

Nutritional and regulatory roles of leucine in muscle growth and fat reduction

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1. ABSTRACT

The metabolic roles for L-leucine, an essential branched-chain amino acid (BCAA), go far beyond serving exclusively as a building block for *de novo* protein synthesis. Growing evidence shows that leucine regulates protein and lipid metabolism in animals. Specifically, leucine activates the mammalian target of rapamycin (mTOR) signaling pathway, including the 70 kDa ribosomal protein S6 kinase 1 (S6K1) and eukaryotic initiation factor (eIF) 4E-binding protein 1 (4EBP1) to stimulate protein synthesis in skeletal muscle and adipose tissue and to promote mitochondrial biogenesis, resulting in enhanced cellular respiration and energy partitioning. Activation of cellular energy metabolism favors fatty acid oxidation to CO₂ and water in adipocytes, lean tissue gain in young animals, and alleviation of muscle protein loss in aging adults, lactating mammals, and food-deprived subjects. As a functional amino acid, leucine holds great promise

to enhance the growth, efficiency of food utilization, and health of animals and humans.

2. INTRODUCTION

Sufficient evidence has pointed out that leucine, a nutritionally essential branched-chain amino acid, plays a unique signaling role in both adipose tissue and skeletal muscle. In these cells, leucine stimulates protein synthesis via the mTOR signaling pathway, mitochondrial biogenesis, and fatty acid oxidation (1, 2). Protein synthesis is the major energy-consuming process in the cell (3). In particular, both mRNA translation and ribosomal biogenesis processes, which are strongly affected by the mTOR pathway, consume high levels of cellular energy (4). Moreover, previous studies indicate that leucine regulates muscle protein synthesis and adipocyte lipid metabolism to provide

an increased flux of lipids to skeletal muscle, thereby supplying energy substrates to support leucine-induced protein synthesis (5). In addition, leucine is most effective in stimulating mTOR among various models (2, 6). In the present review, we firstly focus on the roles of leucine in protein synthesis, which is involved in 1) leucine entering the cells, 2) the mTOR signaling pathway, and 3) insulin's role and endoplasmic reticulum (ER) stress. We then discuss the possible mechanisms of leucine in regulating energy partitioning between skeletal muscle cells and adipocytes.

3. ROLES OF LEUCINE IN PROTEIN SYNTHESIS

3.1. How leucine enters the cell to regulate mTORC1 signaling

L-Leucine has been known as a key activator of protein synthesis in skeletal muscle for more than 30 years (7). The anabolic potential of leucine to stimulate skeletal muscle growth via the mTOR signaling pathway has been examined in various cell models (8). mTOR, a master regulator of cell growth and metabolism, exists in two complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) (9, 10). The differences between mTORC1 and mTORC2 mainly occur in the following two aspects. First, mTORC1 is very sensitive to rapamycin, whereas mTORC2 displays a limited inhibition by this drug. Second, activation of mTORC1 stimulates protein synthesis and cell growth, while mTORC2 is involved in cytoskeleton reorganization and cellular proliferation (11). In mTORC1, mTOR associates with two highly conserved proteins: regulatory associated protein of mTOR (raptor) and mammalian lethal with sec18 protein 8 (mLST8) (12). In the present paper, we focus on mTORC1. When exposed to cells rich in amino acids (AAs), mTORC1 is activated to regulate downstream effectors of mTORC1 to increase protein translation and to inhibit macroautophagy (13). The interaction between mTOR and raptor is mediated in part by changes in amino acid availability. Amino acids promote a shift in the raptor-mTOR complex from a stable, inactive complex to an unstable, active complex, which is essential for activation of the downstream (mainly S6K1 and 4EBP1) of the mTOR pathway (14). Furthermore, numerous metabolic roles of leucine depend on its cellular concentration (15). Therefore, we should first understand the mechanisms by which leucine enters the cell to subsequently activate the mTORC1 pathway.

Amino acid transporters, which are present in an ideal location on membranes to relay nutrient signals to the cell interior, allow the cell to sense amino acid availability, and launch an anabolic response (increased translation and growth) (13, 16). Through three active transporters, leucine enters cells (Figure 1) (16). The primary active transport mechanism is that Na^+ moves from the intracellular side to extracellular side via the hydrolysis of adenosine triphosphate (ATP). The second active transport of leucine is mediated by transporter System A (solute carrier family 38 member 2 (SLC38A2)). Extracellular leucine and Na^+ enter the cytoplasm of the cell through the cell membrane. The tertiary active transport of leucine is carried out by the amino acid transporter hetero-exchange System L (solute carrier family 7 member 5 (SLC7A5)/SLC3A2) (16).

When serum-free DMEM was added to starved HeLa cells, whose mTORC1 signaling was inhibited after deprivation of amino acid and growth factor, activating S6K1 (a key downstream of mTORC1) and caused the phosphorylation of S6K1 and ribosomal protein S6, a key downstream target of S6K1. However, when AAs were added alone to culture medium at the same concentration as found in DMEM, mTORC1 signaling was not activated. In addition to AAs, the remaining components of DMEM (sodium pyruvate, sodium bicarbonate, ferric nitrate, nonessential amino acids and L-Gln) were added alone to the cells, respectively. Likewise, the mTORC1 pathway was not activated. However, the addition of L-Gln to culture medium containing a mixture of EAAs up-regulates mTORC1 signaling, indicating that L-Gln is required for activation of mTORC1 in an AA-rich environment. Following uptake, L-glutamine is effluxed within 1-2 min of adding EAAs to cells, resulting in reciprocal uptake of L-leucine and rapid activation of S6K1. This uptake process requires solute carrier family 1 member 5 (SLC1A5) and SLC7A5/SLC3A2. SLC1A5 is a high affinity L-Gln transporter and its inhibition blocks uptake of L-Gln, resulting in inhibition of the mTORC1 signaling. SLC7A5/SLC3A2 is a bidirectional transporter that regulates the exchange of intracellular L-Gln for extracellular L-leucine. Thus, with the help of L-Gln, SLC1A5 and SLC7A5/SLC3A2, EAAs enter the cells to mediate the mTORC1 signaling (13). In mammalian cells, mTORC1 is acutely responsive to amino acid availability and signals to enhance mRNA translation during periods of plentiful nutrient supply (16).

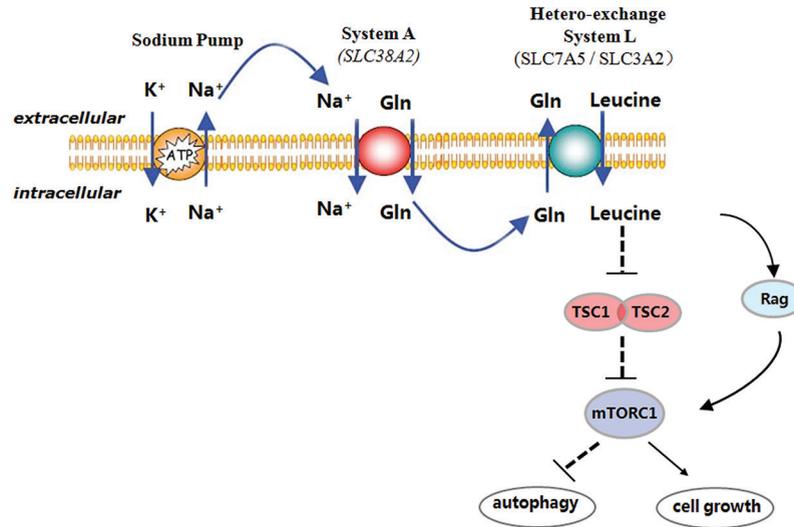


Figure 1. Leucine enters the cells to promote mTORC1 signaling: via amino acid transporters as well as glutamine (Gln). Leucine enters the cell to stimulate mTORC1, which is a master regulator of cell growth and metabolism. Adapted from Reference 16.

3.2. The mechanisms for leucine to stimulate protein synthesis via mTORC1 signaling

Availability of AAs is a key factor in enhancing net protein synthesis (17). As noted previously, BCAAs, especially leucine, are essentially important for the regulation of protein metabolism. The underlying mechanisms include: 1) providing the precursors and metabolic substrates required for polypeptide biosynthesis, 2) regulating the release of endocrine hormones (such as insulin, insulin like growth factor-1(IGF-1)), and 3) modulating the mTOR signaling pathway responsible for protein synthesis (18-20). Numerous studies utilizing various model systems have indicated that AAs stimulate protein synthesis by enhancing the initiation of mRNA translation into protein (21, 22). The rate-limiting step in the process of mammalian translation initiation is the binding of the ribosome to mRNA. Strikingly, almost all of the factors (including the eukaryotic initiation factor (eIF) 4E (eIF4E), eIF4B, and eIF4G that are involved in recruiting the ribosome are phosphoproteins, whose phosphorylation states are directly in proportion to the translation and growth rates of the cell (23). Similarly, 4EBP1, the repressor protein, is also phosphorylated in this process (24). Of considerable interest, the mTOR signaling pathway regulates the phosphorylation of all of these factors, except for eIF4E (23). Our understanding of the molecular mechanisms contributing to the effect of leucine on translation initiation has tremendously

increased in the past few years. Known pathways involve phosphorylation of the mTORC1 and sequential phosphorylation of S6K1, 4EBP1, and eIF4G. S6K1 and 4EBP1 represent important control points in translation initiation (20, 25, 26).

Candidates for mediating the action of leucine to stimulate mTOR signaling include a small GTPase called Rheb (Ras homolog enriched in brain), tuberin (also known as TSC2), and raptor. mTOR signaling is controlled by upstream members of the pathway, such as Rheb, TSC2 and its binding partner, TSC1 (hamartin). Rheb activates mTORC1. However, TSC2-TSC1 complex induces Rheb inactivation by promoting the conversion of Rheb-GTP to Rheb-GDP, which in turn represses mTORC1 activity. Leucine acts on mTORC1 signaling in part through inhibiting the activity of the TSC1-TSC2 complex and subsequently activation of Rheb. Upon activation, mTORC1 phosphorylates downstream components, such as S6K1 and 4EBP1, ultimately activating translation initiation of protein synthesis (11, 25). In particular, previous studies have shown that oral leucine administration results in hyperphosphorylation (inactivation) of 4EBP1 in rats fasted for 18 h (27). However, orally administered leucine causes the phosphorylation (activation) of S6K1 (28). Of particular note, amino acid availability leads to an elevation in intracellular Ca²⁺ levels, which in turn enhances mTORC1 signaling via Ca²⁺/calmodulin-mediated activation of hVPS34, a class III phosphoinositide-3-OH kinase (PI3K) (29).

In addition to mTOR-dependent mechanisms, leucine can also stimulate protein synthesis through mTOR-independent mechanisms (30, 31). Numerous studies show that leucine has a stimulatory effect on the assembly of the eIF4F complex, the phosphorylation status of eIF4G and S6K1, as well as a downstream protein substrate S6 (25). Thus, under mTOR-independent mechanisms, protein synthesis in skeletal muscle is regulated by an ability of leucine to activate eIF4G (30).

3.3. Roles of insulin in leucine-induced protein synthesis in muscle

Elevated levels of leucine are known for their stimulating effect on insulin release from the pancreas. In turn, insulin plays a role in promoting muscle AA uptake in the postprandial period and hindering AA release from skeletal muscle during the post-absorptive period. After meals, the effect of insulin on uptake of AAs by muscle cells activates an anabolic response and enhances protein synthesis (11). Furthermore, the ability of leucine to promote the rate of muscle protein synthesis is attenuated when the elevation in insulin release is prevented (32, 33). Thus, it has been presumed that the anabolic effect of leucine may result from changes in the circulating levels of either leucine or insulin, or a combination of both (34). However, the complex mechanisms by which leucine enhances insulin secretion are not yet fully understood. Nonetheless, a transient increase in insulin is associated with the leucine-induced effect on protein synthesis in skeletal muscle. These studies also show that somatostatin maintains insulin concentrations at the fasting basal level throughout the time course. The transient increase in plasma insulin, which contributes to the leucine-induced phosphorylation of 4EBP1 and S6K1, appears to be permissive to the leucine-mediated stimulation of protein synthesis in skeletal muscle (33). Moreover, studies in rats with experimentally induced diabetes demonstrate that administration of leucine alone has no effect on mTOR signaling to S6K1 or 4EBP1 but rather produces a stimulation of protein synthesis (35). However, administration of leucine in association with insulin infusion is sufficient to enhance mTOR signaling to S6K1 and 4EBP1 (35). Overall, these results indicate that leucine stimulates protein synthesis in skeletal muscle via both insulin-dependent and -independent mechanisms. The insulin-dependent mechanism is associated with signaling through mTOR to S6K1 and 4EBP1, whereas the insulin-independent effect is mediated by an unknown mechanism that may involve phosphorylation of eIF4G and/or its association with eIF4E (25).

Insulin-dependent activation of mTOR is mediated via the PI3K/protein kinase B (also known as Akt) signaling pathway. PI3K is activated by insulin through direct interaction with receptors or through interaction with scaffolding adaptors, such as the insulin receptor substrate proteins. Downstream of PI3K, Akt directly phosphorylates and inactivates the TSC1/TSC2 complex, resulting in the subsequent activation of Rheb, which activates mTOR by binding directly to mTOR and to the mTOR-interacting protein mLST8 (23, 36-38). Upon activation of the mTOR signaling, mTORC1 phosphorylates 4EBP1 and S6K1. Through negative-feedback mechanisms, mTORC1 activity inhibits insulin stimulation of PI3K (39).

All in all, leucine- and insulin- mediated effects on protein synthesis appear to activate different signaling pathways that converge at the mTOR, at least in part through repression of the TSC1-TSC2 complex and consequent inhibition of the GTPase activity of Rheb (11). TSC1/TSC2 protein complex may constitute the convergence point for both leucine- and insulin-related controls of mTOR activity (11, 23). mTOR, which lies downstream of Akt in the PI3K signaling pathway, integrates the two signals and contributes to the regulation of protein synthesis in skeletal muscle (33, 35). It is important to note that insulin signals exert little or no effect on mTORC1 signaling in the absence of AAs (13). However, previous studies report that leucine has a direct action on the PI3K/Akt signal pathway in activating the eIF4E initiation complex independent of insulin (40). This is further supported by studies that have shown that elevations in serum EAAs are responsible for the enhanced protein synthesis in skeletal muscle independent of insulin or IGF-1 changes (41). Also infusion of BCAAs or leucine alone can phosphorylate and activate mTOR in skeletal muscle without activating the PI3K/Akt signaling pathway (42). This raises the possibility that the role of leucine in protein synthesis does not necessarily require an increase in plasma insulin concentrations above fasting values, but does require a minimal amount of insulin.

3.4. Protein synthesis via mTORC1 signaling and endoplasmic reticulum stress

ER is a highly metabolic organelle. The membrane-bound network of ER extends from the nuclear envelope to the periphery of the cell, maintaining vital contact zones with many other cell organelles. Above all, ER regulates

intracellular turnover of proteins (9, 43). Increasing protein synthesis elevates the demand on the ER machinery for protein folding, leading to acute activation of the ER stress (also referred to as the unfolded protein response (UPR)) induced by mTORC1 (9). Ire1- α , PERK, and ATF6- α are three types of ER membrane-embedded proteins. Upon UPR activation, Ire1- α degrades select ER-associated mRNAs to attenuate protein import into the ER. PERK directly phosphorylates the eIF2- α translation initiation factor, lowering overall translation of protein synthesis (43, 44). Overall, the output of UPR signaling is homeostatic adaptation by a variety of mechanisms that primarily aim at lowering the burden of folding substrates, increasing the capacity of the ER folding machinery, and restoring ER function and promote apoptotic cell death (9, 45).

mTORC1 operates upstream as well as downstream of ER stress signals, which can either enhance or antagonize the anabolic output of mTORC1. Upon prolonged ER stress, mTORC1 leads to apoptotic signaling by inhibiting the survival kinase Akt through feedback inhibition. Similarly, chronic ER stress blocks activation of Akt by mTORC2 (9). Overall, inactivation of Akt may be caused by chronic activation of mTORC1 and UPR via the following mechanisms: inhibitory phosphorylation of insulin receptor substrate 1 and mTORC2 by S6K1 downstream of mTORC1; inactivation of mTORC2 by GSK3 β -catalyzed phosphorylation downstream of ER stress; PERK-CHOP-mediated induction of TRB3, which directly binds to Akt and obstructs its activation. Reduced Akt activation results in higher levels of TRAF2, thus triggering the activation of the UPR (9).

4. THE MECHANISMS OF LEUCINE ON ENERGY PARTITIONING BETWEEN SKELETAL MUSCLE AND ADIPOCYTES

4.1. Fatty acid oxidation provides energy required for leucine-induced protein synthesis

In spite of being a major sink for circulating glucose, skeletal muscle takes up a great deal of plasma fatty acids (FA), either for storage (incorporation into intracellular lipids) or for ATP production (oxidation) (46). Skeletal muscle plays a critical role in determining energy metabolism, in the clearance of serum free FA, in lipid utilization, and in whole-body FA oxidation (5, 47). Of note, leucine regulates adipocyte lipid metabolism to provide an

increased flux of free FA to skeletal muscle, thereby supplying energy substrates to support leucine-induced protein synthesis (5). Interestingly, the body fat loss induced by dietary supplementation of leucine is associated with increased protein synthesis (8, 48-53). Taken together, it is speculated that a substantial amount of energy required in the process of leucine-stimulated protein synthesis in muscle cells is mainly provided by FA oxidation. There is emerging evidence supporting this hypothesis. Donate *et al.* (50) reports that body fat in rats receiving a diet containing 1.7.7 percent of leucine after 6 week of food restriction was reduced by 47%, whereas protein nutritional status and protein synthesis capacity in rats were improved. These results suggest that long-term low-dose supplementation of leucine enhances body fat loss, liver protein status, and the capacity of muscle protein synthesis in underfed rats. Zhang *et al.* (54) found a 25% decrease in adiposity in rats receiving a diet with 1.5% L-leucine. Sun and Zemel (55) reported that co-culture of muscle cells with adipocytes or incubation for 48-h with adipocyte-conditioned medium lowered muscle FA oxidation by 62%, but treating adipocytes with leucine attenuated this effect. These results suggest that certain cytokines derived from adipocytes regulate energy metabolism in muscle cells. Leucine significantly increased FA oxidation in C2C12 myotubes, while inhibiting fatty acid synthase and peroxisome proliferator-activated receptor- γ expression in 3T3-L1 adipocytes. These results suggest that leucine can regulate adipocyte lipid metabolism to promote flux of lipid to skeletal and coordinately promote energy partitioning from adipocytes to muscle cells, leading to reduced energy storage in adipocytes and increasing FA utilization in muscle (55). Additionally, Sun and Zemel (5) proposed that energy metabolism in muscle and adipocytes is regulated by leucine at least in part through mitochondrial biogenesis (an increase in mitochondrial mass or number). Recent studies examined the effects of a leucine-rich (4 percent) diet in rats during their most adult life until the onset of old age (56). The results further confirm the findings of previous studies that leucine supplementation promotes a loss in body fat. Accordingly, leucine may have an effect in mediating normal energy metabolism through its role in mitochondrial biogenesis and fat oxidation.

4.2. Mitochondrial biogenesis is involved in the role of leucine in energy metabolism

Mitochondria play a crucial role in regulating adipocyte lipid metabolism and cellular energy

metabolism (57). As noted before, mitochondria involves in mediating energy metabolism of leucine in muscle and adipocytes. Multiple genes expressed in mitochondria, such as peroxisome proliferator-activated receptor gamma co-activator 1-alpha (PGC-1 alpha) and silent information regulator transcript 1 (SIRT-1), may be involved in the modulation of energy metabolism through their regulation of mitochondrial number and FA oxidation (58-60). Further, overexpression of PGC-1 alpha in rodents enables muscle to oxidize fatty acids more efficiently (61). Leucine (0.5. mM) can enhance mitochondrial biogenesis in both C2C12 myocytes and 3T3-L1 adipocytes, and regulate skeletal muscle energy metabolism in part by regulating expression of PGC-1 alpha and SIRT-1(5). In addition, activation of the AMP-activated protein kinase (AMPK) results in phosphorylation and increased activity of PGC-1 alpha, therefore promoting mitochondrial function (1). Overall, leucine plays a unique signaling role in skeletal muscle and adipocytes. Specifically, leucine signaling promotes mitochondrial biogenesis, which enhances cellular respiration and energy partitioning. As a result, oxidation of fatty acids in adipocytes and muscle is increased, and this cellular energy metabolism promotes protein synthesis via mTOR activation within muscle cells (Figure 2) (1).

4.3. Cytokines secreted by adipose and muscle may regulate energy partitioning

Energy repartitioning between skeletal muscle and adipose tissue has been previously demonstrated by many studies (5, 55, 62, 63). Leucine could promote oxygen consumption in myocytes and adipocytes, further supporting the role of leucine in modulation of energy combustion. However, exposure of myocytes to adipocytes via either co-culture or conditioned medium attenuates these effects, suggesting that certain molecules secreted by excess adipose tissue may affect FA oxidation in skeletal muscle by suppressing mitochondrial biogenesis (5). This discovery raises the possibility that cytokines secreted by adipose and muscle tissues may be involved in energy partitioning between these tissues. Cytokines, such as tumor necrosis factor alpha (TNF-alpha), leptin, adiponectin, and Interleukin-15 (IL-15), play a key role in the "cross-talk" between adipose tissue and skeletal muscle. TNF-alpha secreted by adipocytes has been demonstrated to down-regulate mitochondrial biogenesis in both muscle and adipose tissue (64, 65), and to inhibit *in vitro* myoblast differentiation via lowering myoD and myogenin (66). Conversely, two other adipokines, adiponectin and leptin, have

been implicated in increasing the rate of FA oxidation and decreasing muscle lipid content (46). Muoio *et al.*(47) firstly demonstrated that leptin directly alters FA metabolism in skeletal muscle, namely partitioning FA away from intramuscular triglycerides (IMTG) storage towards oxidation. Steinberg *et al.* (67) confirmed these findings by demonstrating that leptin acutely increases IMTG hydrolysis. Of note, leucine increases leptin secretion via mTOR signaling (68). Likewise, adiponectin also has been shown to reduce IMTG, promote FA oxidation (69), and increase various enzymes/proteins involved in FA oxidation (such as fatty acid translocase and fatty acid transport protein 1) (70). Moreover, an increased abundance of mitochondria in adipose tissue enhances adiponectin synthesis (71). However, mitochondrial loss and dysfunction could increase ER stress and decrease adiponectin transcription though activation of N-terminal kinase (71). It is intriguing that the effects of leptin and adiponectin on FA metabolism seem to be regulated at least in part through the activation of AMPK and consequently acetyl-CoA carboxylase (ACC) (46). IL-15, a cytokine highly expressed in skeletal muscle, exerts its anabolic effects on protein dynamics in skeletal muscle both *in vivo* and *in vitro*, and plays a key role in regulating fat deposition (66, 72). Administration of IL-15 reduced adipose tissue deposition by 33% without affecting food intake in growing rats (73, 74). Further, another study with obese rodents showed that sensitivity to the white fat-reducing effects was related to mRNA levels for a key signaling subunit of IL-15 receptor in adipocytes (75). Overall, these results show that IL-15 decreases adipose tissue mass through a direct mechanism. Additionally, IL-15 stimulates secretion of the adipocyte-specific hormone adiponectin by differentiated 3T3-L1 adipocytes (72). In turn, adiponectin greatly enhances IL-15 release from muscle cells (55).

4.4. mTOR and AMPK may regulates leucine-induced energy partitioning

Accumulating evidence has shown that some AA sensors, such as mTOR and AMPK, play critical roles in the modulation of lipid metabolism and energy repartitioning (76). Protein synthesis is the major energy-consuming process in the cell (3). In particular, the processes of mRNA translation and ribosomal biogenesis, which are strongly affected by mTOR pathways, consume high levels of cellular energy (4). Cells exhibit a reduced rate of protein synthesis when there is insufficient AA substrate or energy via a mTOR-dependent mechanism (2). Thus, it is speculated that mTOR activity may be

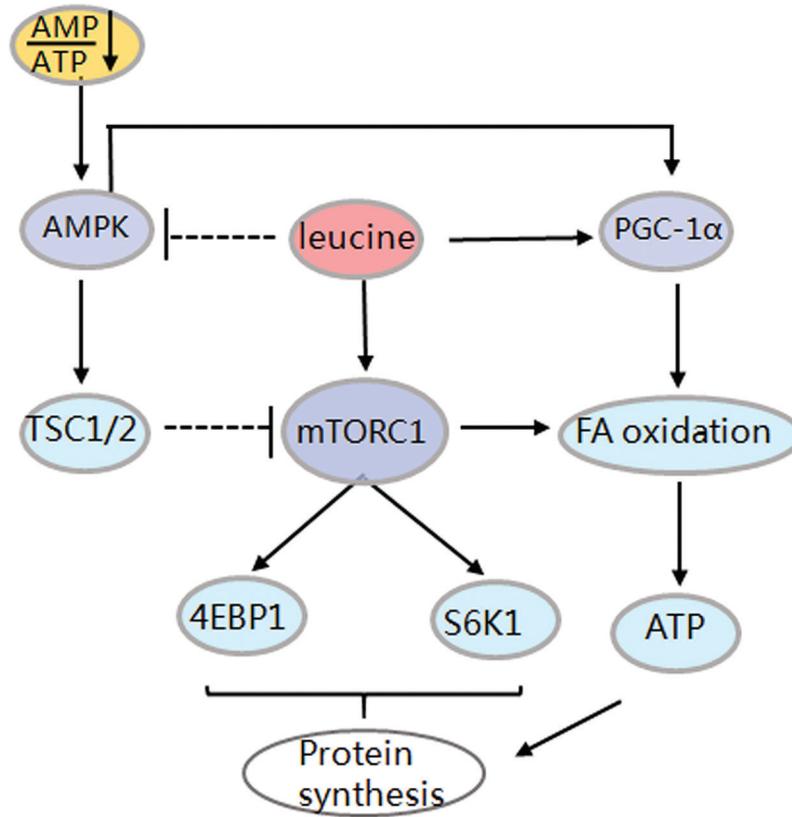


Figure 2. Mechanisms for leucine to regulate protein synthesis and energy partitioning in skeletal muscle and adipocytes. Leucine stimulates protein synthesis via mammalian target of rapamycin (mTOR) signaling, which is involved in the 70 kDa ribosomal protein S6 kinase 1 (S6K1) and eukaryotic initiation factor (eIF) 4E-binding protein 1 (4EBP1). Leucine signaling promotes mitochondrial biogenesis in skeletal muscle and adipocytes, which could enhance cellular respiration and energy partitioning in these cells.

linked to cellular energy status (4). Reduced glucose availability or the suppression of mitochondrial respiration lowers cellular ATP levels and, therefore, impairs the ability of insulin to activate mTOR (4). Thus, cellular energy status has an effect on mTOR activity (4). In other words, mTOR serves as an ATP sensor (2, 4). Consequently, mTOR functions as a checkpoint by which cells sense and decode changes in energy status, which in turn regulates cell growth and proliferation (2). Further, mTOR could promote mitochondrial biogenesis and therefore FA oxidation (Figure 2) (10). Leucine can activate mTOR in various model systems (2, 6). Therefore, the effects of leucine on energy repartitioning between skeletal muscle and adipocytes may be exerted at least, in part, through the role of mTOR in mitochondrial biogenesis.

AMPK is a key regulator of FA metabolism and protein synthesis in skeletal muscle (77). AMPK is regulated by moderate changes in cellular

ATP levels and can sense the ratio of adenosine monophosphate (AMP)/ATP in the cell. Thus, a decline of the intracellular ATP level, accompanied by an increase in the AMP level, results in an elevation of AMPK activity (Figure 2) (46, 78, 79). Additionally, leucine can decrease AMPK activity, which is not mediated by changes in the AMP/ATP ratio but is related to an increase in the lactate/pyruvate ratio, suggesting that leucine could cause an increase in NADH concentrations relative to NAD⁺ availability in muscle (80). Once activated, AMPK phosphorylates multiple downstream substrates with the aim of conserving the existing ATP. On one hand, activation of AMPK increases the supply of ATP by inducing a phosphorylation (deactivation) of ACC and reducing malonyl CoA concentrations (81). Because malonyl-CoA suppresses carnitine palmitoyl transferase-I (CPT-I) activity, the decrease in malonyl-CoA content will relieve CPT-I from its inhibition, resulting in increased uptake of long-chain

fatty acids by mitochondria for oxidation in muscle tissues (82, 83). In contrast, activation of AMPK decreases ATP expenditure by suppressing key enzymes/proteins in biosynthetic pathways, such as mTOR/S6K1 signaling in protein synthesis and glycerol-3-phosphate acyltransferase and ACC in lipid synthesis (80, 84). Thus, AMPK activation enables adipocytes to release more FA into the circulation for the metabolic needs of muscle and liver. Furthermore, AMPK is also a mediator of the effects of adipocyte-derived hormones on fatty acid oxidation in peripheral tissues (77). Leptin could activate AMPK in muscle, which is essential for its modulation of energy homeostasis (85, 86). Decreasing pro-inflammatory cytokines, such as interleukin-6 and TNF- α , activates AMPK in adipose tissue and indirectly increases adiponectin secretion (87).

mTOR not only promotes protein synthesis but also suppresses autophagy in the presence of mitogens and available AA. Additionally, mTOR may be one of the downstream targets of AMPK. Upon depletion of ATP, AMPK activation leads to suppression of mTOR signaling via activation of TSC2, thereby inhibiting protein synthesis in cells. Inhibition of protein synthesis via AMPK is an important pathway by which AMPK conserves cellular energy during low energy states, implying that energy metabolism and protein synthesis are tightly coupled via AMPK and TSC2 (23, 77). Previous studies report that S6K1 is suppressed by treatment with mitochondrial inhibitors, suggesting that mitochondrial dysfunction caused by mitochondrial inhibitors may suppress S6K1 by activating AMPK (84). In addition, catabolism of leucine forms isovaleryl CoA and NADH by metabolic enzymes, including branched-chain amino acid aminotransferase and branched-chain α -keto acid dehydrogenase complex (6). Therefore, it has been speculated that leucine activates S6K1 via the mTOR signaling pathway, in part by serving both as a mitochondrial fuel through oxidative decarboxylation and an allosteric activation of glutamate dehydrogenase (88). This further supports the notion that leucine regulates mTOR function, in part by regulating mitochondrial function and AMPK (6). Thus, it can be surmised that mTOR senses the intracellular level of ATP through AMPK (84).

5. SUMMARY AND PERSPECTIVES

Our basic knowledge of the regulation of protein synthesis and energy metabolism by

leucine has been greatly expanded over the past years. A growing body of evidence has shown that AA sensors (such as mTOR and AMPK) and mitochondrial biogenesis are involved in this modulation. Of note, understanding the important relationship between protein synthesis and energy metabolism may provide new strategies for favorably affecting body composition in animals and humans. Reducing the loss of lean body mass in mammals and birds during catabolic states, which occurs in specific physiological conditions (e.g. starvation, aging, and lactation) (89-94), while promoting the loss of excessive white adipose tissue (95-99), is important for both human health and animal agriculture. Key questions include the following. First, because activation of AMPK favors FA oxidation over FA synthesis providing energy and inhibiting protein synthesis via suppressing mTOR, how does leucine regulate AMPK during the process of protein synthesis? Second, what is the most suitable amount of leucine supplementation in order to coordinate effectively with insulin for stimulating protein synthesis? Third, although Wu and co-workers recently proposed optimal patterns of dietary amino acids for gestating, lactating, and growing swine (100-103), what is the optimal ratio of dietary BCAAs for protein synthesis and energy partitioning in skeletal muscles and adipocytes? Further studies are essential to clearly address these questions. Like other functional AA (e.g. 104-126), a suitable ratio of dietary BCAAs is essential to build a reasonable AA model for requirements, decrease dietary crude-protein levels, promote growth performance of animals, and protect the environment by rational utilization of AAs and the reduction of nitrogen discharge. Sources of supplemental leucine in diets can be synthetic leucine and leucine-rich animal products (127-131). Thus, leucine holds great promise for optimizing growth and health in animals and humans (132-135).

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Abbreviations: 4EBP1, 4E-binding protein 1; AA, amino acid (s); ACC, consequently

Acetyl-CoA carboxylase; AMP, adenosine monophosphate; AMPK, AMP-activated protein kinase; ATP, adenosine triphosphate; BCAA, branched-chain amino acid; CPT-I, carnitine palmitoyl transferase-I; eIF, eukaryotic initiation factor; ER, endoplasmic reticulum; FA, fatty acid; Gln, glutamine; mLST8, mammalian lethal with sec18 protein 8; IGF-1, insulin like growth factor-1; IL-15, Interleukin-15; IMTG, intramuscular triglycerides; mTOR, mammalian target of rapamycin; mTORC1, mTOR complex 1; mTORC2, mTOR complex 2; PI3K, phosphoinositide-3-OH kinase; raptor, regulatory associated protein of mTOR; PGC-1 alpha, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; Rheb, Ras homolog enriched in brain; S6K1, the 70 kDa ribosomal protein S6 kinase 1; SIRT-1, silent information regulator transcript 1; SLC38A2, solute carrier family 38 member 2; SLC7A5, solute carrier family 7 member 5; TNF-alpha, Tumor necrosis factor alpha; UPR, unfold protein response

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