

Leucine and Protein Synthesis: mTOR and Beyond

Martha H. Stipanuk, PhD

The effects of amino acid intake on protein synthesis in the intact rat appear to be mediated almost entirely by a single amino acid: leucine. The effect of leucine on protein synthesis appears to be closely associated with eIF4G phosphorylation and its association with eIF4E, but whether eIF4G phosphorylation actually mediates the effects of leucine or is merely associated with these events has not been elucidated. Additional research is needed to determine whether leucine effects eIF4G phosphorylation, whether eIF4G phosphorylation is essential for the effect of leucine on protein synthesis, and whether mTOR (mammalian target of rapamycin) or another component of the mTOR complex is somehow involved in leucine-specific signaling.

Key words: amino acids, insulin, leucine, mTOR, mammalian target of rapamycin, protein synthesis

© 2007 International Life Sciences Institute
doi: 10.1301/nr.2007.mar.122–129

INTRODUCTION

The synthesis of protein in tissues is rapidly stimulated after oral intake of nutrients. The importance of an adequate supply of amino acids for feeding-induced changes in muscle protein synthesis has been demonstrated by observations that, whereas ingestion of a mixed meal stimulates skeletal muscle protein synthesis in food-deprived animals, consumption of a protein-deficient meal does not elicit this response.^{1–4} Of all of the amino acids present in dietary protein, one particular amino acid, leucine, appears to mediate most of the effects of protein/amino acid intake on protein metabolism. The central role of leucine in mediating the anabolic effects of protein/amino acids on muscle protein metabolism was obvious by the mid-1970s,^{5–9} but the mechanisms by which leucine increases protein synthesis have begun to emerge only since the turn of the century.

The anabolic effect of leucine on protein synthesis is of great interest nutritionally both in terms of the reduction of loss of lean body mass in various disease states and in the maintenance/enhancement of lean body mass in healthy individuals.

A number of studies have been conducted to elucidate the basis of this anabolic effect of leucine. One major contender over the past few years has been the mTOR (mammalian target of rapamycin) pathway, which mediates the effects of insulin on protein synthesis. However, it now seems clear that leucine stimulates protein synthesis largely through insulin-independent mechanisms, although basal insulin levels are important for a maximal effect of leucine on protein synthesis, and the possible role of mTOR in leucine signaling is uncertain. The objective of this review is to summarize much of the work addressing the role of leucine in the regulation of protein synthesis.

THE STIMULATION OF PROTEIN SYNTHESIS BY AMINO ACIDS IS NOT CAUSED SIMPLY BY AN INCREASED CONCENTRATION OF SUBSTRATE

Of the nutrients provided by a complete meal, amino acids are important because they provide substrate for enhanced protein accretion. However, the increase in protein synthesis that follows intake of a meal containing protein or amino acids cannot be explained by the consequent increase in amino acid concentrations. Vary et al.¹⁰ identified four lines of evidence suggesting that amino acids do not augment protein synthesis merely by increasing substrate availability. First, tRNAs are essentially fully charged with their respective amino acids at the intracellular amino acid concentrations found in tissues of fasting animals. Thus, it seems unlikely that increasing substrate availability by provision of a meal would result in further charging of the tRNAs. Second, whereas one might predict that an increase in substrate (i.e., aminoacyl-tRNA) concentration would accelerate protein synthesis by enhancing peptide-chain elongation, experimental evidence clearly indicates that amino acids primarily exert their effects on protein synthesis by accelerating peptide chain initiation rather than elonga-

Dr. Stipanuk is with the Division of Nutritional Sciences, 227 Savage Hall, Cornell University, Ithaca, NY 14853; Phone: 607-255-2683; Fax: 607-255-1033; E-mail: mhs6@cornell.edu.

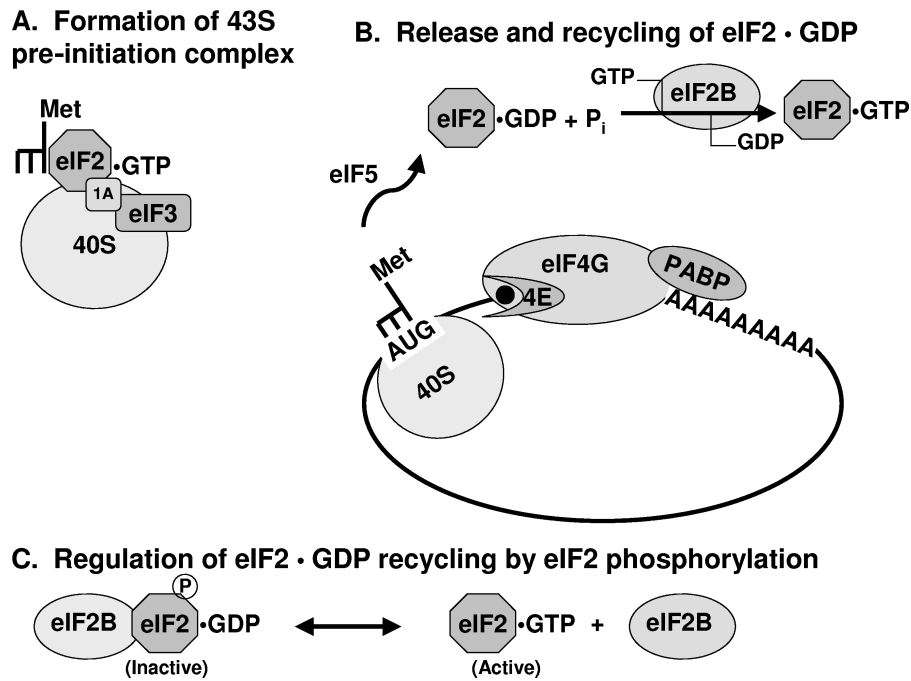


Figure 1. A, Step 1 of the pathway for initiation of eukaryotic mRNA translation: formation of the 43S preinitiation complex. B, Release and recycling of eIF2-GDP upon successful engagement of Met-tRNA_i with the start codon. C, Regulation of eIF2-GDP recycling by eIF2 phosphorylation.

tion. Third, removal of leucine can prevent the overall stimulation of protein synthesis by amino acids, yet removal of methionine is without effect. Fourth, nor-leucine, a structural analogue of leucine, is able to stimulate protein synthesis even though it cannot be incorporated into the growing polypeptide chain during protein synthesis.

MAMMALIAN PROTEIN SYNTHESIS IS REGULATED AT TWO STEPS OF THE TRANSLATION INITIATION PHASE

The regulation of protein synthesis in mammalian tissues has been demonstrated to occur primarily through modulation of two of the steps in this complex pathway. These two steps are both involved in the initiation phase of translation—the formation of a ribosome binding site/translation start site—rather than in the elongation phase.

Step 1: eIF2-Dependent Formation of the 43S Pre-Initiation Complex

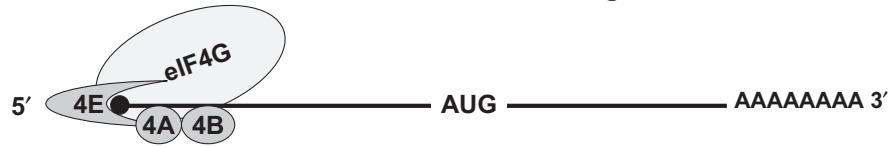
One of the regulated steps in translation initiation is the binding of methionyl-tRNA_i (Met-tRNA_i) to the 40 S ribosomal subunit to form the 43 S pre-initiation complex (Figure 1A). This step is mediated by eukaryotic initiation factor 2 (eIF2), which is a heterotrimeric GTP-

binding protein. In its GTP-bound state, eIF2 interacts with Met-tRNA_i to form an eIF2-GTP·Met-tRNA_i ternary complex, which in association with other factors, binds to the 40 S ribosomal subunit. The resulting 40S·Met-tRNA_i·eIF2-GTP complex then binds to mRNA near the cap structure by interaction of a central domain of eIF4G with eIF3 (see Figure 2B and subsequent text).

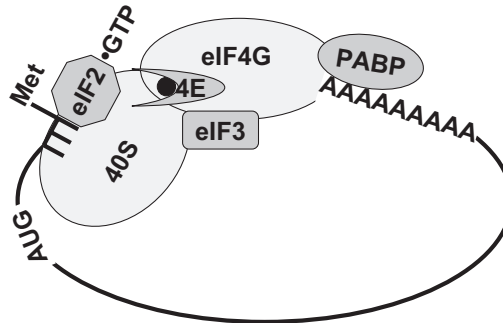
eIF2 Kinase Controls eIF2-GDP Recycling

Following successful engagement of Met-tRNA_i with the start codon, the GTP is hydrolyzed to GDP and Pi, a process that also involves eIF5 (a GTPase activator protein), and the eIF2-GDP complex leaves the ribosome (Figure 1B). For eIF2 to be used to form a new ternary complex, the eIF2-GDP must be recycled back to the active eIF2-GTP complex. Recycling of eIF2-GDP is the function of eIF2B, the guanine nucleotide exchange factor for eIF2. The overall process of Met-tRNA_i binding is regulated via phosphorylation of eIF2's α -subunit by any one of four mammalian eIF2 kinases, which are activated under specific stress conditions. Phosphorylation of eIF2 both enhances the association of eIF2 with eIF2B and potently inhibits the GDP/GTP exchange activity of eIF2B (Figure 1C). One of the eIF2 kinases, GCN2 (general control nonderepressible 2), is activated by a lack of one or more essential amino acids.

A. eIF4E-dependent recognition of the m⁷GTP mRNA cap structure and formation of a ribosome binding site



B. Binding of the 43S ribosomal (pre-initiation) complex



C. Regulation of eIF4E availability by phosphorylation of 4E-BP1

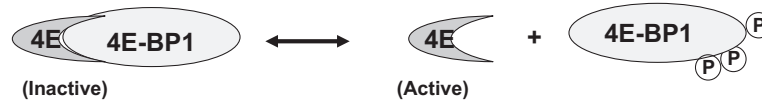


Figure 2. A, Step 2 of the pathway for initiation of eukaryotic mRNA translation: eIF4E-dependent recognition of the m⁷GTP mRNA cap structure and formation of a ribosome binding site. B, Binding of the 43S ribosomal (preinitiation) complex. C, Regulation of eIF4E availability for cap recognition by phosphorylation of eIF4E-BP.

Step 2: eIF4-Dependent Recognition of the m⁷GTP mRNA Cap Structure

The second of the two regulated steps in translation initiation involves recognition of the cap structure and formation of a ribosome binding site/translation start site selection (Figure 2A). This step is mediated by a heterotrimeric complex of eukaryotic factors referred to as eIF4F. The three subunits of eIF4F are eIF4A, eIF4E, and eIF4G. The triggering event for translation start site selection is the binding of the eIF4E subunit of eIF4F to the m⁷GTP cap structure present at the 5'-end of essentially all eukaryotic mRNAs. The eIF4A subunit, an ATP-dependent RNA helicase, unwinds a secondary structure in the 5'-untranslated region of the mRNA to facilitate small ribosomal subunit binding. As shown in Figure 2B, eIF4G, a large, multi-domain protein, mediates a series of protein-protein interactions that culminate in the recruitment of the 43S complex to the mRNA 5'-end. In addition to binding the eIF4E and eIF4A subunits of the eIF4F complex, eIF4G serves as a scaffold for recruitment of eIF3, which also binds the small (40S) ribosomal subunit (i.e., the 40S·Met-tRNAi·eIF2-GTP complex described above in step 1 of the translation initiation process). eIF4G also binds the poly(A)-binding protein (PABP), allowing circularization of the mRNA, which may allow for more efficient translation. eIF4G also has a binding site for the Mnk1 protein kinase,

which phosphorylates eIF4E, and assembly of the eIF4F complex may be stimulated through phosphorylation of eIF4E and eIF4G, although the mechanisms are poorly defined.

Phosphorylation-Dependent Formation of the eIF4 Complex

Changes in the availability of eIF4E to form the active eIF4E·eIF4G complex occur through modulation of the association of eIF4E with eukaryotic initiation factor 4E-binding protein (4E-BP1), which acts as a translational repressor. The binding of eIF4E to 4E-BP1 is regulated through phosphorylation of 4E-BP1 (Figure 2C). In its hypophosphorylated state, 4E-BP1 binds eIF4E tightly. Because 4E-BPs bind to the same site on eIF4E as does eIF4G, the association of eIF4E with 4E-BP1 blocks the ability of eIF4E to bind to eIF4G. Phosphorylation of 4E-BP on multiple residues relieves translational repression by favoring the dissociation of the eIF4E·4E-BP complex, freeing eIF4E to bind to eIF4G and, hence, to form the translationally active eIF4F complex.

Leucine Stimulates Assembly of the eIF4F Complex

Anthony et al.¹¹ investigated the mechanisms involved in the stimulatory effect of leucine on protein

synthesis in skeletal muscle of rats. Leucine had no effect on the Met-tRNAⁱ binding step in translation initiation, as assessed by the phosphorylation status of eIF2 α -subunit (Ser⁵¹) and by the guanine nucleotide exchange activity of eIF2B. In contrast, leucine had a stimulatory effect on assembly of the eIF4F complex, a key component in the mRNA binding step in translation initiation, as assessed by the phosphorylation status of the eIF4B binding protein 4E-BP1 and by the association of eIF4E with 4E-BP1 and eIF4G.

MANY OF THE EFFECTS OF LEUCINE ARE ASSOCIATED WITH THE ACTIVATION OF mTOR AND ARE SIMILAR TO THE EFFECTS OF INSULIN

The discovery in mammalian cells of mTOR, a homolog of the yeast protein serine/threonine kinase known as target of rapamycin (TOR), in the mid-1990s provided a very significant breakthrough that has furthered our understanding of the role of both leucine and insulin in the regulation of protein synthesis. Named for its sensitivity to inhibition by the antifungal/immunosuppressive agent rapamycin, mTOR is a large, 290-kD protein kinase whose catalytic domain resembles lipid kinases such as phosphatidylinositol (PI) 3-kinase (PI3K), although it functions as a serine/threonine kinase. mTOR signals through two physically distinct multiprotein complexes, but nutrient- and hormonal-mediated effects on cell growth are mediated primarily by TOR complex 1 (TORC1), which contains raptor and LST8 in addition to mTOR.

The discovery of mTOR was followed by the observation in CHO-RI cells that the response to rapamycin (an inhibitor of mTOR) resembled the response to amino acid withdrawal. Selective phosphorylation of the 70-kD S6 protein kinase (S6K1 or p70S6k) and of 4E-BP1 occurred in response to both amino acid withdrawal and rapamycin treatment, suggesting that amino acid sufficiency and mTOR may signal through a common effector, which could be mTOR itself or a protein downstream of mTOR.¹² This report was followed by the 1999 report of Kimball et al.¹³ that rapamycin prevented the leucine-induced stimulation of phosphorylation of both 4E-BP1 and S6K1 in L6 myoblasts. Animal studies have also indicated that the effects of intake of leucine on protein synthesis in liver, skeletal muscle, adipose tissue, and other tissues (as assessed by S6K1 and S6 phosphorylation, hyperphosphorylation of 4E-BP1, or an increase in ribosomal proteins being actively translated on polyosomes) may be linked to an mTOR-mediated, rapamycin-sensitive pathway.¹⁴⁻¹⁶ These effects of leucine were similar to the observed insulin-induced increases in ribosomal protein S6 phosphorylation and 4E-BP1 phosphorylation, which had already been shown to be medi-

ated by the mTOR signal transduction pathway.¹⁷ Because leucine is known to act as an insulin secretagogue, this raised questions about whether the effects of leucine on protein synthesis are mediated by increases in insulin.

Leucine Stimulates Protein Synthesis Largely Through Insulin-Independent Mechanisms

The effects of insulin on mTOR signaling are largely due to upstream control of mTOR through the TSC1 (tuberous sclerosis complex 1)-TSC2 protein complex, and this upstream regulation of mTOR signaling through the TSC1-TSC2 complex is largely mediated by activation of protein kinase B (PKB or Akt) through a phosphatidylinositol (PI) 3-kinase-dependent pathway (Figure 3). Signal transduction from TSC2 to mTOR is mediated by a G protein called Rheb (Ras homolog enriched in brain). In its active state, Rheb is bound to GTP and interacts with a variety of effector proteins including mTOR. However, TSC2 has GTPase-activating protein properties toward Rheb and can convert Rheb to its inactive Rheb-GDP form. The activity of TSC2 is impaired when TSC2 is phosphorylated by PKB in response to insulin. Thus, in the presence of insulin, TSC2 does not hydrolyze the GTP associated with Rheb and Rheb-GTP is able to activate mTOR. Several proteins involved in the translational machinery, including the 4E-BP1 and the S6 kinases that phosphorylate multiple sites in the C-terminus of the 40 S ribosomal protein S6, are controlled by mTOR. Additionally, unknown targets for mTOR signaling appear to be involved in modulating translation of a group of mRNAs known as 5'-TOP (terminal oligopyrimidine tract) mRNAs, many of which code for ribosomal proteins and translation factors.

Jefferson et al.^{11,14,15,18,19} conducted a series of studies aimed to elucidate the insulin-dependent versus insulin-independent mechanisms through which leucine affects protein synthesis. Anthony et al.^{11,14} administered, via oral gavage, saline, carbohydrate, leucine, isoleucine, valine, or a combination of carbohydrate plus leucine to rats that had been deprived of food for 18 h. The amount of leucine, isoleucine, or valine administered (approximately 1.35 mg/kg) was equivalent to the typical intake of leucine over 24 h by rats fed ad libitum, and the amount of carbohydrate (approximately 13 mg/kg provided as a mixture of glucose and sucrose) was equivalent to about 15% of the daily energy intake of rats fed ad libitum. In this model, protein synthesis in skeletal muscle (gastrocnemius/plantaris) was reduced in the food-deprived rats to 65% of the rate observed in control animals fed ad libitum. Administration of leucine to the food-deprived rats stimulated protein synthesis to

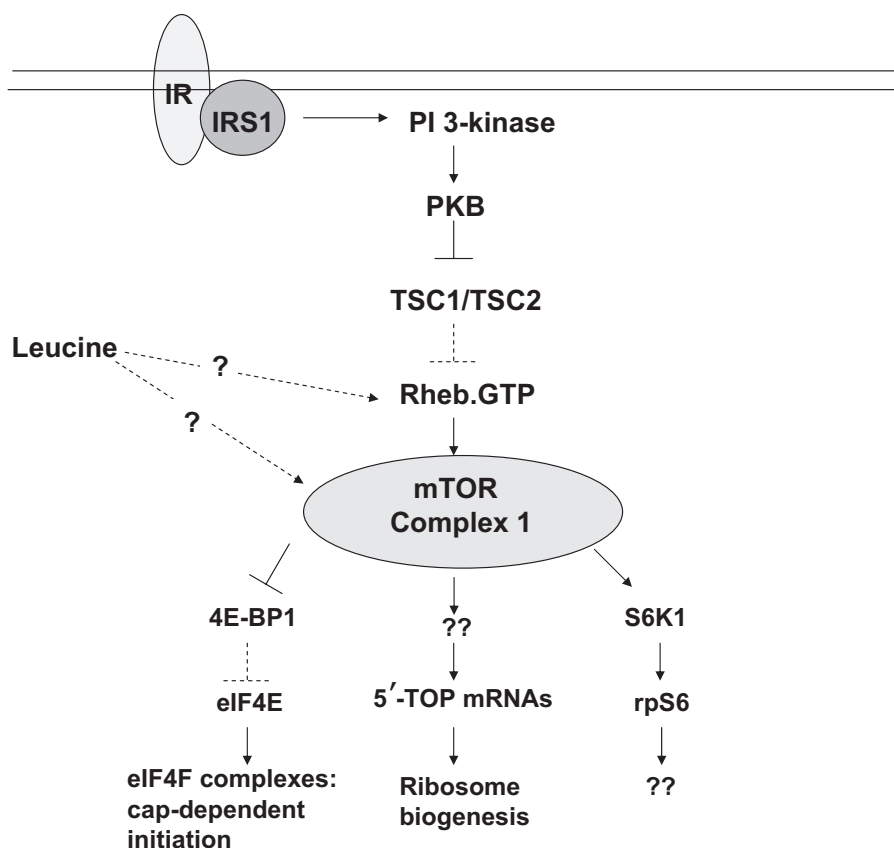


Figure 3. The pathway for activation of mTOR (mammalian target of rapamycin) by insulin, through PKB (protein kinase B) and the inactivation of TSC1-TSC2 (tuberous sclerosis complex 1 and 2), and downstream signaling events from mTOR that impinge upon the translational machinery. (Adapted from Proud, 2006.³²)

136%¹¹ or 165%¹⁴ of the untreated saline control value within 60 min, whereas administration of carbohydrate alone had no effect. Leucine plus carbohydrate had the same effect as leucine alone, and administration of either isoleucine or valine alone had no effect. Leucine administration produced only a slight, transient rise in the plasma insulin concentration at 30 min, whereas carbohydrate caused a much greater increase that was maintained at 60 min.¹⁸ Using a meal-feeding model, Lynch et al.¹⁵ confirmed the stimulatory effect of leucine on tissue protein synthesis in gastrocnemius, kidney, and adipose tissue, whereas a carbohydrate meal had no effect despite a robust increase in plasma insulin in response to carbohydrate ingestion. Protein synthesis was associated with an increase in 4E-BP1 phosphorylation, a decrease in association of 4E-BP1 with eIF4E, an increase in association of eIF4G with eIF4E, and an increase in S6K1 phosphorylation.

Additional studies by this same group of investigators addressed the response of protein synthesis to leucine administration when a change in insulin concentration was blocked. The first approach was to use somatostatin, an inhibitor of pancreatic hormone release. Anthony et al.¹⁸ administered somatostatin prior to the

administration of leucine to maintain insulin concentrations at the fasting basal level throughout the 60-min time course. When insulin concentrations were maintained at fasting basal levels via administration of somatostatin, the effect of leucine on protein synthesis was attenuated, leading to the conclusion that the transient increase in plasma insulin in response to leucine administration to food-deprived rats may be permissive for the leucine-induced stimulation of protein synthesis. The leucine-induced increases in 4E-BP1 and S6K1 phosphorylation were also attenuated and the increase in S6 phosphorylation was completely blocked, but the association of eIF4E and eIF4G was not diminished by somatostatin treatment. The relation between insulin and leucine-induced stimulation of protein synthesis in skeletal muscle was further explored using food-deprived rats with experimentally induced diabetes and food-deprived nondiabetic control rats.¹⁹ As expected, protein synthesis was reduced (by 65%) in muscle of diabetic rats compared with nondiabetic control rats. Administration of leucine to the diabetic rats stimulated protein synthesis by about 50%, but the resulting rate of protein synthesis was still markedly lower than that observed in saline-treated control, nondiabetic rats. The stimulatory

response to leucine was enhanced in diabetic rats treated acutely with insulin, although the rate of protein synthesis was still substantially less than the value for nondiabetic rats treated with leucine. Remarkably, in the diabetic rats, leucine administered alone had no effect on mTOR signaling to 4E-BP1 (including eIF4G association with eIF4E) or S6K1 in skeletal muscle, but nonetheless stimulated protein synthesis.

Comparison of the effects of norleucine, a leucine analogue that does not act as an insulin secretagogue, to those of leucine also supported an insulin-independent mechanism for leucine signaling. Lynch et al.¹⁵ found that norleucine stimulated protein synthesis, without affecting plasma insulin concentrations, in gastrocnemius, heart, adipose tissue, kidney, and liver of rats that had been food deprived for 18 h. The effect of norleucine on protein synthesis was similar to that produced by supplementation with an equimolar amount of leucine (approximately 1.35 mg/kg), whereas norleucine typically was less effective than leucine in increasing S6K1 phosphorylation and 4E-BP1 phosphorylation.

To assess the responses to lower amounts of leucine, food-deprived rats were administered (by oral gavage) leucine in amounts ranging from 0.068 to 1.35 g/kg body weight.¹⁶ Results showed that stimulation of protein synthesis by leucine 30 min after its administration reached a maximal value of 135% of the untreated control rate with 0.135 g/kg of the amino acid (i.e., 10% of the amount used in the studies in the food-deprived rat model that were discussed above). The response in protein synthesis paralleled those of eIF4G phosphorylation and eIF4G association with eIF4E, whereas signaling through mTOR to 4E-BP1 and S6K1 continued to increase in proportion to the increasing amounts of leucine administered and its plasma concentrations. An increase in the plasma insulin concentration was observed only at the highest amounts of leucine administered (i.e., 50% and 100% of the amount used in the earlier studies described above). Thus, this study suggested that the maximal effect of leucine on protein synthesis could be obtained without an increase in plasma insulin. Furthermore, this study showed a closer correlation of protein synthesis to eIF4G phosphorylation and its association with eIF4E (which reached a plateau with an increase in leucine dose) than with signaling through mTOR to 4E-BP1 and S6K1 (e.g., phosphorylation of 4E-BP1 and S6K1 continued to increase as the leucine dose increased). In a different model in which overnight fasted rats were infused intravenously with leucine, lipids, or both to alter rates of protein synthesis, Lang²⁰ similarly showed a close correlation of changes in muscle protein synthesis to changes with eIF4G phosphorylation and with the association of eIF4G with eIF4E, whereas changes in 4E-BP1 phosphorylation, S6K1 phosphoryla-

tion, and ribosomal protein S6 phosphorylation were not closely correlated.

Overall, these results suggest that leucine stimulates protein synthesis in skeletal muscle largely through insulin-independent mechanisms, but the pathway and mechanism by which leucine exerts its effects are still uncertain.

WHAT MEDIATES THE EFFECTS OF LEUCINE ON PROTEIN SYNTHESIS?

If the effects of leucine on protein synthesis are mediated largely via an insulin-independent mechanism, what is the pathway for leucine signaling? Several studies have implicated eIF4G phosphorylation as a possible mediator of the effects of leucine on protein synthesis. As mentioned above for studies with food-deprived rats given leucine, when insulin concentrations were maintained at fasting basal levels via administration of somatostatin, the leucine-induced increases in 4E-BP1 and S6K1 phosphorylation were attenuated and the increase in S6 phosphorylation was completely blocked, but the association of eIF4E and eIF4G was not diminished.¹⁸ Furthermore, the leucine dose-response study¹⁶ and the leucine/lipid study²⁰ showed that the response of protein synthesis closely paralleled those of eIF4G phosphorylation and eIF4G association with eIF4E. In addition, studies in perfused hind-limb preparations from postabsorptive rats demonstrated a 60% to 70% increase in protein synthesis when the perfusate leucine concentration was increased to 10 times that in the basal medium, and this increase in protein synthesis was associated with no change in mTOR signaling to 4E-BP1 or S6K1.²¹ Instead, the higher leucine concentration caused phosphorylation of eIF4G on Ser¹¹⁰⁸ and increased association of eIF4G with eIF4E.

Vary and Lynch^{22,23} further examined the promotion of protein accretion in rat skeletal muscle and heart in response to meal intake. Rats were trained to consume a meal (non-purified diet) when it was presented for 3 h beginning 30 min after the beginning of the dark cycle. Meal feeding enhanced the assembly of the active eIF4G·eIF4E complex, and this was associated with a 10-fold rise in phosphorylation of eIF4G (Ser¹¹⁰⁸) and a decreased assembly of the inactive 4E-BP1·eIF4E complex, which returned to basal levels within 3 h of removal of food (i.e., 6 h after meal provision). The reduced assembly of 4E-BP1·eIF4E complex was associated with a 75-fold increase in phosphorylation of 4E-BP1 in the γ -form (i.e., the most highly phosphorylated isoform resolved upon electrophoresis of 4E-BP) during feeding. Similar results were obtained in rat hearts. Although meal feeding had a clear effect on eIF4G and 4E-BP1 phosphorylation, its effects on mTOR signaling were less

clear. In skeletal muscle, meal feeding promoted phosphorylation of mTOR (Ser²⁴⁴⁸ and Ser²⁴⁸¹, neither of which is necessarily responsible for mTOR activity and downstream signaling) and of S6K1. In heart, phosphorylation of mTOR and phosphorylation of S6K1 were not significantly altered by meal feeding.

Anand and Gruppuso²⁴ studied liver growth in rats over a 24-h refeeding period following 48 h of food deprivation. Refeeding resulted in accumulation of liver protein, increased phosphorylation of S6K1 and 4E-BP1, diminished association of 4E-BP1 with eIF4E, and an increased abundance of ribosomal proteins. Administration of rapamycin to the starved/refed rats potently inhibited the phosphorylation of S6K1 and S6K2 and of 4E-BP1, markedly increased the association of eIF4E with 4E-BP1, and diminished translation of 5'-TOP mRNAs (ribosomal proteins) compared with saline-injected starved/refed control rats. In spite of the marked inhibition of mTOR signaling pathways by rapamycin, the net gain in liver protein over the 24 h refeeding period was essentially the same for rapamycin- and saline-injected rats. In addition, refeeding resulted in a rapid increase in eIF4G association with eIF4E that was insensitive to rapamycin.

CONCLUSIONS AND QUESTIONS

At this point, it seems clear that most of the effects of amino acids on protein synthesis are mediated by leucine. Additionally, it seems clear that leucine stimulates protein synthesis largely through insulin-independent mechanisms, although basal insulin levels are important for a maximal effect of leucine on protein synthesis. In general, the effects of leucine do not appear to depend upon the pathways used by insulin signaling or upon mTOR activation above basal levels. Additional work is required to resolve the question of whether mTOR, another component of the mTOR complex, or an mTOR-independent pathway is involved in leucine-specific signaling.

The effect of leucine on protein synthesis appears to be closely associated with eIF4G phosphorylation and its association with eIF4E, but it is not clear whether eIF4G phosphorylation is mediated by a leucine-specific activation of mTOR that is insensitive to rapamycin and distinct from the insulin-mediated activation of mTOR or by a signaling pathway that does not involve mTOR. Furthermore, although eIF4G phosphorylation and the association of eIF4G with eIF4E appear to be highly correlated with enhancement of protein synthesis in various experimental models, whether eIF4G phosphorylation actually mediates the effects of leucine or is merely associated with these events has not been elucidated. Additional research is needed to determine if leucine

mediates eIF4G phosphorylation and if eIF4G phosphorylation is essential for the effect of leucine on protein synthesis.

With regard to these questions, the recent report by Wang et al.²⁵ that amino acids and insulin regulate different sets of phosphorylation sites in 4E-BP1, and that the insulin-modulated ones are sensitive to rapamycin (i.e., Ser64/65, Thr69/70) whereas the amino acid-modulated ones (i.e., Thr36/37, Thr36/37) are largely insensitive, is of much interest. It is possible that mTOR itself may undergo differential phosphorylation at various sites that affects its downstream functions/targets. Long et al.²⁶ showed that binding of Rheb to mTOR is promoted by the presence of amino acids and inhibited by withdrawal of amino acids or just leucine, but it seems unlikely that this mechanism could account for the specific physiological effects of leucine in intact animals. On the other hand, other kinases have been shown to be activated in response to leucine administration. Phosphorylation of PKC ϵ on its catalytic domain autophosphorylation site (Ser⁷²⁹) in skeletal muscle and heart increased in response to meal feeding and to norleucine or leucine administration but not in response to insulin or carbohydrate alone.^{27,28} Furthermore, perfusion of rat hind limb with buffer containing elevated concentrations of leucine stimulated phosphorylation of PKC ϵ , adding further evidence that leucine could mediate the enhanced phosphorylation of PKC ϵ .

As work in this area proceeds, it will also be important to address inter-species differences. The prominent effect of leucine administration in humans appears to be a reduction of protein breakdown rather than an increase in protein synthesis, a situation that seems to be analogous to the results found for insulin in human versus animal experiments (i.e., a reduction in proteolysis in humans and a stimulation of protein synthesis in animals).^{29,30} Nevertheless, leucine supplementation has been shown to increase the fractional synthesis rate for myofibrillar proteins from vastus lateralis biopsies of healthy male subjects consuming an adequate diet.³¹ Whether these apparent differences reflect experimental approaches and measurements or true differences in physiological responses and regulatory mechanisms needs to be resolved.

REFERENCES

1. Garlick PJ, Millward DJ, James WP. The diurnal response of muscle and liver protein synthesis *in vivo* in meal-fed rats. *Biochem J.* 1973;136:935-945.
2. Garlick PJ, Fern M, Preedy VR. The effect of insulin infusion and food intake on muscle protein synthesis in postabsorptive rats. *Biochem J.* 1983;210:669-676.
3. Garlick PJ, Millward DJ, James WP, Waterlow JC.

- The effect of protein deprivation and starvation on the rate of protein synthesis in tissues of the rat. *Biochim Biophys Acta*. 1975;414:71–84.
4. Yoshizawa F, Kimball SR, Vary TC, Jefferson LS. Effect of dietary protein on translation initiation in rat skeletal muscle and liver. *Am J Physiol*. 1998;275:E814–E820.
 5. Buse MG, Reid SS. Leucine. A possible regulator of protein turnover in muscle. *J Clin Invest*. 1975;56:1250–1261.
 6. Buse MG, Weigand DA. Studies concerning the specificity of the effect of leucine on the turnover of proteins in muscles of control and diabetic rats. *Biochim Biophys Acta*. 1977;475:81–89.
 7. Fulks RM, Li JB, Goldberg AL. Effects of insulin, glucose, and amino acids on protein turnover in rat diaphragm. *J Biol Chem*. 1975;250:290–298.
 8. Li JB, Jefferson LS. Influence of amino acid availability on protein turnover in perfused skeletal muscle. *Biochim Biophys Acta*. 1978;544:351–359.
 9. Tischler ME, Desautels M, Goldberg AL. Does leucine, leucyl-tRNA, or some metabolite of leucine regulate protein synthesis and degradation in skeletal and cardiac muscle? *J Biol Chem*. 1982;257:1613–1621.
 10. Vary TC, Goodman S, Kilpatrick LE, Lynch CJ. Nutrient regulation of PKCepsilon is mediated by leucine, not insulin, in skeletal muscle. *Am J Physiol Endocrinol Metab*. 2005;289:E684–E694.
 11. Anthony JC, Anthony TG, Kimball SR, Vary TC, Jefferson LS. Orally administered leucine stimulates protein synthesis in skeletal muscle of postabsorptive rats in association with increased eIF4F formation. *J Nutr*. 2000;130:139–145.
 12. Hara K, Yonezawa K, Weng QP, Kozlowski MT, Belham C, Avruch J. Amino acid sufficiency and mTOR regulate p70 S6 kinase and eIF-4E BP1 through a common effector mechanism. *J Biol Chem*. 1998;273:14484–14494.
 13. Kimball SR, Shantz LM, Horetsky RL, Jefferson LS. Leucine regulates translation of specific mRNAs in L6 myoblasts through mTOR-mediated changes in availability of eIF4E and phosphorylation of ribosomal protein S6. *J Biol Chem*. 1999;274:11647–11652.
 14. Anthony JC, Yoshizawa F, Anthony TG, Vary TC, Jefferson LS, Kimball SR. Leucine stimulates translation initiation in skeletal muscle of postabsorptive rats via a rapamycin-sensitive pathway. *J Nutr*. 2000;130:2413–2419.
 15. Lynch CJ, Patson BJ, Anthony J, Vaval A, Jefferson LS, Vary TC. Leucine is a direct-acting nutrient signal that regulates protein synthesis in adipose tissue. *Am J Physiol Endocrinol Metab*. 2002;283:E503–E513.
 16. Crozier SJ, Kimball SR, Emmert SW, Anthony JC, Jefferson LS. Oral leucine administration stimulates protein synthesis in rat skeletal muscle. *J Nutr*. 2005;135:376–382.
 17. von Manteuffel SR, Dennis PB, Pullen N, Gingras AC, Sonenberg N, Thomas G. The insulin-induced signalling pathway leading to S6 and initiation factor 4E binding protein 1 phosphorylation bifurcates at a rapamycin-sensitive point immediately upstream of p70s6k. *Mol Cell Biol*. 1997;17:5426–5436.
 18. Anthony JC, Lang CH, Crozier SJ, et al. Contribution of insulin to the translational control of protein synthesis in skeletal muscle by leucine. *Am J Physiol Endocrinol Metab*. 2002;282:E1092–E1101.
 19. Anthony JC, Reiter AK, Anthony TG, et al. Orally administered leucine enhances protein synthesis in skeletal muscle of diabetic rats in the absence of increases in 4E-BP1 or S6K1 phosphorylation. *Diabetes*. 2002;51:928–936.
 20. Lang CH. Elevated plasma free fatty acids decrease basal protein synthesis, but not the anabolic effect of leucine, in skeletal muscle. *Am J Physiol Endocrinol Metab*. 2006;291:E666–E674.
 21. Bolster DR, Vary TC, Kimball SR, Jefferson LS. Leucine regulates translation initiation in rat skeletal muscle via enhanced eIF4G phosphorylation. *J Nutr*. 2004;134:1704–1710.
 22. Vary TC, Lynch CJ. Meal feeding enhances formation of eIF4F in skeletal muscle: role of increased eIF4E availability and eIF4G phosphorylation. *Am J Physiol Endocrinol Metab*. 2006;290:E631–E642.
 23. Vary TC, Lynch CJ. Meal feeding stimulates phosphorylation of multiple effector proteins regulating protein synthetic processes in rat hearts. *J Nutr*. 2006;136:2284–2290.
 24. Anand P, Gruppuso PA. Rapamycin inhibits liver growth during refeeding in rats via control of ribosomal protein translation but not cap-dependent translation initiation. *J Nutr*. 2006;136:27–33.
 25. Wang X, Beugnet A, Murakami M, Yamanaka S, Proud CG. Distinct signaling events downstream of mTOR cooperate to mediate the effects of amino acids and insulin on initiation factor 4E-binding proteins. *Mol Cell Biol*. 2005;25:2558–2572.
 26. Long X, Ortiz-Vega S, Lin Y, Avruch J. Rheb binding to mammalian target of rapamycin (mTOR) is regulated by amino acid sufficiency. *J Biol Chem*. 2005;280:23433–23436.
 27. Anthony JC, Anthony TG, Kimball SR, Jefferson LS. Signaling pathways involved in translational control of protein synthesis in skeletal muscle by leucine. *J Nutr*. 2001;131:856S–860S.
 28. Lynch CJ, Hutson SM, Patson BJ, Vaval A, Vary TC. Tissue-specific effects of chronic dietary leucine and norleucine supplementation on protein synthesis in rats. *Am J Physiol Endocrinol Metab*. 2002;283:E824–E835.
 29. Matthews DE. Observations of branched-chain amino acid administration in humans. *J Nutr*. 2005;135:1580S–1584S.
 30. Garlick PJ. The role of leucine in the regulation of protein metabolism. *J Nutr*. 2005;135:1553S–1556S.
 31. Rieu I, Balage M, Sornet C, Giraudet C, Pujos E, Grizard J, Mosoni L, Dardevet D. Leucine supplementation improves muscle protein synthesis in elderly men independently of hyperaminoacidaemia. *J Physiol*. 2006;575:305–315.
 32. Proud CG. Regulation of protein synthesis by insulin. *Biochem Soc Trans*. 2006;34:213–216.

Copyright of Nutrition Reviews is the property of International Life Sciences Institute and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.