characterized by a decrease of muscle mass associated with a decrease in postprandial anabolism. This study was performed to gain a better understanding of the intracellular mechanisms involved in the stimulation of muscle protein synthesis by amino acids and their role in the decrease of muscle sensitivity to food intake during aging. The effects of amino acids or leucine alone were assessed in vitro on epitrochlearis muscle from young, adult and old rats. Protein synthesis was assessed by incorporation of radiolabeled phenylalanine into protein and p70 S6 kinase activity by incorporation of $^{32}$P into a synthetic substrate. Amino acids, at physiologic concentrations, stimulated muscle protein synthesis ($P < 0.05$) and leucine reproduced this effect. The intracellular targets of amino acids were phosphatidylinositol 3 kinase and the rapamycin-sensitive pathways mammalian target of rapamycin (mTOR)/p70 S6 kinase. In old rats, the sensitivity of muscle protein synthesis to leucine was lower than in adults ($P < 0.05$) and this paralleled the lesser ability of leucine to stimulate the rapamycin-sensitive pathways ($P < 0.05$). We demonstrated that amino acids and leucine stimulate muscle protein synthesis and that aging is associated with a decrease in this effect. However, because aged rats are still able to respond normally to high leucine concentrations, we hypothesize that a nutritional manipulation increasing the availability of this amino acid to muscle could be beneficial in maintaining the postprandial stimulation of protein synthesis. J. Nutr. 130: 2630–2635, 2000.

KEY WORDS: • rats • protein synthesis • leucine • age • muscle

During aging, a progressive loss of muscle mass occurs in both humans (Forbes 1976) and rodents (Hollosy et al. 1991, Klitgaard et al. 1989). This loss of protein results from an imbalance between protein synthesis and degradation rates. The imbalance is not clearly apparent when basal rates of protein turnover are measured (Dardevet et al. 1994, Goldspink et al. 1987, Mosoni et al. 1993). A reduction in the stimulation of muscle protein synthesis was detected in rats during the postprandial period (Mosoni et al. 1995), suggesting that the “meal signal” was altered with age. The origin of this alteration remains obscure because muscle protein synthesis responds normally if amino acids are perfused continuously into old rats (Mosoni et al. 1993). Similarly, Volpi et al. (1999) showed that muscle protein synthesis was still able to respond positively to an increase of amino acid availability in elderly humans after oral amino acid administration. Moreover, a recent study of Arnal et al. (1999) demonstrated that the response of protein turnover was restored in elderly humans if a “protein-pulse feeding” pattern (80% of dairy proteins in one meal) was used instead of “spread-protein feeding” (dairy proteins equally distributed). Taken together, these results suggest that aged muscle is less sensitive to the stimulatory effect of amino acids at physiologic concentrations but is still able to respond if the increase in amino acidemia is sufficiently large.

Amino acids play an important role in regulating muscle protein synthesis [see May and Buse (1989) for a review]. Many mechanisms have been proposed to explain this effect, including regulation of cell volume (Lobley et al. 1998) and regulation of gene expression (Bruhat et al. 1997, Jousse et al. 1998). Recently, several studies conducted in vitro showed that in addition to being the precursors of protein synthesis, amino acids also mimic the effect of insulin and modulate translation initiation. Incubation of cells [Fao and H4IIE hepatocytes (Patti et al. 1998), CHO (Wang et al. 1998) and adipocytes (Fox et al. 1998)] without amino acids caused a very rapid decrease in p70 S6 kinase (p70$^{S6K}$) activity, dephosphorylation of eukariotic initiation factor 4E (eIF-4E) binding protein (eIF-4E-BP1) and an increased binding of initiation factor eIF-4E to the inhibitory regulator protein 4E-BP1. These effects were rapidly reversed by supplying a mixture of amino acids, but blocked by inhibitors of phosphatidylinositol 3’ kinase (PI3 kinase) and the mammalian target of rapamycin (mTOR). This suggested a role for these two kinases in the...
signaling pathway linking amino acids with the control of p70S6K and these translation factors. However, no measurement of protein synthesis was performed simultaneously in the presence of rapamycin, which blunts the regulation of the eIF-4E/4E-BP1; thus, there is no evidence that these initiation factors represent the limiting step of the stimulation of protein synthesis by amino acids. In skeletal muscle, few data are available concerning the role of amino acids in modulating intracellular factors and kinases. Only the works of Svanberg et al. (1997) and Yoshizawa et al. (1998) have shown that amino acids are also potent regulators of the 4E-BP1/eIF-4E complexes in vivo.

In this study, we hypothesized that the defect in the postprandial stimulation of muscle protein synthesis recorded in old rats might be the consequence of an alteration in the signaling cascade responsible for the amino acid–induced stimulation of protein synthesis in skeletal muscle. To assess and further understand the physiologic role of amino acids and the signaling pathways activated by their regulation in the regulation of protein synthesis, we performed this study on rat epitrochlearis muscle in vitro to demonstrate the direct effects of amino acids and also to eliminate the interactions with other in vivo potent regulators of protein synthesis such as insulin. To address this question, we measured the effect of inhibitors of selected intracellular kinases (PD98059, LY294002, rapamycin) on amino acid–stimulated protein synthesis in young rat muscle and the effect of amino acids or a single amino acid on p70 S6 kinase activity in young, adult and old rats.

MATERIALS AND METHODS

Animals. The experiments were conducted in accordance with NIH guidelines (NRC 1985). Young (4–5 wk old; 120–150 g), adult (6–8 mo old; 550–650 g) and old (20 mo old; 600–650 g) male Wistar rats were housed under controlled environmental conditions (temperature, 22°C; 12-h dark period starting at 1800 h). Rats had free access to a commercial laboratory diet (22% protein, 4.3% lipid, 53% carbohydrate; UAR, Villemoisson-sur-orge, France) and water. They were deprived of food overnight before the incubation experiment. Rats were anesthetized with sodium pentobarbital (6.0 mg/100 g body) and epitrochlearis muscles were dissected intact for incubation. Rats were deprived of food overnight before the incubation experiment. They were deprived of food overnight before the incubation experiment. Rats had free access to a commercial laboratory diet (22% protein, 4.3% lipid, 53% carbohydrate; UAR, Villemoisson-sur-orge, France) and water. They were deprived of food overnight before the incubation experiment.

Effect of amino acids on muscle protein synthesis in the presence of PD98059, LY294002 and rapamycin. Muscles were preincubated for 30 min in Krebs-Henseleit buffer (KHB) (NaCl, 120 mmol/L; KCl, 4.8 mmol/L; NaHCO3, 25 mmol/L; CaCl2, 2.5 mmol/L; KH2PO4, 1.2 mmol/L; and MgSO4, 1.2 mmol/L; pH 7.4) supplemented with 5 mmol/L HEPES, 5 mmol/L glucose, 1 g/L BSA and 5,5-dimethyl-1-pyrroline N-oxide (DMPO, 100 mmol/L: inhibitor of the mitogen-activated protein kinase (MAP) kinases pathways). Muscles were then transferred into fresh medium of the same composition for 2 h in the presence or absence of an amino acid mixture that was representative of the arterial postabsorptive (PA) or postprandial (PP) status found in control rats (Table 1). Muscles were then incubated for an additional 75 min in fresh medium containing 0.5 mmol/L L-[1-14C]phenylalanine (8.5 MBq/L). In an additional experiment, the effects of leucine, arginine and histidine alone were compared with that of the total amino acid mixture (PP) or in terms of the dose-response curves at the concentrations indicated. Viability of such muscle preparations has been assessed previously. ATP, phosphocreatine and lactate levels were similar in muscles of young, adult and old rats and remained constant throughout the incubation period (Dardevet et al. 1994). At the end of the incubation, muscles were blotted and homogenized in 0.61 mol/L trichloroacetic acid (TCA). Samples were centrifuged at 10,000 X g for 10 min at 4°C and TCA-insoluble material was washed 3 times with 0.61 mol/L TCA. The resultant pellet was solubilized in 1 mol/L NaOH at 37°C for determination of protein and radioactivity incorporated into muscle proteins. Tissue protein mass was determined using the bicinchoninic acid procedure (Pierce Chemical, Rockford, IL) and protein-bound radioactivity was measured using scintillation counting. Protein synthesis was calculated by dividing the protein-bound radioactivity by the specific activity of free phenylalanine in the incubation medium and expressed as nanomoles of phenylalanine incorporated per milligram protein per 75 min.

The effect of inhibitors was assessed by preincubating epitrochlearis muscle in KHB supplemented with 5 mmol/L HEPES, 5 mmol/L glucose, 1 g/L BSA in the presence of LY294002 (100 μmol/L: inhibitor of PI3 kinase), rapamycin (200 nmol/L: inhibitor of mTOR) or PD98059 (20 μmol/L: inhibitor of the mitogen-activated protein kinase (MAP) kinases pathways). Muscles were then transferred into fresh medium of the same composition with or without supplementation of the amino acid mixture (PP). Incubation and determination of protein synthesis were performed as described previously.

Effect of amino acids and leucine on p70S6K activation. Epitrochlearis muscles were preincubated for 45 min in KHB supplemented with 5 mmol/L HEPES, 5 mmol/L glucose, 1 g/L BSA and transferred into fresh medium of the same composition in the presence or absence of amino acid mixture (PP) or leucine (200 μmol/L) for 60 min. Pilot experiments established that maximal phosphorylation of p70S6K was observed 40 min after the addition of either PP or leucine (data not shown). Muscles were homogenized in ice-cold extraction buffer [50 mmol/L Tris-acetate, 50 mmol/L NaCl, 2.5 mmol/L EDTA, 1 mmol/L EGTA, 5 mmol/L sodium pyrophosphate, 5 mmol/L β-glycerophosphate, 1 mmol/L Na3VO4, 2 mmol/L dithiothreitol (DTT), 1 mmol/L benzamidine, 4 μg/mL leupeptin and 0.5% Triton X-100; pH 7.2] and centrifuged at 10,000 X g for 10 min at 4°C. The activity of p70S6K was assessed by an immune complex kinase assay. Normalized amounts of muscle proteins (100 and 200 μg for young and adult-old rats, respectively) were incubated for 4 h at 4°C with 3 μL of p70S6K antibodies preabsorbed to protein A-agarose beads. The immune complexes were washed three times with 56 kinase assay buffer [25 mmol/L 2-(N-morpholino)propanesulfonic acid, 15 mmol/L MgCl2, 1 mmol/L DTT, 0.1% BSA; pH 7.2]. The beads were then resuspended in 50 μL of S6 kinase assay buffer containing 0.1 mmol/L of the S6 peptide RRRLSSLR, 2 μmol/L phosphate inhibitor of cAMP-dependent protein kinase and 100 μmol/L ATP (specific activity 3000 dpm/pmol). After a 60-min incubation at 30°C, the phosphorylation reaction was stopped with 10 μL of 50

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1 PA, postabsorptive concentration; PP, postprandial concentration.
mmol/L unlabeled ATP. The reaction mixture was spotted onto Whatman P-81 phosphocellulose filter paper squares (Whatman, Kenilworth, UK); after three washes in 75 mmol/L phosphoric acid, the squares were counted in a β-scintillation counter. Pilot experiments showed that $^{32}$P incorporation into S6 substrate was linear for at least 60 min and proportional to the amount of protein used (100–200 μg).

**Statistical analysis.** Data are expressed as means ± SD. Statistical evaluation of the data was performed using Student’s t test or by ANOVA when appropriate. When differences were detected, a Student-Newman-Keuls post-hoc test was performed to determine pair-wise differences. Differences among means were considered significant when $P < 0.05$.

**RESULTS**

**Effect of amino acids on muscle protein synthesis.** Addition of amino acids to the incubation medium significantly increased the rate of muscle protein synthesis in epitrochlearis muscle from young rats ($0.224 ± 0.009, 0.267 ± 0.012, 0.302 ± 0.007$ nmol phenylalanine/(mg protein · 75 min) for basal, PA and PP, respectively; $P < 0.05$, PA and PP vs. basal; $P < 0.05$, PP vs. PA). The maximal stimulation of protein synthesis was systematically recorded with the PP mixture, whereas the PA mixture gave variable stimulations between basal and PP values. Among the amino acids, branched-chain amino acids, and leucine in particular, were the most efficient at increasing the rate of basal muscle protein synthesis in epitrochlearis muscle from young rats ($0.224$ vs. $0.091$ nmol phenylalanine/(mg protein · 75 min), respectively). In muscles of adult rats, the addition of leucine to the incubation medium significantly increased protein synthesis (Fig. 1). The other amino acids tested (arginine and histidine) did not significantly stimulate muscle protein synthesis when added at the postprandial concentration. Increasing leucine concentration progressively increased muscle protein synthesis, and the maximal effect was recorded at 200 μmol/L, which is similar to the arterial postprandial leucinemia (May and Buse 1989). In our muscle preparations, leucine alone, at the same concentration as that present in PP, was as potent as all amino acids combined in stimulating muscle protein synthesis (Fig. 1). The other amino acids tested (arginine and histidine) did not significantly stimulate muscle protein synthesis when added at the postprandial concentration. Increasing leucine concentration progressively increased muscle protein synthesis, and the maximal effect was recorded at 200 μmol/L, which is similar to the arterial postprandial leucinemia (Fig. 2). The half-maximum effect (IC$_{50}$) was recorded with a leucine concentration close to the normal postabsorptive concentration.

In muscles of adult rats, the addition of leucine to the incubation medium significantly increased protein synthesis (Fig. 3). As observed in young rats, maximal stimulation was recorded at a leucine concentration close to 200 μmol/L and the IC$_{50}$ occurred at 110–120 μmol/L leucine. However, when the effect of leucine was considered in term of incremental increase, its effect was lower in adult than in young rats ($0.025$ vs. $0.091$ nmol phenylalanine/(mg protein · 75 min), respectively). In muscles of old rats, leucine also stimulated protein synthesis to the same extent as in adult rats, but the maximal response was recorded at a leucine concentration of ~400 μmol/L, with an IC$_{50}$ at 200–250 μmol/L leucine (Fig. 3).

**Effects of PD98059, LY294002 and rapamycin on maximal amino acid–stimulated protein synthesis in young rat muscle.** The addition of PD98059 (an inhibitor of MAP-kinase pathways) into the incubation medium did not modify the rate of basal muscle protein synthesis ($0.281 ± 0.013$ vs. $0.297 ± 0.015$ nmol phenylalanine/(mg protein · 75 min) without or with the inhibitor, respectively, not shown) nor the stimulatory effect of amino acids ($0.373 ± 0.025$ vs. $0.387 ± 0.017$ nmol phenylalanine/(mg protein · 75 min) without or with the inhibitor, respectively) (Fig. 4). Therefore the MAP-kinase pathways are not involved in the acute regulation of muscle protein synthesis by amino acids.

The presence of LY294002 (inhibitor of the PI3 kinase) in the

![Figure 1](image1.png)  **FIGURE 1** Effect of total amino acids (PP) or a single amino acid (leucine 200 μmol/L = Leu; arginine 300 μmol/L = Arg; histidine 100 μmol/L = His) on epitrochlearis muscle protein synthesis measured in vitro in young rats. Protein synthesis was expressed as a percentage of the basal values (means ± SD, n = 6–8). Means not sharing a superscript are different; $P < 0.05$.

![Figure 2](image2.png)  **FIGURE 2** Effect of increasing leucine concentrations on epitrochlearis muscle protein synthesis measured in vitro in young rats. Incubation was performed with leucine concentrations indicated. The half-maximum effect (IC$_{50}$) was estimated graphically. Values are means ± SD, n = 9–10.

![Figure 3](image3.png)  **FIGURE 3** Effect of increasing leucine concentrations on epitrochlearis muscle protein synthesis measured in vitro in adult and old rats. Incubation was performed with leucine concentrations indicated. The half-maximum effect (IC$_{50}$) was estimated graphically. Values are means ± SD, n = 10–12.
incubation medium resulted in a significant decrease in the basal rate of protein synthesis \(0.260 \pm 0.010\ vs. 0.161 \pm 0.006\ \text{nmol phenylalanine/(mg protein \cdot 75 min)}\) without or with the inhibitor, respectively. Amino acid–induced stimulation was completely inhibited in the presence of the inhibitor \(0.171 \pm 0.008\ \text{nmol phenylalanine/(mg protein \cdot 75 min)}\), demonstrating that the activation of this kinase was necessary for amino acid–induced stimulation of muscle protein synthesis.

The addition of rapamycin to the incubation medium did not affect basal protein synthesis \(0.279 \pm 0.012\ vs. 0.280 \pm 0.010\ \text{nmol phenylalanine/(mg protein \cdot 75 min)}\) without or with the inhibitor, respectively but completely abolished the stimulatory effect of amino acids on muscle protein synthesis (Fig. 4). Similarly, rapamycin also completely inhibited stimulation of muscle protein synthesis when leucine alone was added to the incubation medium (data not shown).

**Effects of amino acids and leucine on p70\text{S6K} activity.**

The addition of amino acids to the incubation medium stimulated p70\text{S6K} activity. When leucine alone was added, p70\text{S6K} was stimulated but to a lesser extent than with the total amino acid mixture (Fig. 5). Thus, p70\text{S6K}, which is part of the rapamycin-dependent pathways, might be responsible in part for the effects of amino acids on muscle protein synthesis. However, because leucine alone was less effective than total amino acids, this suggests that other amino acids have minor effects on this kinase activity. In adults, leucine also stimulated the activity of p70\text{S6K} with a maximal effect at 200 \(\mu\text{mol/L}\) (Fig. 6). The basal activity of p70\text{S6K} was not different in muscles of old and adult rats, but the maximal stimulation in muscle of old rats was at a higher leucine concentration (400 \(\mu\text{mol/L}\)). For protein synthesis, p70\text{S6K} activity in muscle of old rats was less sensitive to leucine compared with muscle of adults (Fig. 6).

**DISCUSSION**

In our study on epitrochlearis muscle, amino acids increased protein synthesis in vitro and this stimulation occurred at physiologic concentrations. In addition, maximal stimulation was obtained with the normal postprandial amino acid concentrations. Among amino acids, leucine alone reproduced the effect of total amino acids on muscle protein synthesis, and this effect also occurred at physiologic concentrations \(IC_{50} = 110\ \mu\text{mol/L}\). However, we cannot exclude the possibility that the two other branched-chain amino acids, valine and isoleucine, may also have had an effect, although Lynch et al. (2000) showed that leucine was the more potent amino acid in stimulating 4E-BP1 phosphorylation in adipocytes.

It seems likely that leucine is involved in the regulation of protein synthesis in vivo. A recent study of Anthony et al. (2000) showed that orally administered leucine stimulated muscle protein synthesis independently of insulin in vivo. We found that a modest increase in plasma leucine above postabsorptive levels had no significant effect, whereas a twofold increase stimulated whole-body protein synthesis in healthy humans (Bergström et al. 1990, Elia et al. 1989, Giordano et al. 1996). A similar conclusion could be drawn from experiments using intravenous amino acid infusion in rats. Only a sustained large hyperaminoacidemia

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**FIGURE 4** Effect of PD98059 and rapamycin on epitrochlearis muscle protein synthesis stimulated by total amino acids in young rats. Muscles were incubated in Krebs-Henseleit buffer in the presence (+) or absence (−) of amino acids (PP concentration), PD98059 or rapamycin at a concentration of 20 \(\mu\text{mol/L}\) and 200 \(\mu\text{mol/L}\), respectively. Values are means ± so, \(n = 7–12\). Means not sharing a superscript are different, \(P < 0.05\).

**FIGURE 5** Effect of total amino acids (PP) and leucine (leucine 200 \(\mu\text{mol/L} = \text{Leu}\)) on p70 S6 kinase activity in muscles of young rats. Values are means ± so, \(n = 9–11\). Means not sharing a superscript are different, \(P < 0.05\).

**FIGURE 6** Effect of increasing leucine concentrations on muscle p70 S6 kinase activity in vitro in adults and old rats. Incubations were performed with the leucine concentrations indicated. The half-maximum effect \(IC_{50}\) was estimated graphically. Values are means ± so for \(n = 4–8\). *\(P < 0.05\) vs. adult value at the same leucine concentration.
(leucine increased two- to threefold) stimulated muscle protein synthesis (Garlick and Grant 1988, McNulty et al. 1993, Mosoni et al. 1993, Sinaud et al. 1999). Moreover, the postprandial stimulation of translation of muscle protein synthesis in rats originated mainly from absorbed amino acids because this stimulation was observed after feeding a high protein meal but not after an isonenergetic protein-free meal (Toshizawa et al. 1998). It is important to emphasize that amino acids or feeding affects plasma insulin, which also participates in the stimulation of muscle protein synthesis, especially in young growing animals (Garlick and Grant 1988). Dose-response curves of insulin action showed a very high sensitivity in vivo. Indeed, the effect of exogenous insulin was visible only in the postabsorptive state and not during the postprandial state when insulin levels are already elevated (Garlick and Grant 1988). The acute decrease of postprandial insulinemia below the postabsorptive levels due to diazoxide treatment greatly impaired muscle protein synthesis (Sinaud et al. 1999). The present experiment was therefore performed in vitro in the absence of insulin to analyze specifically the effect of total amino acids and leucine.

Because leucine alone reproduced the effect of total amino acids on muscle protein synthesis in our experiment, it might be hypothesized that this effect was not dependent on the amino acid concentration itself but on a specific signal initiated by leucine. However, to our knowledge, the effect of inhibitors of specific kinases has not been investigated on amino acid–stimulated protein synthesis itself in isolated muscles. We demonstrated, for the first time, that inhibition of PI3 kinase by LY294002 completely abolished amino acid–stimulated protein synthesis in isolated muscles. We presented results in rat skeletal muscle. Moreover, the MAP kinase pathway was not involved in this stimulation because PD98059, an inhibitor of the upstream activator of MAP kinases, MEK, had no effect. We found that amino acids stimulated muscle protein synthesis through intracellular kinases, which are PI3 kinase dependent. The downstream events linked to PI3 kinase remain poorly defined; in several cell lines, however, (Cheatham et al. 1994, Cross et al. 1994, Welsh et al. 1994) as well as in rat skeletal muscle (Dardevet et al. 1996), PI3 kinase activation was required for stimulation of p70S6K. This kinase has been shown to be partially involved in the regulation of protein synthesis in rat epimysial muscle and inhibited by rapamycin (Dardevet et al. 1996). Although rapamycin caused a deactivation of p70S6K, the direct target of this inhibitor is in fact the protein known as mTOR (mammalian target of rapamycin). The role of mTOR as the rapamycin target responsible for inhibition of p70S6K was demonstrated by Brown et al. (1995) who showed that mutant TOR, which lack the ability to bind rapamycin, prevent the inhibition of p70S6K by the drug. Our results clearly showed that addition of rapamycin completely inhibited the stimulation of muscle protein synthesis by amino acids or leucine alone and that mTOR represents the downstream target of amino acid–stimulated PI3 kinase in skeletal muscle. We also demonstrated that amino acids or leucine alone at physiologic concentrations stimulated p70S6K activity. However, leucine alone seemed to be less effective than total amino acids in increasing p70S6K activity, suggesting that other amino acids might have minor stimulating effects on this kinase. Very recently, p70S6K activation by amino acids was investigated in culture cells and it was shown that in Fao hepatocytes (Patti et al. 1998), CHO cells (Hara et al. 1998, Wang et al. 1998) and L6 myoblasts (Kimball et al. 1999), amino acids are also potent stimulators of this kinase. As we found, leucine is the major amino acid responsible for this effect. Activation of p70S6K by leucine has been reported to be very specific; the structural requirement of this amino acid to induce p70S6K activation is very strict and precise (Shigemitsu et al. 1999). However, to our knowledge, the target of leucine that would initiate the activation of the PI3 kinase/mTOR pathways is not known.

It was shown previously that stimulation of muscle protein synthesis was lower in old rats than in adults (Mosoni et al. 1995). Therefore, we investigated whether a decrease in the sensitivity of muscle protein synthesis to amino acids with age may explain the defect in the postprandial anabolism. We found that muscle protein synthesis still responded to the leucine signal in old rats, but the IC50 was observed with amino acid levels two to three times greater than in young or adult rats. This indicated that at postprandial amino acid concentrations, muscle protein synthesis was maximally stimulated in adult rats but poorly stimulated in old rats. Indeed, we measured the plasma amino acid concentrations in old and adult rats during the postabsorptive and postprandial states and no difference of aminoacidemia was found (data not shown). Similarly, Volpi et al. (1999) showed that amino acid concentrations were not different in adult and elderly humans in both basal and fed states. However, it is important to note that muscle protein synthesis was stimulated to the same extent in old and adult rats when leucine levels reached supraphysiologic concentrations, demonstrating that muscle from old rats did not lose the ability to respond to the amino acid signal. Moreover, these results suggest that a difference in postprandial stimulation of muscle protein synthesis with age will be detectable only if the plasma leucine concentration is moderately increased (1.5–2 times) as observed after a standard oral feeding (Bergström et al. 1990). This observation might explain why amino acid perfusion, which induced a threefold increase in plasma leucine, stimulated muscle protein synthesis to the same extent in adult and old rats (Mosoni et al. 1993), whereas a defect in this stimulation was observed after “normal” feeding. A similar conclusion could be reached from the study of Volpi et al. (1999) who observed a similar effect of oral amino acid administration on muscle protein synthesis in adult and elderly humans. Indeed, arterial leucine concentrations were increased threefold in both age groups. Our hypothesis is also in agreement with the work of Arnal et al. (1999) who showed that protein-pulse feeding improved protein retention in elderly women, whereas protein-spread feeding did not. In these experiments, plasma amino acid concentrations were not determined; we assume, however, that amino acid availability (i.e., leucine) to peripheral tissues was higher with protein-pulse feeding than with protein-spread feeding, and reached the level that would significantly stimulate muscle protein synthesis in elderly humans.

The decreased sensitivity of muscle protein synthesis to leucine in aged rats suggested that the signaling pathway that carries the leucine signal to the protein translation machinery was less responsive to the amino acid. Our study demonstrated that p70S6K activity was stimulated by leucine in both adult and old rats, but as observed with protein synthesis, this activation occurred with higher, supraphysiologic levels of leucine (IC50 110 vs. 260 µmol/L, respectively). Thus, for full activation, postprandial leucine concentrations are sufficient in adult rats, whereas up to 400 µmol/L is required in old rats. Our results showed a similar alteration of the sensitivity of muscle protein synthesis and sensitivity of p70S6K activation to leucine. This confirms that the signaling pathway PI3 kinase/mTOR/p70S6K is involved in the stimulation of muscle protein synthesis by leucine.

In conclusion, we showed that amino acids directly stimulated muscle protein synthesis in vitro, independently of insulin. Furthermore, among the amino acids, leucine alone
reproduced this effect, suggesting that this branched-chain amino acid acted as a mediator of muscle protein synthesis stimulation. To date, no studies have been performed on skeletal muscle in vitro to identify the signaling pathways involved. We demonstrated that the amino acid signal occurred through the stimulation of PI3 kinase and its downstream substrate, mTOR. We also demonstrated that amino acids were potent stimulators of p70S6K activity.

Moreover, our results suggested that the defect of postprandial muscle protein anabolism during aging may result from a decrease of protein synthesis sensitivity to amino acids, particularly leucine. This defect was associated with the inability of leucine to stimulate p70S6K. Because old rats remain able to respond normally to higher leucine concentrations, it is possible that increasing plasma leucine may be beneficial in maintaining the postprandial stimulation of muscle protein metabolism. However, it has been demonstrated by Boirie et al. (1997) that the first-pass splanchnic uptake of leucine increases with age and may limit the availability of amino acids to peripheral tissues. Recently, Volpi et al. (1999) confirmed this observation but showed that despite this phenomenon, the delivery of amino acids to the leg increased to the same extent in both adult and elderly humans. Thus, an oral leucine supplementation may increase leucine availability to peripheral tissues in aged animals and may restore normal postprandial anabolism.

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LITERATURE CITED


Bergstrom, J., Furst, P. & Vinnars, E. (1990) Effect of a test meal, without and with supplementation may increase leucine availability to peripheral tissues. Recently, Volpi et al. (1999) confirmed this observation but showed that despite this phenomenon, the delivery of amino acids to the leg increased to the same extent in both adult and elderly humans. Thus, an oral leucine supplementation may increase leucine availability to peripheral tissues in aged animals and may restore normal postprandial anabolism.