Leucine as a pharmaconutrient to prevent and treat sarcopenia and type 2 diabetes

Marika Leenders and Luc JC van Loon

Amino acids function as precursors for de novo protein synthesis. In addition, however, they play a key role as nutritional signals that regulate multiple cellular processes. There is ample in vitro and in vivo evidence showing that muscle tissue responds to increases in amino acid availability via signal transduction pathways that are also regulated by insulin, glucagon, growth hormone, and insulin growth factor 1. The increased amino acid availibility results in the upregulation of mRNA translation, thereby increasing muscle protein synthesis, which, in turn, leads to greater net muscle protein accretion. These findings have been particularly pronounced for the amino acid leucine. Furthermore, leucine has the ability to act as a strong insulin secretagogue. Consequently, it has been suggested that leucine represents an effective pharmaconutrient for the prevention and treatment of sarcopenia and type 2 diabetes. In accordance, recent in vivo studies in humans show that free leucine ingestion can reverse the blunted response of muscle protein synthesis to amino acid/protein intake in the elderly. Although short-term studies suggest that leucine supplementation can stimulate muscle mass accretion in the elderly, there are no long-term nutritional intervention studies to confirm this or the other proposed benefits of leucine as a pharmaconutrient.

INTRODUCTION

Besides their function as precursors for de novo protein synthesis, amino acids are also involved in regulating numerous cellular processes. The essential amino acids seem to play a key role in regulating the synthesis and breakdown of skeletal muscle protein. In vitro studies show that the branched-chain amino acids (BCAAs), i.e., leucine, isoleucine, and valine, act as potent nutritional signaling molecules that regulate the rate of protein synthesis and degradation. Leucine seems to represent a unique amino acid in this regard,[1–4] as it can stimulate mRNA translation initiation via insulin-dependent and -independent pathways, thereby stimulating muscle protein synthesis.[5] As a consequence, leucine has been identified as a pharmaconutrient with the potential to promote muscle hypertrophy. More detailed information on the impact of leucine on the activation of the mammalian target of rapamycin (mTOR) signaling pathway and the subsequent initiation of mRNA translation is provided later in this review.

Aging is accompanied by a progressive decline in muscle mass and strength, or sarcopenia. This loss of muscle mass and strength results in a decline in functional capacity and predisposes to the development of chronic metabolic diseases such as obesity and type 2 diabetes.[6] Recent work suggests that the elderly show a blunted response of muscle protein synthesis to food ingestion.[7–9] It has been suggested that the blunted response of muscle protein synthesis to food intake can be compensated for by increasing the leucine content of a meal.[8,10] Consequently, leucine supplementation might

Affiliations: *M Leenders* and *LJC van Loon* are with the Top Institute Food and Nutrition (TIFN), Wageningen, the Netherlands, and the Department of Human Movement Sciences, Nutrition and Toxicology Research Institute Maastricht (NUTRIM), Maastricht University Medical Centre, Maastricht, the Netherlands.

Correspondence: *LJC van Loon*, Department of Human Movement Sciences, Faculty of Health, Medicine and Life Sciences, Maastricht University Medical Centre, PO Box 616, 6200 MD Maastricht, the Netherlands. E-mail: l.vanloon@maastrichtuniversity.nl, Phone: +31-43-3881397, Fax: +31-43-3670972.

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represent an interesting approach to prevent or reverse the progressive loss of muscle mass that occurs with aging. Furthermore, leucine has also been identified as a potent insulin secretagogue when administered in combination with carbohydrate and protein. Co-ingestion of additional leucine increases postprandial insulin release and stimulates blood glucose disposal. Several studies support the hypothesis that administration of protein and additional leucine represents an effective dietary strategy to improve glycemic control in patients with type 2 diabetes.[11–16] This review article evaluates whether leucine represents an effective pharmaconutrient in the prevention and treatment of sarcopenia and type 2 diabetes. The impact of the BCCAs, and of leucine in particular, on muscle protein metabolism and endogenous insulin release are addressed with regard to the existing literature.

IN VITRO EVIDENCE OF THE ANABOLIC PROPERTIES OF LEUCINE

Amino acids function not only as building blocks for de novo protein synthesis; they also play a key role as nutritional signals that regulate multiple cellular processes.[17] In the 1970s, several laboratories performed in vitro studies to investigate the potential of amino acids to regulate muscle protein metabolism. Since then, numerous studies have reported that amino acids can stimulate muscle protein synthesis[18–21] and inhibit proteolysis.[20,22] Other studies further investigated the proposed anabolic properties of amino acids and observed that the BCAAs are mainly responsible for stimulating muscle protein synthesis and inhibiting protein degradation.[1,23] Classic studies by Buse and Reid[4] describe the incorporation of lysine into muscle protein in isolated rat diaphragms following administration of BCCAs. Rates of muscle protein synthesis were 20% greater in muscle tissue incubated with the BCAAs. After testing each of the BCAAs separately, it became evident that leucine was solely responsible for stimulating protein synthesis and reducing proteolysis. This has since been confirmed by several other groups.[1,4,23]

Much of the work in this area has focused on the molecular mechanisms that might be responsible for the leucine-induced stimulation of muscle protein synthesis. Acute changes in muscle protein synthesis generally occur well before increases in mRNA content become evident.[24] As such, activation of muscle protein synthesis must be controlled by a post-transcriptional mechanism.[25] The post-transcriptional regulation of protein synthesis involves mRNA translation, elongation, termination, and post-translational modification. The initiation of mRNA translation is thought to represent one of the more important levels of control of muscle protein

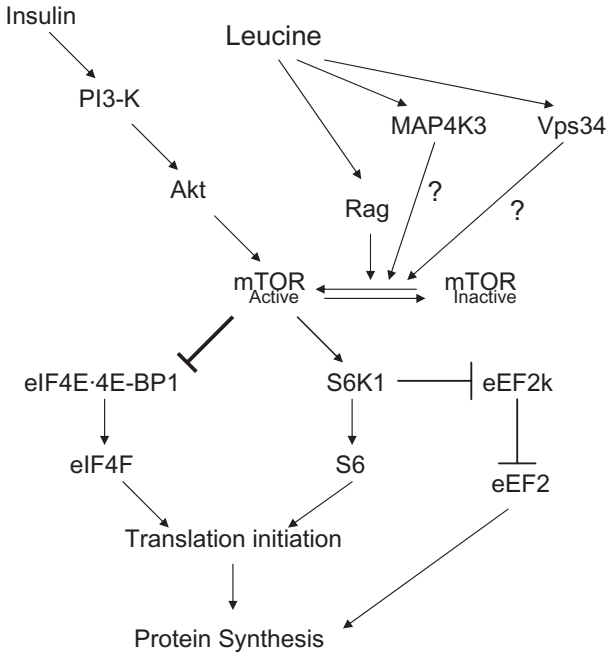

Figure 1 **Overview of the proposed mechanisms by which insulin and leucine can regulate protein metabolism by modulating the signal transduction pathways that regulate mRNA translation.** Besides the insulin-mediated signaling cascade that leads to activation of the mammalian target of rapamycin (mTOR) signal transduction pathway, leucine activates mTOR in an insulin-independent manner through Ras-related GTPase (Rag), Vps34, and MAP4K3. Activation of mTOR leads to an increase in phosphorylation of ribosomal protein S6 kinase 1 (S6K1) and the eukaryotic initiation factor 4E-binding protein (4E-BP1). Phosphorylation of 4E-BP1 prevents binding with eIF4E, thereby enhancing the assembly of the eIF4F complex. Both these processes initiate translation and stimulate protein synthesis. Leucine also has the potential to regulate mRNA translation through the phosphorylation of the eukaryotic elongation factor 2 (eEF2). *Abbreviations*: Akt, protein kinase B; eEF2k, eukaryotic elongation factor 2 kinase; PI3-K, phosphoinositide 3-kinase.

synthesis.[26,27] The mTOR signal transduction pathway plays a major role in regulating the initiation of mRNA translation.[27,28] Leucine stimulates the initiation of mRNA translation via activation of mTOR[27,29] and the subsequent phosphorylation and activation of eukaryotic initiation factor 4E-binding protein 1 (4E-BP1), ribosomal protein S6 kinase 1 (S6K1), and ribosomal protein S6, thereby increasing the rates of muscle protein synthesis (Figure 1). mTOR is thought to serve as a convergence point for leucine-mediated effects on the initiation of mRNA translation.[27,30]

Recent studies reveal that a small subfamily of GTPases, i.e., Rags, play an important role in the regulation of mTOR activation.[31,32] Rag GTP levels are upregu-

lated by leucine stimulation, suggesting that the Rag complex may function as a sensor for nutrients to modulate mTOR activity.[31,32] In addition to Rags, it is postulated that Vps34 (a PI3 kinase) and MAP4K3 (a germinal center kinase-related kinase) are involved in the regulation of mTOR activation in response to amino acids.[33–35] MacKenzie et al.[36] recently reported that mVps34 (a PI3 kinase) becomes phosphorylated when intramuscular leucine levels increase, which is followed by the phosphorylation and activation of mTOR.[36] Furthermore, in vitro work has identified MAP4K3 as a regulator of S6K and 4E-BP1 activity in response to leucine.[34] In addition to increased signaling through mTOR, an increase in essential amino acid availability results in a decreased phosphorylation state of the eukaryotic initiation factor 2.[37] This cascade also results in increased activation of translation initiation. An overview of the signaling pathways and the (un)known signaling properties of leucine are illustrated in Figure 1. Recently published reviews provide a more detailed description of the molecular pathways regulating muscle protein synthesis.[37–39] However, it should be acknowledged that a close association between the activation status of signaling proteins regulating mRNA translation initiation and the rates of muscle protein synthesis is not always evident in vivo.[40] Consequently, based on the proposed properties of leucine to stimulate protein synthesis in vitro, many research groups have since started to assess the impact of leucine administration on in vivo muscle protein synthesis and breakdown.

IN VIVO EFFECTS OF LEUCINE ADMINISTRATION IN RODENTS

Administration of BCAAs or leucine has the ability to stimulate protein synthesis and inhibit myocellular protein degradation in vitro.[1,3,23] So far, most in vivo animal models seem to confirm these findings.[18,41–45] Garlick and Grant[18] studied the effects of intravenous infusions with various combinations of insulin and amino acids on rates of muscle protein synthesis in rodents. They showed that infusing food-deprived rats with an amino acid mixture for 1 hour increased rates of muscle protein synthesis by 15%. A similar increase in rates of protein synthesis was observed when these food-deprived rats were infused with merely the amount of BCAAs present in the same amino acid mixture (9 mg leucine, 7.5 mg isoleucine, and 7.3 mg valine over 1 hour).[18] The specific relevance of the stimulatory properties of leucine became evident when Anthony et al.[41] reported that oral free leucine administration directly stimulated skeletal muscle protein synthesis during post-exercise recovery in rats. Exercised rats were fed leucine (270 mg) immediately after exercise, which resulted in a steep approximately 18% rise in the rates of muscle protein synthesis. In a subsequent study,[46] they tried to determine whether leucine is unique among the BCAAs to stimulate skeletal muscle protein synthesis. In this follow-up experiment, food-deprived rats received 1.35 g of valine, isoleucine, or leucine per kilogram body weight (providing approximately 270 mg of amino acid). Leucine was the only BCAA that stimulated in vivo skeletal muscle protein synthesis. Consequently, it was concluded that leucine is of key importance in regulating skeletal muscle protein synthesis.[46]

To establish the minimal dose of leucine required to stimulate muscle protein synthesis, Crozier et al.[42] measured the incorporation rate of [^{3}H]-labeled phenylalanine into muscle protein 30 minutes after oral administration of saline or leucine with doses ranging from 0.068 g to 1.35 g of leucine/kg body weight (providing from 14 mg to up to 270 mg). Even relatively small amounts of leucine (i.e., 0.135 g/kg body weight, or 27 mg) were shown to increase rates of muscle protein synthesis by as much as 30%.[42] Whereas some investigated the impact of the administration of free leucine, others tried to assess the anabolic properties of leucine when provided in combination with mixed meals. Dardevet et al.[43] reported that the addition of 445 mg leucine with a mixed meal further increased rates of postprandial muscle protein synthesis in vivo by as much as 19% in older rats. In an effort to examine whether the acute benefits of leucine supplementation on postprandial muscle protein anabolism persist when leucine is supplemented for more prolonged periods, Rieu et al.[44] provided these older rats with similar leucine-enriched meals over a 10-day period. Again, rates of muscle protein synthesis were considerably higher (+24%) in the rats fed the leucine-supplemented meals (providing 445 mg leucine per day). Moreover, the higher rates of postprandial muscle protein synthesis following ingestion of the leucine-enriched meals persisted over the subsequent 10 days. Thereafter, the same research group continued their work by assessing the anabolic properties of various dietary protein sources that differed in leucine content (providing from 250 mg to up to 300 mg per day) supplemented over a prolonged intervention period, i.e., a 30-day period.[45] In accordance with the results of free leucine feeding,[44] rates of postprandial muscle protein synthesis were approximately 25% greater following ingestion of the protein sources with the greater leucine content (i.e., 300 mg/day versus 250 mg/day). Furthermore, the greater response of protein synthesis persisted even after 30 days of intervention.[45] These studies by Rieu et al.[44,45] strongly suggest that the stimulatory properties of leucine ingestion, provided either as free leucine or via dietary protein with a higher leucine content, on postprandial muscle protein synthesis persist over time. As a

consequence, it was speculated that leucine supplementation with each main meal over a prolonged period of time represents an effective nutritional intervention strategy to stimulate net muscle protein accretion and to counteract muscle loss with aging. However, despite the observed increase in the rates of postprandial muscle protein synthesis, none of the studies detected increases in muscle mass or muscle strength.[44,45] This apparent discrepancy might be attributable to the relatively short timeline of these nutritional intervention studies, but it might also be due to the fact that the acute response of postprandial muscle protein synthesis does not necessarily translate into net muscle protein accretion. Other factors, such as changes in the hormonal milieu,[47] the activation, proliferation, and differentiation of satellite cells,[48] and neuromuscular alterations,[49] may all play an important role in regulating the more long-term increases in muscle mass accretion.

In short, there is ample evidence to support the claim that leucine administration represents an effective strategy to stimulate postprandial muscle protein synthesis in vivo in rodents. However, it is still unknown whether prolonged leucine supplementation can be applied effectively to augment skeletal muscle mass and strength. Evidence of this augmentation would further strengthen the proposal of leucine as an effective pharmaconutrient and could set the stage for leucine intervention studies in vivo in humans.

ANABOLIC PROPERTIES OF LEUCINE IN VIVO IN HUMANS

Although leucine administration has been shown to stimulate muscle protein synthesis and inhibit protein breakdown in in vitro[1,3,23] and in vivo studies in rodents,[18,41–46] in vivo studies in humans generally report inhibition of muscle protein breakdown following intravenous leucine administration, with no apparent effect on the rates of muscle protein synthesis.[50,51] Louard et al.[50] studied the impact of continuous intravenous BCAA administration on whole-body and skeletal muscle amino acid kinetics in healthy, young subjects aged 23 ± 1 years. Combining the use of the arteriovenous balance model across the forearm with the application of stable isotope tracers, i.e., labeled phenylalanine and leucine, they assessed the acute effects of BCAA infusion (providing 0.035 g valine, 0.040 g leucine, and 0.040 g isoleucine per kilogram body weight within a 3-hour period) on rates of muscle protein synthesis and breakdown. Whole-body phenylalanine flux, used as an index of proteolysis, was suppressed by approximately 40% following intravenous administration of BCAAs. However, administration of BCAAs did not increase rates of muscle protein synthesis.[50,52] Furthermore, Nair et al.[51] studied the impact of intravenous infusion of leucine (0.14 g/kg body weight over a 7-hour period) on rates of muscle protein synthesis and breakdown in postabsorptive, healthy subjects aged 25 ± 2 years. Arteriovenous differences across the leg showed that valine release and phenylalanine release were reduced, suggesting an approximate 35% decline in protein degradation following leucine administration. However, in accordance with Louard et al.,[50] intravenous administration of leucine did not seem to elevate the rates of muscle protein synthesis.[51] Collectively, these studies[50,51] do not report any effect of BCAA or leucine administration on rates of muscle protein synthesis in vivo in humans.

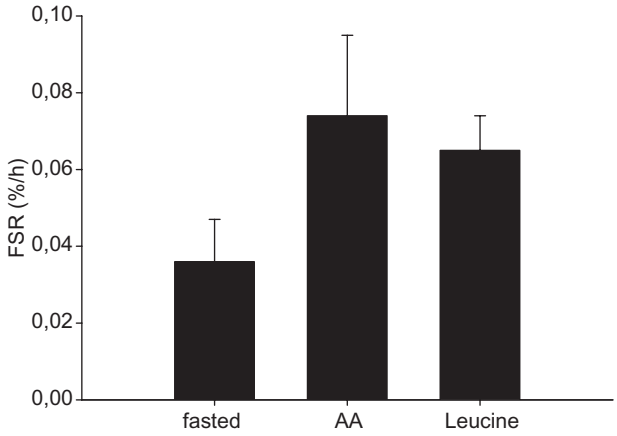

Figure 2 **Muscle protein fractional synthetic rates (FSR) in healthy subjects assessed under various conditions by Bennet et al.[53] and Smith et al.[54,55]** Fasted: fractional synthetic rates in the overnight fasted state.[54] AA: fractional synthetic rates following continuous infusion of 0.33 g/kg mixed amino acids.[53] Leucine: fractional synthetic rates following intravenous infusion of a flooding dose of 0.05 g/kg leucine.[55] Data expressed as means ± SD.

In contrast, other groups reported substantial increases (between 35% and 50%) in rates of muscle protein synthesis following intravenous administration of amino acid in vivo in humans.[53–55] An overview of some of these studies is provided in Figure 2. Bennet et al.[53] measured rates of muscle protein synthesis following continuous infusion of 0.33 g mixed amino acids/kg body weight, providing 0.032 g leucine/kg body weight. Rates of mixed muscle protein synthesis averaged 0.055 ± 0.008% per hour in the fasted state and increased by more than 35% during administration of amino acid (up to 0.074 ± 0.021% per hour). During administration of amino acid, the rates of whole-body protein synthesis increased by approximately 13%, and rates of whole-body protein breakdown declined by approximately 12%. These findings show that an increase in plasma amino acid availability can reverse the whole-body protein balance from negative to positive.[53] In line with the in

vivo data from rodent studies,[41–45] leucine seems to represent the essential amino acid with the greatest anabolic properties. Smith et al.[55] observed an approximate 50% increase in rates of muscle protein synthesis following administration of a large flooding dose of leucine (0.05 g/kg body weight). In fact, this response was not much different from the approximately 35% greater response of muscle protein synthesis after administration of 0.33 g mixed amino acids per kilogram body weight (providing 0.032 g leucine/kg body weight).[53] However, it should be noted that similar increases in rates of muscle protein synthesis were also reported following flooding doses (0.05 g/kg body weight) of other essential amino acids.[54] A more complete and detailed assessment of the stimulatory properties of various other amino acids on muscle protein synthesis is still lacking. The apparent discrepancy between the studies that did and did not observe the proposed stimulatory effects of intravenous leucine administration on the rates of muscle protein synthesis in vivo in humans might be attributable to the relatively large bolus of leucine that was administered over a short period of time[55] as opposed to the other studies that applied a more continuous infusion protocol over time.[50,51] More work is warranted to establish the specific relevance of a (more) rapid increase in free leucine concentration in plasma or tissue as an anabolic stimulus.

Besides studies that investigated the effects of intravenous administration of amino acid, numerous other studies have reported the stimulatory properties of ingestion of amino acid and protein on postprandial muscle protein synthesis.[8,10,15,19,20,50,51,56–59] Volpi et al.[60] reported an approximate 80% increase in the rates of muscle protein synthesis in vivo following ingestion of 40 g mixed amino acids.[60] They continued their work by studying the impact of ingesting a single bolus of essential amino acids (18 g) with or without an additional 22 g of nonessential amino acids on postprandial muscle protein synthesis.[56] As there was no difference in the anabolic response between treatments, the authors concluded that the essential amino acids are responsible for the observed increase in postprandial muscle protein synthesis.[56] These earlier findings show that the essential amino acids play a key role in the regulation and, more specifically, in the stimulation of postprandial muscle protein synthesis (Table 1).

Besides amino acid administration, muscle contraction, i.e., physical activity, strongly stimulates muscle protein synthesis.[61–65] Many studies addressed the impact of amino acid/protein administration as a nutritional strategy to further increase muscle protein accretion during and after exercise.[66–70] Physical activity and exercise not only increase rates of muscle protein synthesis but also increase rates of muscle protein breakdown, although the latter occurs to a lesser extent.[61,71] As a consequence, net muscle protein balance improves, but in the absence of nutrient intake, muscle protein balance will remain negative.[61] Protein and amino acid administration with and without carbohydrate strongly increases rates of mixed muscle protein synthesis (ranging between 35% and 65%) and improves net muscle protein balance both at rest[19,57,58] and during postexercise recovery.[72] The improved protein balance has been associated with an increase in intra- and extra-cellular leucine concentrations, now believed to form the main stimulus driving the postprandial response of muscle protein synthesis.[27] It has been speculated that ingestion of additional leucine during postexercise recovery could further accelerate postexercise rates of muscle protein synthesis. In accordance, Dreyer et al.[67] recently reported that ingestion of a leucine-enriched essential amino acid and carbohydrate mixture (providing 7 g leucine, 20 g essential amino acids, and 35 g carbohydrate) enhances mTOR signaling and increases muscle protein synthesis during postexercise recovery in vivo in humans. However, previous observations showed no additional value of additional leucine supplementation (0.18 g leucine/kg body weight or approximately 12.6 g over a 6-hour period) on the acute postexercise response of muscle protein synthesis in both young (20 ± 1 years)[57] and elderly (74 ± 1 years)[57,58] subjects when ample amounts of dietary protein were already ingested (72 g protein, providing 13.5 g protein-bound leucine; see Table 1). It may be speculated that additional leucine does not further increase postexercise muscle protein synthesis when ample amounts of leucine (>10 g) are already ingested. Furthermore, it is not unlikely that the stimulatory properties of physical activity, including muscle contraction and increased muscle perfusion, sensitize the muscle to such an extent that the anabolic stimulus of leucine is no longer of any additional value.

In summary, intravenous as well as oral administration of essential amino acids, and of leucine in particular, strongly stimulates postprandial rates of muscle protein synthesis in vivo in humans. Although the essential amino acids, and leucine in particular, seem essential in maximizing the postprandial response of muscle protein synthesis, there seems to be a limit to the surplus benefits of leucine when supplemented above a certain level or when additional leucine is provided during the initial stages of postexercise recovery.

LEUCINE AS A PHARMACONUTRIENT IN THE ELDERLY

One of the factors playing an important role in the loss of functional performance and, therefore, the capacity to maintain a healthy, active lifestyle is the progressive loss of skeletal muscle mass with aging. Lean muscle mass generally contributes up to approximately 50% of total

Table 1 **Effect of leucine on muscle protein synthesis and muscle mass accretion.**

Reference	Study participants	Exercise	Source of leucine	Corresponding leucine (g/day)	Duration	Outcomes
Acute leucine administration studies						
Volpi et al. (2003)[56]	14 elderly men and women (70 ± 2 years)	–	18 g EAA versus 40 g AA	3.2 g leucine 3.2 g leucine	3 h	↑ FSR in both groups. No significant difference
Koopman et al. (2006)[57]	8 elderly men (75 ± 1 years) 8 young men (20 ± 1 years)	30 min, moderate intensity	CHO + 71 g whey + leucine versus CHO	0 versus 13 g leucine	6 h	Protein balance is negative in CHO group. ↑ FSR in both groups for CHO + PRO + Leu. No significant difference between groups
Koopman et al. (2008)[58]	8 elderly men (73 ± 1 years)	30 min, moderate intensity	86 g whey protein versus 86 g whey protein + leucine	No extra leucine versus 12.78 g additional leucine	6 h	No additional effect of leucine on FSR
Paddon-Jones et al (2004)[19]	7 elderly men (67 ± 2 years) 6 young men (34 ± 4 years)	–	15 g EAA	2.8 g leucine	4 h	↑ FSR in both groups. No significant difference
Katsanos et al.(2005)[84]	11 elderly men (68 ± 2 years) 8 young men (31 ± 2 years)	–	6.7 g EAA	1.7 g leucine	3.5 h	6.7 g EAA results in diminished muscle protein accretion in elderly persons when compared with young persons
Katsanos et al. (2006)[8]	20 elderly men and women (66 ± 2 years)	–	6.7 g EAA, 26% leucine versus 41% leucine	1.7 g leucine versus 2.8 g leucine	3.5 h	Increased FSR only with 41% leucine (+ 47%)
Rieu et al. (2006)[10]	20 elderly men (70 ± 1 years)	–	Semiliquid diet versus semiliquid diet + leucine	No extra leucine versus 3.6 g additional leucine	5 h	↑ FSR (+ 56%) with extra leucine
Long-term intervention studies						
Bohrsheim et al. (2008)[90]	12 men and women (67 ± 6 years) with glucose intolerance	–	2 × 11 g EAA + arg (36% leucine) versus baseline	7.9 g leucine	4 months	↔ body weight, ↑ LBM (+1.3%), ↔ lean leg mass, ↔ fat mass ↑ leg strength (+ 22%), ↑ physical function (6–12%)
Dillon et al. (2009)[92]	14 elderly women (68 ± 2 years)	–	2 × 7.5 g EAA versus placebo	2.78 g leucine versus placebo	3 months	↑ Basal FSR (15%), ↔ body weight, ↑ LBM (+ 3.9%) ↔ leg strength
Verhoeven et al.(2009)[89]	30 elderly men (71 ± 4 years)	–	3 × 2.5 g leucine versus placebo	7.5 g leucine versus placebo	3 months	↔ LBM, ↔ fat mass, ↔ lean leg mass

Abbreviations: AA, amino acid; arg, arginine; CHO, carbohydrates; EAA, essential amino acids; FSR, fractional synthetic rate; LBM, lean body mass; Leu, leucine; PRO, protein, ↔, no change.

body weight in young adults but declines to approximately 25% when the age of 75–80 years is reached.[73,74] The loss of muscle mass is typically offset by gains in fat mass. The loss of muscle mass is most notable in the lower limb muscle groups, with the cross-sectional area of the vastus lateralis being reduced by as much as 40% between the ages of 20 and 80 years.[75] The age-related loss of skeletal muscle mass is facilitated by a combination of factors that include a less-than-optimal diet[76–78] and a sedentary lifestyle.[79] The decline in muscle tissue with aging must be attributed to a disruption in the regulation of skeletal muscle protein turnover, leading to a structural imbalance between muscle protein synthesis and protein degradation. Though some studies suggest that this is at least partly attributable to lower basal rates of muscle protein synthesis in senescent muscle,[64,74,80–82] other studies do not show any differences in basal rates of muscle protein synthesis between the young and the elderly.[7–9,19,60,83,84] As a consequence, many research groups have started to focus on potential age-related differences in the response of muscle protein synthesis to the main anabolic stimuli, i.e., dietary protein intake and physical activity. It has been hypothesized that skeletal muscle tissue is less responsive to the anabolic properties of dietary protein intake[9,85] and physical activity[86] in the elderly.

Cuthbertson et al.[9] assessed the response of muscle protein synthesis to the ingestion of different amounts of essential amino acids in both young (28 ± 6 years) and elderly (70 ± 6 years) men under conditions in which insulin was clamped at approximately 10 mIU/L following intravenous infusion of both (the somatostatin analog) octreotide and exogenous insulin. The elderly showed a blunted response of muscle protein synthesis to the ingestion of 10–20 g essential amino acids when compared with their younger controls. Rates of postprandial muscle protein synthesis increased threefold when compared with basal fasting levels in the young, whereas the increase was only twofold in the elderly. The latter finding implies the presence of some level of anabolic resistance to amino acid/protein intake in senescent muscle. However, these findings seem to be at odds with previous work in which similar amounts of amino acids were provided in a more physiological setting without modulating the postprandial endocrine response.[56] Volpi et al.[56] reported similar increments (circa 100% increase) in young (28 ± 2 years) and elderly (69 ± 2 years) subjects in rates of mixed muscle protein synthesis following ingestion of 40 g mixed amino acids or 18 g essential amino acids. In agreement, Paddon-Jones et al.[19] observed no significant differences between young (34 ± 4 years) and elderly (67 ± 2 years) subjects in rates of postprandial muscle protein synthesis following ingestion of 15 g essential amino acids (providing approximately 2.8 g leucine) (0.103 ± 0.011% per hour in young versus 0.088 ± 0.011% per hour in elderly subjects). More recently, no differences were observed in the postprandial response of muscle protein synthesis following the ingestion of 20 g or 35 g intrinsically labeled casein (providing approximately 1.7 g or 2.8 g leucine) between healthy young (<24 years) and elderly (>65 years) males (0.062 ± 0.007% per hour versus 0.056 ± 0.004% per hour and 0.063 ± 0.006% per hour versus 0.054 ± 0.004% per hour, respectively).[87,88]

So far, there is only one study that reports an impaired postprandial response of muscle protein synthesis in the elderly versus the young, without modulation of the associated endocrine response.[84] Katsanos et al.[84] reported an attenuated postprandial response of muscle protein synthesis following the ingestion of a small, meal-like amount of essential amino acids (6.7 g with a 26% leucine content, providing 1.7 g leucine) in the elderly (68 ± 2 years) versus the young (31 ± 2 years). As a consequence, it is now speculated that anabolic resistance in the elderly becomes relevant when small, meal-like amounts of protein are ingested. Interestingly, with leucine being of particular relevance to the stimulation of muscle protein synthesis, Katsanos et al.[8] demonstrated that the blunted response to amino acid ingestion in the elderly could be compensated for by increasing the leucine content of the amino acid mixture from 26% to 41% (from 1.7 g to 2.8 g leucine). The higher content stimulated postprandial muscle protein synthesis by approximately 20% in the elderly (66 ± 2 years) when compared with the postprandial response to the ingestion of the 26% leucine mixture, resulting in a response that no longer differed from that observed in the young (30 ± 2 years). The authors proposed that increasing the leucine content of a meal might represent an effective dietary strategy to normalize the response of muscle protein synthesis in the elderly. These observations are supported by Rieu et al.,[10] who evaluated the impact of meals enriched with leucine on muscle protein synthesis in the elderly (70 ± 1 years). Subjects received semiliquid meals administered over a 5-hour period, with 50 mL provided every 20 minutes. The leucine diet was supplemented with 0.052 g leucine per kilogram body weight (providing an additional ~3.6 g leucine compared with 2.2 g protein-bound leucine). Leucine supplementation increased the muscle protein fractional synthetic rate, measured at the end of the feeding period, from 0.053 ± 0.009% per hour in the control group to 0.083 ± 0.008% per hour in the leucine-supplemented group. The proposed anabolic properties of the leucine-supplemented diet were attributed to the approximately 130% increase in the leucine concentration in plasma, as only plasma-free leucine concentrations differed between groups.[10] These recent studies in humans strongly suggest that leucine supple-

mentation with each main meal might represent an effective nutritional strategy to improve skeletal muscle mass and function in the elderly.[89] However, such an effect will depend on the amount of leucine that is provided by the meal and the extent of additional supplementation. So far, there are no dose-effect studies that have particularly addressed the postprandial response of muscle protein synthesis to the ingestion of various amounts of leucine. However, the above-reported data suggest that approximately 3 g leucine is sufficient to maximize the postprandial response of muscle protein synthesis in the elderly. For an overview of the amounts of leucine that were provided in the key studies mentioned, see Table 1.

Long-term studies of leucine supplementation are warranted to address whether the proposed anabolic properties of leucine co-ingestion will translate into clinically relevant gains in muscle mass and strength in the elderly. So far, only a few studies have addressed the potential impact of prolonged leucine supplementation in the elderly.[89–92] Borsheim et al.[90] studied the effects of 16 weeks of essential amino acid supplementation (11 g essential amino acids containing 2.8 g leucine, twice daily) on muscle mass and strength in elderly subjects (approximately 67 years of age). They reported a 22.2 ± 6.1% increase in muscle strength. Dillon et al.[92] performed a similar study over 12 weeks, during which they supplemented healthy elderly women (approximately 68 years of age) with 15 g essential amino acids per day (providing 4.0 g leucine per day). After 12 weeks, lean mass had increased by approximately 4% (representing 1.7 kg lean tissue). As neither study reported data on total energy intake or habitual diet, it can only be speculated whether the benefits of essential amino acid supplementation were attributable to an increase in total amino acid/protein intake or to the anabolic properties of leucine or other specific essential amino acids.

A recent study investigated the impact of 3 months of leucine supplementation with each main meal on muscle mass and strength in healthy, elderly males (71 ± 4 years).[89] Thirty healthy men were randomly assigned to either placebo or leucine supplementation for a 12-week intervention period. Subjects were administered 2.5 g leucine (or placebo) with each main meal (3 times 2.5 g [7.5 g] leucine or placebo per day). No changes in skeletal muscle mass or strength were observed over time in either the leucine-supplemented or the placebo group. Extrapolation of the acute stimulatory properties of leucine ingestion (2.8 g) on postprandial muscle protein synthesis reported by Katsanos et al.[8] towards the impact of prolonged leucine supplementation with each main meal should translate into a gain of at least 1.7 kg lean muscle mass over a 3-month intervention period. However, no such changes in body composition or lean tissue mass were detected.[89] There is no clear explanation for the apparent discrepancy between the acute and more prolonged effects of leucine supplementation on muscle protein metabolism. It might be speculated that 3 months of leucine supplementation is insufficient to maximize the proposed benefits of prolonged leucine supplementation on muscle mass accretion. Furthermore, healthy elderly men who habitually consumed ample amounts of protein in their diet (approximately 1.0 g/kg/day, resulting in a leucine intake of 8–15 g per day) were selected. This might explain why a further increase in leucine intake did not result in net muscle mass accretion. However, in line with the in vivo studies in rodents, it should also be considered that a greater postprandial response of muscle protein synthesis does not necessarily translate into structural skeletal muscle hypertrophy during more prolonged intervention, as many other factors contribute to the regulation of muscle mass maintenance.[47–49] It could be speculated that long-term leucine supplementation is of greater clinical relevance in frail and malnourished elderly or in specific clinical subpopulations. In elderly patients with type 2 diabetes, a more rapid decline in muscle mass has been observed with aging. Because of its insulinotropic properties, leucine might be even more relevant as a pharmaconutrient in elderly patients with type 2 diabetes.

LEUCINE AS A PHARMACONUTRIENT IN PATIENTS WITH TYPE 2 DIABETES

Epidemiological studies and preliminary intervention studies showed that postprandial hyperglycemia represents a direct and independent risk factor for the development of cardiovascular disease.[93] Importantly, the rapid postprandial increase in blood glucose concentrations, or "hyperglycemic spikes," seem to be even more relevant to the onset of cardiovascular complications than merely elevated fasting plasma glucose levels.[94] The glycemic instability is a severely underestimated problem in patients with type 2 diabetes. Even in patients whose type 2 diabetes is well controlled with oral blood-glucose-lowering medication, hyperglycemia is still prevalent throughout the greater part of the day.[95,96] The capacity to maintain good glycemic control is generally evaluated on the basis of the glycemic and insulinemic response to the ingestion of a single bolus of carbohydrate.[97] However, it should be noted that carbohydrate is not the only macronutrient that strongly increases endogenous insulin release following food intake. Dietary protein and free amino acids can have strong insulinotropic effects, especially when co-ingested with carbohydrate.[98–100] In accordance, co-ingestion of protein plus leucine represents an effective nutritional strategy to strongly stimulate postprandial insulin release, augment blood glucose disposal,

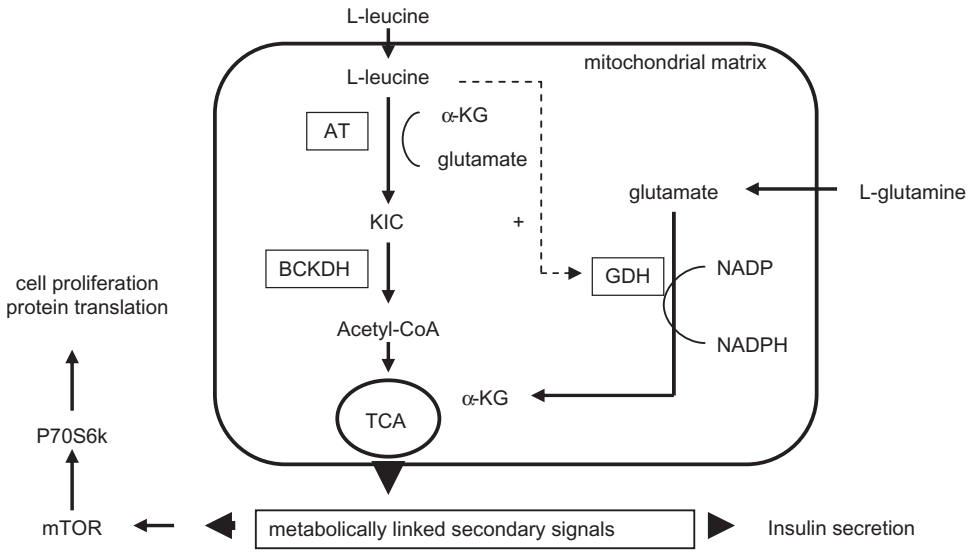

Figure 3 **Simplified overview of the mechanisms by which leucine stimulates insulin secretion in the pancreatic β-cell.** Leucine-induced insulin secretion is mediated by leucine's oxidative decarboxylation as well as by allosteric activation of glutamate dehydrogenase. Both the generation of acetyl coenzyme A (Acetyl-CoA) and α-ketoglutarate (α-KG) are needed for leucine to fully stimulate mitochondrial activity in the pancreatic β-cell. The metabolically linked secondary signals that subsequently lead to insulin exocytosis have yet to be established and seem responsible for the leucine-induced activation of the mammalian target of rapamycin (mTOR) signaling pathway. *Abbreviations:* α-KG, α-ketoglutarate; AT, aminotransferase; BCKDH, branched-chain α-keto-acid dehydryogenase; GDH, glutamate dehydrogenase; KIC, α-ketoisocaproate; P70S6k, P70S6 kinase; TCA, tricarboxylic acid cycle (adapted from van Loon et al.[132]).

and attenuate the postprandial rise in blood glucose concentration in patients with type 2 diabetes.[11,13,15] These findings imply that the insulin secretory capacity of the compromised β-cell remains highly functional when responding to stimuli other than glucose, like amino acids.

From both in vitro and in vivo studies in humans, it has become evident that leucine functions as a strong insulin secretagogue. Recent in vivo observations show a two- to four-fold increase in endogenous insulin release following ingestion of relatively small amounts of free leucine (3.75 g) with carbohydrate and protein.[72,101] Leucine stimulates insulin release in the pancreas via its mitochondrial oxidative decarboxylation as well as by allosterically activating glutamate dehydrogenase in the β-cell[102–104] (Figure 3). Besides the acute effects of leucine co-ingestion on β-cell function, Xu et al.[102] suggested that a more prolonged exposure to leucine might also contribute to enhanced β-cell function through improved maintenance of β-cell mass. In accordance, Zhang et al.[105] reported improvements in glycemic control by increases in insulin sensitivity (homeostasis model of insulin resistance [HOMA-IR] index was 50% lower) and approximately 50% lower glucagon levels following 14 weeks of leucine supplementation (via drinking water containing 1.5% leucine) in mice fed a high-fat diet. These findings support the hypothesis that leucine co-ingestion with each main meal, containing both carbohydrate and protein, might represent an effective nutritional strategy to increase postprandial insulin release and, as such, improve glycemic control.

Generally, improvements in postprandial blood glucose homeostasis are accompanied by improvements in the blood lipid profile.[105] Besides the improvements in glycemic control, Zhang et al.[105] reported a 27% decrease in plasma total cholesterol concentration and a 53% lower level of low-density lipoprotein cholesterol following 14 weeks of leucine supplementation in mice fed a high-fat diet.[105] Their data imply that leucine supplementation protects against the harmful effects of a high-fat diet, providing further support for leucine as a promising pharmaconutrient in the prevention and treatment of chronic metabolic disease. The proposed clinical benefits of leucine supplementation in reducing both hyperglycemia and hypercholesterolemia remain to be investigated in an in vivo setting in humans.

Besides disturbances in glucose homeostasis, there is a two- to three-fold greater risk of injurious falls[106] and physical disability[107–110] in elderly adults with type 2 diabetes. In accordance, elderly patients with type 2 diabetes generally show a more pronounced decline in skeletal muscle mass and strength when compared with age-matched, normoglycemic controls.[6] In accordance, Park et al.[6] showed that muscle quality, defined as muscle

strength per unit of regional muscle mass, is consistently lower in elderly adults with type 2 diabetes. In fact, leg muscle quality was reported to be approximately 8% lower in the elderly with diabetes compared with the normoglycemic elderly (14 ± 3 versus 15 ± 3 Nm/kg, respectively). In another study by Park et al., a more rapid decline in skeletal muscle mass was reported in patients with previously undiagnosed type 2 diabetes.[111] The mechanisms responsible for the accelerated loss of skeletal muscle mass in elderly patients with type 2 diabetes remain to be elucidated. It is likely that the metabolic abnormalities associated with the type 2 diabetic state impair muscle protein metabolism.[112] It could be speculated that the anabolic resistance of muscle protein synthesis to food intake[113,114] is even more pronounced in elderly patients with type 2 diabetes. Although it has been proposed that circulating insulin levels (above 15 μU/mL) are rather permissive instead of modulatory to allow muscle protein synthesis to be increased,[9,115] it seems evident that this might not be the case in an insulin-resistant state. Postprandial insulin release is severely blunted in patients with longstanding type 2 diabetes[116] and, combined with peripheral insulin resistance, might impair the postprandial response of muscle protein synthesis. This possible impairment of the postprandial response of muscle protein synthesis can be attributed to a reduced capacity of insulin to stimulate postprandial muscle perfusion,[117,118] thereby lowering amino acid delivery to the muscle[119–121] and attenuating myocellular anabolic signaling.[9,85,122] Consequently, increasing postprandial insulin release, e.g., by ingesting leucine (2–4 g) with a mixed meal, might represent an effective nutritional strategy to improve postprandial muscle protein synthesis and counteract the anabolic resistance to feeding in insulin-resistant muscle. Furthermore, the impact of greater postprandial insulin release might also inhibit muscle protein breakdown in the type 2 diabetic state. Though more research is warranted, it seems evident that nutritional strategies could be defined that will improve postprandial muscle protein synthesis in elderly patients with longstanding type 2 diabetes. Such an improvement might attenuate or reverse the accelerated loss of muscle mass and function in aging patients with type 2 diabetes.

CONCERNS OF USING LEUCINE

Despite the proposed benefits of leucine supplementation on muscle hypertrophy and glycemic control, the potential concerns about the use of leucine as a nutritional supplement must be considered. Leucine supplementation generally induces a decline in the plasma concentration of the other BCCAs, i.e., valine and isoleucine.[52] It has been suggested that such a decline might negate the anabolic properties of leucine administration.[123] However, it should be noted that lower plasma concentrations of valine and isoleucine are observed only in a postabsorptive resting condition and not in a postprandial situation, when large increases in the plasma concentrations of virtually all amino acids become apparent. Therefore, the stimulatory properties of the ingestion of leucine (2–3 g) with a mixed meal are unlikely to be attenuated by a relative lowering of the basal plasma concentration of valine or isoleucine. Furthermore, despite the observed decreases in basal fasting plasma concentrations of valine (approximately 10–20%) and isoleucine (approximately 0–10%), plasma levels still fall well within the normal physiological range. Consequently, there do not seem to be any major concerns associated with the lowering of the basal plasma concentration of valine or isoleucine following leucine supplementation with the main meals.

As leucine stimulates muscle hypertrophy, it has been questioned whether leucine could also have an unwanted impact on tumor growth. This is supported by the findings of Vogt,[124] who showed that signaling through the PI3-kinase and mTOR pathway is increased in some forms of cancer. In accordance, McNurlan et al.[125] reported a similar increase in the rates of fractional protein synthesis in both muscle and tumor tissue following amino acid infusion prior to colorectal tumor surgery. However, further increasing the fraction of BCCAs in the amino acid mixture did not result in a further increase in rates of protein synthesis. This suggests that further increasing the leucine content in the diet does not necessarily affect tumor growth.

The potential safety limits for (free) amino acid supplementation are subject to speculation. De Lorenzo et al.[126] provided 10 healthy males with 14.4 g/day BCAAs for 30 days, without reporting any side effects or adverse reactions. In agreement, Marchesini et al.[127] treated 20 patients with chronic hepatic encephalopathy for 6 months with an enteral supplement that provided 240 mg/kg/day BCAAs, without reporting any side effects or adverse reactions. Furthermore, patients with sepsis, stress, or injury have been treated with parental solutions containing up to 50% of the amino acid nitrogen as BCAA, without apparent side effects.[128] It seems that large dietary excess intake of an individual BCAA (>8 g/day) is well tolerated when consumed in a diet containing surfeit levels of protein and, therefore, the other BCAAs.[129] In agreement, based on the work to date, Matthews[52] concludes that leucine and the other BCAAs can be safely consumed in large amounts relative to the other amino acids in protein. Finally, it should be noted that the addition of free leucine in consumer food products can severely affect taste. Though this generally does not preclude the use of leucine-containing sports nutrition supplements in athletes seeking ergogenic benefits, it

might affect satiety and impair food intake in more compromised clinical subpopulations, where insufficient food intake generally constitutes a major problem.

FUTURE RESEARCH

Over the past decade, there has been an enormous gain in insight into the role of essential amino acids in regulating skeletal muscle protein synthesis and breakdown. However, there are still discrepancies between the results obtained in vivo in rodents and humans. In the in vivo rodent studies, an increase in the rates of postprandial muscle protein synthesis was observed, but not one of the studies referenced was able to detect increases in muscle mass or muscle strength.[44,45] So far, one human intervention study[92] has reported an increase in muscle mass (from 43.5 ± 2.8 to 45.2 ± 3.0 kg lean body mass), whereas another study[90] reported an increase in leg strength (from 127 ± 21 kg to 146 ± 19 kg, sum of individual knee extensors and flexors) following prolonged essential amino acid supplementation. It seems imperative to use the human model to explore the true functional role of leucine in the regulation of muscle metabolism in the elderly. There is ample evidence to support the ability of leucine to stimulate protein synthesis in rodents.[18,41–45] In accordance, there are also strong indications that leucine plays a key role in regulating muscle protein synthesis in vivo in humans.[8,10,55] However, apart from the observed increase in muscle protein synthesis following the use of the flooding dose technique with labeled amino acids,[55] there is limited evidence of the differential anabolic properties of the various amino acids in vivo in humans. Comparisons of leucine versus other (essential) amino acids using established methods for measuring muscle protein synthesis and breakdown are required to elucidate the acute anabolic effects of amino acid administration on muscle protein turnover. Furthermore, the use of leucine co-ingestion as a means to improve net muscle protein balance should be assessed in a more practical, postprandial condition.

Besides studying the acute effects of leucine administration on muscle protein metabolism, it is imperative to assess whether these proposed acute effects are maintained during more prolonged leucine supplementation protocols in vivo in humans. Furthermore, more long-term intervention studies are warranted to investigate whether the acute effects of leucine administration on muscle protein synthesis can be translated into measurable and clinically relevant increases in muscle mass, strength, and functional capacity. So far, there is little evidence to support the proposed clinical benefits of prolonged leucine supplementation in healthy elderly subjects.[89] It could be speculated that the clinical benefits of leucine supplementation are more relevant in more compromised elderly subpopulations. Research is warranted to assess whether leucine co-ingestion can promote postprandial muscle protein accretion under conditions in which food intake is suboptimal, i.e., in malnourished and/or frail elderly. Assessing the clinical benefits of prolonged leucine supplementation on preventing or attenuating the greater loss of muscle mass and strength in these clinical subpopulations could be of greater clinical relevance.

Due to its insulinotropic properties, leucine might represent an even more interesting pharmaconutrient for elderly patients with type 2 diabetes.[11,13,15] The greater postprandial insulin release would likely augment the protein synthesis response in insulin-resistant muscle[118,130] and also improve postprandial glucose and lipid handling. So far, there is little evidence to support the proposed beneficial effects of prolonged leucine supplementation on blood glucose levels and lipid profiles in rodents.[105] Both short- and long-term intervention studies are warranted to assess the efficacy of leucine as an effective pharmaconutrient in the prevention and treatment of type 2 diabetes in vivo in humans. Other clinical subpopulations that might benefit from leucine administration include patients with accelerated muscle wasting and cachexia, as occurs in cancer, chronic obstructive pulmonary disease, and intensive care unit (ICU) patients.[131] Whether acute leucine administration can effectively modulate muscle protein synthesis and proteolysis in these conditions remains to be established in vivo in humans. Subsequently, prolonged leucine supplementation studies should be performed to confirm the proposed anabolic properties of leucine in muscle-wasting disease and cachexia.

CONCLUSION

Leucine administration stimulates muscle protein synthesis and inhibits protein degradation via insulin-dependent and insulin-independent pathways. Recent studies report that increasing the leucine content of a meal to a level exceeding 3 g increases rates of postprandial muscle protein synthesis in vivo in elderly men, thereby normalizing the blunted response of muscle protein synthesis to food ingestion. Furthermore, due to its insulinotropic properties, free leucine (2–5 g) ingested with a mixed meal stimulates endogenous insulin release and attenuates the rise in postprandial blood glucose concentrations in patients with type 2 diabetes. Consequently, leucine supplementation has been suggested to represent an effective nutritional strategy to prevent and treat the loss of muscle mass with aging as well as to improve postprandial glycemic control in patients with type 2 diabetes. Though promising, there is no evidence that dietary supplementation with leucine can augment

muscle mass or strength or improve glycemic control. More prolonged nutritional intervention studies in vivo are warranted to assess the proposed clinical benefits of leucine supplementation in elderly individuals or in patients with chronic metabolic disease or muscle-wasting conditions.

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