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REVIEW ARTICLE

An overview of the therapeutic effects of leucine supplementation on skeletal muscle under atrophic conditions

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Abstract The characterization of the mechanisms underlying skeletal muscle atrophy under different conditions has been a constant focus of research. Among anti-atrophic therapies, amino acid supplementation, particularly with leucine, has received a lot of attention. Supplementation has been shown to have remarkable effects on muscle remodeling through protein turnover modulation. This may then impact physiological parameters related to muscle function, and even quality of life. In light of this, leucine supplementation could be a useful therapy for mitigating the atrophic effects of catabolic conditions. The purpose of this review is to present the major results of human studies evaluating the effects of leucine supplementation on structure and function of skeletal muscle in atrophic conditions such as muscle disuse, sarcopenia, and cancer.

Keywords Leucine · Branched-chain amino acids · Skeletal muscle · Atrophy · Disuse

Introduction

Organic homeostasis can be defined as the process by which a cell or an organism as a whole (e.g., the human body) maintains a stable internal environment. In homeostasis, inter-related mechanisms are responsible for the maintenance of many variables within certain limits that keep the organism alive. Metabolic stress, variations in body temperature, salinity, pH and nutrient concentrations are situations that are known to disrupt organic homeostasis. Such alterations in steady state can have both positive and negative effects and lead to adaptation (Macias 2004).

Skeletal muscle, like other organ systems, is in a constant balance between anabolism and catabolism, known as "skeletal muscle protein turnover". By definition, skeletal muscle protein turnover is the ratio between protein synthesis and degradation rates during a given period of time (Burd et al. 2009; Rodriguez et al. 2007; Rose and Richter 2009). Thus, protein turnover is neutral whenever the daily rates of protein synthesis and degradation are equal, which results in muscle mass maintenance. In contrast, protein turnover is positive: (a) when protein synthesis is higher than degradation or (b) when there is a reversal or attenuation of the degradation process without any change in the rate of synthesis. Negative protein degradation occurs: (a) when the protein degradation rate is greater than the rate of protein synthesis; (b) when synthesis is decreased without any change in degradation rate or (c) if there is a decline in protein synthesis only. In a healthy sedentary adult, we can assume that daily protein turnover is neutral since there are no disturbances in synthesis and degradation and food intake adequately meets physiological needs for muscle mass maintenance. By contrast, if the same individual is subjected to resistance exercise or if he/she consumes a supraphysiological amount of protein or amino acids protein turnover will be shifted from a neutral to a positive state (Burd et al. 2009; Phillips et al. 2009). Protein turnover will be negative under catabolic conditions (e.g., cancer, AIDS, sepsis, burns, disuse, aging, and the

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long-term use of glucocorticoids) (Lemoine et al. 2009; Tesch et al. 2008). Although all of these conditions are characterized by a negative balance between protein synthesis and degradation, it is important to note that each atrophic state has its own specific features, i.e., the specific mechanisms and pathways that lead to muscle mass loss are different.

There is currently no treatment for skeletal muscle atrophy that is totally safe and effective, so it is essential that new therapies are found. In fact, the number of scientific studies addressing this concern has increased over the last few years. The anti-catabolic effects of protein supplementation on skeletal muscle mass were shown in the study by Buse and Reid (1975). Because of its isolated action, leucine is considered not only a single amino acid that constitutes proteins, but also an amino acid with physio-pharmacological properties that can promote significant anti-catabolic effects. Specifically, it can cause attenuation of muscle protein catabolism (Zanchi et al. 2008). Leucine supplementation has consistently been shown to mitigate muscle loss when administered intravenously (Kee et al. 2003; Kobayashi et al. 2006; Louard et al. 1995; Nair et al. 1992; Tessari et al. 1987), when incubated with muscle tissue (Buse and Reid 1975; Busquets et al. 2000) and muscle cells (Nakashima et al. 2005), and when ingested through standard oral feeding (Anthony et al. 2000a, b; Combaret et al. 2005). Leucine has been recently demonstrated to possess unique features, as a highly selective amino acid that plays an important role in muscle remodeling. Supplementation might be effective in promoting muscle remodeling in some conditions, as is the case when it is combined with resistance exercise (Tipton et al. 2009). However, leucine supplementation may also act as a negative modulator of muscle mass as demonstrated during glucocorticoid treatment (Rieu et al. 2004). This selective characteristic underlines the fact that each catabolic state has different mechanisms that converge to cause skeletal muscle loss.

Thus, this review discusses the potential therapeutic effects of leucine supplementation on regulation of muscle remodeling in some atrophic states. There is a lack of consensus in the literature, thus an overview of the physiological responses to leucine supplementation in atrophic states could promote a better understanding of its potential therapeutic effects. The catabolic conditions addressed are the ones mainly discussed in the past years and represent a non pathological state (disuse, which takes a few days to occur), a pathological (cancer and cachexia, which take several weeks or months to occur) and a physiological one (aging, which takes years to occur). Although our main objective was to emphasize the clinical aspects of leucine supplementation on attenuation of muscle mass, this was not entirely possible since some atrophic models were mostly that were studied were developed in animals and primarily addressed cellular mechanisms. Additionally, discussion of the molecular mechanisms underlying the effects of supplementation was out of the scope of this review. However, for the reader's benefit, they were briefly addressed in some scenarios in order to facilitate a better understanding of the phenomenon.

Leucine as an anti-atrophic agent

Leucine can be considered a regulatory amino acid with unique characteristics. It plays several roles in muscle metabolism regulation, which include translational control of protein synthesis (Norton and Layman 2006) and glucose homeostasis (Nair et al. 1987). Biologically, the use of leucine as an anti-atrophic agent is justified based on evidence showing its ability to act as a critical regulator of the activity of a number of cytoplasmatic proteins that are involved in the initiation of translation of skeletal muscle protein synthesis and the insulin signaling pathway. Also, leucine has been demonstrated to be a nitrogen donor for the synthesis of muscle alanine and glutamine (Norton and Layman 2006). Additionally, there is evidence suggesting that leucine is able to interact with proteolytic machinery and attenuate skeletal muscle wasting. These results have been observed in both in vitro and in vivo studies, and this interaction is likely to occur in a dose-dependent manner (Zanchi et al. 2008). More specifically, some studies with experimental models have observed that muscle incubated with leucine at physiological and supraphysiological levels might have increased protein synthesis and attenuated protein degradation, respectively. The same effects are believed to occur in vivo as well, since incubation assays were performed to mimic leucine plasma concentrations. Recent studies have shown that protein-rich meals promote an increase of $\sim$0.2 mM in leucine plasma levels. Under normal conditions, this concentration is able to fully saturate the synthetic machinery (Bohe et al. 2003; Dardevet et al. 2000).

In humans, however, there are few studies evaluating the isolated effects of leucine supplementation on skeletal muscle mass in atrophic states, and whether or not a dose–response effect can be observed. Some studies have used different mixtures of essential and non-essential amino acids (e.g., whey protein) and the amount of leucine present in these mixtures, despite being significative, can elicit different response on muscle protein synthesis in comparison with the dose shown to yield this maximum effect (i.e., 0.135 g/kg/day) (Crozier et al. 2005). However, independently of the mixture used, it should be considered that the rise of leucine in the plasma compartment can be achieved by distinct routes of administration and factors

such as protein source and digestibility can influence it directly. Even though studies in vitro and in vivo involving animals produced interesting results, they may not be an appropriate model for studying human skeletal muscle protein metabolism, and they may not mimic the mechanisms underlying muscle wasting in humans. Hence, caution should be exercised when extrapolating results from these studies to humans. For example, both muscle protein synthesis and degradation rates are higher in rodents than in humans (Thomason et al. 1989; Waterlow et al. 1978) and the in vitro environment does not reproduce the real physiological conditions observed in vivo (Waterlow et al. 1978). Furthermore, humans appear to show more specific responses than rodents to atrophic mechanisms according to the catabolic state. This means that each atrophic state probably has distinct mechanisms for triggering the common final response of muscle loss (Table 1). Therefore, based on the above studies, it is important to consider that besides a possible dose–response effect, leucine supplementation might have other distinct effects on human skeletal muscle protein turnover.

Considering that leucine (Fajans et al. 1967) and its metabolite α-ketoisocaproate (Hutton et al. 1980) are potent stimulants of insulin secretion, leucine supplementation might not only have a direct effect but also an indirect effect on net protein synthesis by causing insulin release. However, this information should be carefully interpreted because there is come controversy about the direct effects of insulin on muscle protein accretion (Denne et al. 1991; Gelfand and Barrett 1987; Louard et al. 1992).

Leucine supplementation in atrophic states

Skeletal muscle disuse

Skeletal muscle disuse can be defined as the lack of movement and/or overload on a particular limb. In humans, the most common models for disuse are the absence of gravity, suspension, bed rest, and immobilization. The magnitude of muscle loss varies according to the experimental regimen applied. For lower limb muscles (particularly the quadriceps muscle) bed rest is the favored model as it induces the greatest loss of cross-sectional area (CSA) (Alkner and Tesch 2004; Andersen et al. 1999; Biolo et al. 2004; Brooks et al. 2008; de Boer et al. 2007; Ferrando et al. 1997; Paddon-Jones et al. 2004a, 2006; Trappe et al. 2007). Cast immobilization elicits a lower atrophic response when compared to bed rest (Carrithers et al. 2002; Gibson et al. 1987; Hespel et al. 2001; Hortobagyi et al. 2000; Jones et al. 2004, while limb suspension (Carrithers et al. 2002; de Boer et al. 2007; Trappe et al. 2002) and knee immobilization result in skeletal muscle atrophy somewhat lower than that observed in the first two conditions, respectively (Deschenes et al. 2002; Glover et al. 2008; Yasuda et al. 2005). Although the magnitude of muscle loss differs among these atrophic states, all of them lead to significant acute and chronic structural (contractile proteins) and functional (strength) deficits. Under these conditions, the skeletal muscle deficit is due to an imbalance between protein synthesis and degradation, even though each condition leads to the imbalance via unique pathways. In theory, skeletal muscle disuse can promote a negative protein turnover, not only by increasing protein degradation but also by decreasing protein synthesis (Phillips et al. 2009). Two recent studies from Phillips's group reported an increase in ubiquitin-conjugated proteins after 2 days of quadriceps immobilization (Glover et al. 2010) whilst no increase in polyubiquitinated proteins was seen after 14 days of immobilization (Glover et al. 2008). de Boer et al. (2007) also reported a significant rise in MuRF-1 and Atrogin-1/MAFBx gene expression after 10 days of unilateral lower limb suspension. His response, however, was blunted after 21 days of disuse. Together, these data suggest that proteolysis occurs in the early stage of muscle disuse. Then, it returns to baseline levels and afterwards a subsequent decrease in protein synthesis is observed. Recent studies have shown that a decrease in protein synthesis, without significant changes in protein degradation may be the major catabolic response in disuse-induced atrophy. This is believed to be the case since the human body continues to receive anabolic stimulus in the form of meals (postabsorptive and postprandial states) during the day, and skeletal muscle disuse does not increase protein degradation (Adams et al. 2003; Glover et al. 2008).

The phenomenon called "anabolic resistance" was recently described in the literature (Cuthbertson et al. 2006; Rennie et al. 2004). According to these authors, the anabolic response of disused skeletal muscle to exogenous nutrient stimulation, especially to protein and amino acids, is less sensitive than at the basal state. This results in lower protein accretion than is observed in a control (basal) situation as a result of feeding (Phillips 2004; Rennie et al. 2004). Basically, this phenomenon supports the hypothesis that the primary cause of skeletal muscle atrophy during catabolic states, including muscle disuse (Biolo et al. 2004; de Boer et al. 2007; Glover et al. 2008), is the decrease in protein synthesis. The study of Glover et al. (2008) demonstrated no impairment in intestinal absorption of amino acids during muscle disuse. Thus, despite the uptake of amino acids by skeletal muscle remaining unchanged, its responsiveness to amino acids, especially to leucine, is markedly decreased, characterizing a resistance of muscle to leucine (or leucine resistance). This suggests that skeletal muscle remodeling is sensitive to extracellular

Table 1 Human studies investigating the effects of leucine or amino acids mixture supplementation on atrophic conditions

Study	Experimental design	Supplementation protocol	Duration	Results
Skeletal muscle disuse				
Paddon-Jones et al. (2004a, b)	Randomized	15 g EAAs (3.1 g leucine)	28 days of bed rest	↑ PP FSR at day 28 compared to control group (∼70%); ↔ lean leg mass throughout bed rest; ↓ strength loss in supplemented group
Trappe et al. (2007)	N/R	3.6 g leucine + 1.8 g isoleucine + 1.8 g valine	60 days of bed rest at −6° of declination	↓ quadriceps muscle mass at 4% after leucine supplementation; ↓ food intake during bed rest
Glover et al. (2008)	Randomized	Acute infusion of a low (43 mg/kg/h) or high dose (261 mg/kg/h) of EAAs	Infusion after 14 days of immobilization	↓ PA MPS at 27% in immobilization; ↓ MPS in immobilized leg compared to non-immobilized leg after 4 h of EAAs infusion
Trappe et al. (2008)	N/R	3.6 g leucine + 1.8 g isoleucine + 1.8 g valine	60 days of bed rest at −6° of declination	No deleterious effects of leucine supplementation were observed
Cancer				
Daly et al. (1983)	Randomized; Blinded	25 or 45% BCAA of the total amino acids of isonitrogenous and isoproteic diets	8 days postoperative of intestine cancer	↑ nitrogen balance in 45% BCAA group
Tayek et al. (1986)	Randomized; Crossover	Parenteral nutrition formula containing 19 or 50% BCAA enriched		↑ total serum protein in both groups; ↑ 1.7 g of protein/kg of body weight in the rate of WBPS in 50% BCAA-enriched group
Meng et al. (1999)	Randomized	Normal Diet + AA mixture containing BCAA	12 weeks in cirrhotic patients	↓ hospital stay and bilirubin level; ↑ hemoglobin level, sodium level, albumin level
May et al. (2002)	Randomized; Double-blind; Nitrogen-controlled	AA mixture containing HMβ (3 g); L-arginine (14 g) and L-glutamine (14 g); AA mixture containing L-alanine (11 g), L-glutamic acid (1.75 g), L-glycine (6.10 g), and L-serine (4.22 g)	24 weeks	↑ FFM in the HMB/Arg/Gln-supplemented group that was maintained over 24 weeks
Poon et al. (2004)	Randomized	100 g AA mixture/day (4.0 g leucine, 3.8 g isoleucine, 3.2 g valine)	29 months	↓ morbidity (17.1 vs. 37.2%) and ↑ isometric maximal strength in BCAA supplemented group
Sarcopenia				
Paddon-Jones et al. (2004a, b)	N/R	15 g EAAs (2.79 g leucine through drink beverage)	Acute (Supplementation through 1 drink)	↑ muscle PA FSR by 0.04%
Katsanos et al. (2005)	Placebo-controlled	6.7 g EAAs (1.72 g leucine)	Acute (Supplementation through 1 meal)	↑ ∼22% in delivery of phenylalanine to the leg muscle after intake of EAAs.
Katsanos et al. (2006)	Randomized	6.7 g EAAs (1.72 or 2.79 g leucine)	Acute (Supplementation through 1 meal)	↑ ∼47% increase in muscle PA FSR after 41% Leucine-enriched meal
Rieu et al. (2006)	Nitrogen-controlled	0.052 g/kg leucine + 0.0116 g/kg isoleucine + 0.0068 g/kg valine or 0.071 g/kg Alanine as placebo	Acute (Supplementation through semi-liquid diet)	↑ FSR (∼55%) after 5 h of leucine-supplemented meals
Verhoeven et al. (2009)	Randomized; double-blind; placebo-controlled	7.5 g/day leucine or placebo through enriched meals	12 weeks	↓ Valine plasma concentration after leucine supplementation

BCAA branched-chain amino acids, *EAAs* essential amino acids, *FFM* fat-free mass, *FSR* fractional synthetic rate, *HMβ* β-hydoxy-β-methylbutyrate, *MPS* muscle protein synthesis, *PA* postabsorptive, *PP* postprandial, *WBPS* whole-body protein synthesis

concentrations of amino acids. Specifically, some studies postulate that there are membrane receptor(s) in skeletal muscle that are sensitive to leucine (Bohe et al. 2003). These membrane transceptors (transporters and receptors) may modulate proteins involved in intracellular signaling pathways (Akt, mTOR, Vps34, 4E-BP1, and eukaryotic initiation factors) (Hundal and Taylor 2009). If a membrane sensor that is specific to leucine really exists, skeletal muscle disuse would attenuate this atrophic response, even when the amino acid concentration is high, such as in the absence of mechanical stimuli and/or overload.

Recently, Glover et al. (2008) demonstrated that subjects immobilized for 14 days showed anabolic resistance to amino acids administered by intravenous infusion of low and high doses of essential amino acids at the end of the 14-day period. Although the measurement of this phenomenon was done using intravenous infusion methodology instead of orally, the use of amino acids as anti-atrophic tools has not been evaluated. The authors observed that only the quadriceps muscle had a lower anabolic responsiveness than the control (contralateral) limb to amino acids after 14 days of knee immobilization. In contrast, Paddon-Jones et al. (2004a, b) showed that supplementation with essential amino acids during 28 days of bed rest preserved skeletal muscle mass and significantly sustained the rate of myofibrillar protein synthesis. As described above, bed rest is the model that induces the greatest catabolic response. Therefore, the responsiveness of skeletal muscle to amino acids should be, at least theoretically, attenuated rather than preserved, thus demonstrating the anti-atrophic role of amino acids. Trappe et al. (2007) evaluated the effects of leucine supplementation, administered through enriched meals, on thigh and calf muscle mass in adult women subjected to bed rest at a six degree angle of inclination. The supplemented group received 3.6 g/day of leucine distributed in meals throughout the day for 60 days. Surprisingly, the group that received leucine supplements had a greater muscle mass loss ($\sim$4%) than the control group at the end of the protocol. The authors of this study concluded that if leucine has negative effects on skeletal muscle mass, these effects would be fiber-specific, and could possibly be a result of stimulation of protein catabolism after supplementation. While this was the only study that showed a catabolic effect of leucine ingestion on skeletal muscle, evidence consistently shows that leucine does not cause an increase in any proteolytic marker during disuse-induced muscle atrophy in humans (Glover et al. 2008). Other points that require further exploration are the differential responses to the time and the amount of leucine administered. Some studies have explored the relative (g/kg) amount of leucine needed to achieve the maximum stimulatory effect on protein synthesis [0.135 g/kg (Crozier et al. 2005)]. In the study by Trappe et al. (2007), subjects should ingest $\sim$8 g/day of leucine, an amount twice as high as the amount that was administered in their supplementation protocol. Another study evaluated the responsiveness of the soleus muscle to both bed rest and leucine supplementation (of note, the soleus is one of the most sensitive to bed rest-induced skeletal muscle atrophy) and found no structural changes due to leucine compared to the placebo group (Trappe et al. 2008). Corroborating these data, Paddon-Jones et al. (2004a, b) evaluated the same dose of leucine ($\sim$3 g/day), offered in an essential amino acids mixture during 28 days of bed rest. Instead of finding an impaired atrophic response, they found a significant increase in muscle protein synthesis (0.061%/h^{-1}) when compared to the placebo group.

Literature interprets the incomplete restoration of basal protein synthesis as "anabolic resistance", i.e., in skeletal muscle disuse, amino acids promote an increase in protein synthesis but do not restore it to the basal control levels. So, despite the anabolic response being less sensitive in disused muscle, amino acid stimulation as an anti-atrophic strategy can still attenuate skeletal muscle mass loss by continuously stimulating protein synthesis. Thus, leucine supplementation can both exert significant anti-atrophic therapeutic effects and interact with protein synthesis/degradation pathways. It is also important to note that the measurement of functional and clinical parameters, such as muscle strength, is necessary since muscle disuse leads to substantial deficits in these markers (Hespel et al. 2001; Hortobagyi et al. 2000; Rozier et al. 1979; Veldhuizen et al. 1993). There are very few human studies that look at the effects of leucine supplementation alone on functional parameters. Although the studies by Trappe et al. (2007) and Trappe et al. (2008) did not show improvements in isometric and dynamic strength with leucine supplementation compared to placebo, these results should be interpreted with some caution. This is because the absence of mechanical stimuli/overload, besides very detrimental to muscle function, is characterized by low neural activity (Kraemer and Ratamess 2004). Thus, amine compounds do not act directly on these parameters and any possible interaction between leucine supplementation and muscle function would be indirect. Specifically, leucine could contribute to the process by attenuating volume loss and consequently increasing muscle strength.

Data available in the literature so far are inconsistent, thus the effectiveness of leucine supplementation in different disuse models has not been proven. Based on some experimental evidence, it appears that leucine might have a positive effect on skeletal muscle mass in disuse-induced atrophic states. Although there is a study (Trappe et al. 2007) that shows that leucine increases skeletal muscle loss, these data were not replicable by the same research

group using the same experimental protocol (Trappe et al. 2008).

Cancer

Cachexia is observed in more than 50% of cancer patients (Wong et al. 2001) and ~45% of the cancer patients lose more than 10% of their prediagnostic weight (Argiles 2005). It has been associated with increased morbidity and mortality, poor quality of life, decreased responsiveness to chemotherapy, increased susceptibility to chemotherapy-associated toxic effects and a higher incidence of postsurgical complications (Argiles 2005). The most prominent characteristics of cachexia are the progressive loss of weight, normally accompanied by remarkable skeletal muscle wasting as well as by the atrophy of adipose tissue, and decreased plasma free amino acid concentrations (Norton et al. 1985). The most pronounced reductions in amino acids are observed in the branched-chain amino acids (BCAA) (Beck and Tisdale 1989). Cachectic patients may lose up to 75% of their total initial muscle mass (Wigmore et al. 1997) and 85% of their prediagnostic body fat (Fearon 1992). Cachexia-associated skeletal muscle atrophy seems to be caused by different mechanisms than other atrophic conditions involving both reduced protein synthesis and increased protein degradation. Control of the pathways for protein synthesis and breakdown may be altered by direct effects of the tumor cells on skeletal muscle and by an increase in plasma inflammatory mediators (e.g., TNF-α and IL-6). Plasma inflammatory mediators trigger signaling pathways that stimulate protein breakdown and inhibit protein synthesis (Argiles 2005). An increase in proteolysis-inducing factor (PIF), angiotensin II and reactive oxygen species (ROS) have also been observed in cancer patients (Rous and Kidd 1941).

This evidence has led researchers to believe that BCAA, especially leucine or its active metabolite HMβ (β-hydroxy-β-methyl-butyrate), supplementation could have beneficial effects on cachexia-related skeletal muscle wasting. In fact, several studies have supported this hypothesis in both animal models for cachexia (Baracos and Mackenzie 2006) and cancer patients (Poon et al. 2004; Tayek et al. 1986). Briefly, BCAA supplementation in cancer patients was demonstrated to increase whole-body protein synthesis (Tayek et al. 1986) and nitrogen balance (Tayek et al. 1986; Daly et al. 1983). It has also been shown to improve body composition (i.e., increase body weight and fat-free mass) (May et al. 2002) and reduce the number of days of hospitalization, and the associated risks of hospitalization (Poon et al. 2004). These parameters described above can be considered very relevant to the patient and the clinical prognosis of their disease.

Tayek et al. (1986) supplemented terminal adenocarcinoma patients with parenteral BCAA. The patients were divided into two groups, both of which consumed an isonitrogenous diet. In one group, 19% of the total amino acids were supplied as BCAA while the other group received 50% of their total amino acids as BCAA. Both groups had increased total serum protein, but the rate of whole-body protein synthesis was 1.7 g of protein/kg of body weight per day greater in the group consuming higher doses of BCAA than the group receiving the diet with 19% BCAA. Likewise, nitrogen balance was also improved in the group receiving high BCAA diet. Similarly, Daly et al. (1983) supplemented two groups of colon cancer patients, receiving isonitrogenous and isoproteic diets, with 25 and 45% of BCAA, respectively. The group ingesting a diet with 45% BCAA had a much greater increase in their nitrogen balance than the second group (-1.4 ± 7 g vs. -14.2 ± 4 g). According to these authors, the improvement in nitrogen balance was due to a higher BCAA uptake by skeletal muscle. The effects of BCAA supplementation on several clinical parameters have also been investigated. In a study done by Poon et al. (2004) on 88 cancer patients undergoing chemotherapy, subjects were divided into two groups: one group was supplemented with a solution containing 27 g of protein (3.2 g valine, 4.0 g leucine and 3.8 g isoleucine) while the control group received a standard diet. The group ingesting BCAA presented better clinical parameters (e.g., 20% less cancer-associated morbidity) than the control group. Importantly, the authors have shown an increase in isometric maximal strength, a relevant clinical marker of muscle function directly related with morbidity and mortality (Metter et al. 2002), in the group supplemented with BCAA after 3 and 6 months of treatment.

Further evidence of the positive effects of BCAA was found by May et al. (2002) who administered a solution combining the leucine metabolite HMβ (3 g/day) with L-arginine (14 g/day) and L-glutamine (14 g/day) to 32 cachectic patients divided into a control group (standardized diet only) and a supplemented group (standardized diet plus amino acids). The results showed that the supplemented group had an increase in body weight of ~1 kg in 4 weeks, while the control group had lost ~0.3 kg during the same period. This increase in body weight was accompanied by a slight increase in lean body mass by approximately 1.1 kg. Twenty-four weeks after the treatment was initiated, the same parameters were re-evaluated and the increase in body weight persisted in the supplemented group as did the decrease in the control group. However, it is not possible to affirm that HMβ per se was responsible for such effects in skeletal muscle, as the experimental design did not test its isolated effects. Nonetheless, May et al. speculate that the results observed

could be attributed to HMβ because it is strongly associated with improved muscle protein turnover. Indeed, more studies are needed to confirm this hypothesis.

The results of animal studies agree with these findings in cancer patients, providing more specific evidences on the protective effects of BCAA supplementation (particularly leucine and HMβ) on cachexia-induced muscle wasting According to data by Eley and Tisdale (2007), of the three BCAA, only leucine and valine were able to elicit a significant reduction in weight loss and protein degradation as well as increase in protein synthesis in rats with Walker 256 tumor. In addition, leucine was the only amino acid that promoted an increase in the weight of the soleus muscle (Eley and Tisdale 2007). Similarly, Gomes-Marcondes et al. (2003) demonstrated that addition of leucine (3% of the total dietary protein) to a diet caused attenuation of muscle mass loss in the gastrocnemius. Moreover, the study by Caperuto et al. (2007) showed that HMβ supplementation (320 mg/kg of body weight for 7 days) increased the survival time of rats with walker 256 tumors by up to 100% independent of the site of inoculation.

Considering the above discussed evidence from both human and animal studies, one can conclude that BCAA play an important role in attenuating muscle wasting in situations of severe catabolic stress, such as cancer-related cachexia. Of all the BCAA, leucine probably has the most anti-catabolic and pro-anabolic properties, and its effects are probably mediated by HMβ. Such effects make BCAA, leucine and HMβ potential therapeutic nutritional agents for the treatment of some of the most debilitating metabolic effects of cancer. Treatment with these agents may increase survival time, reduce length of hospital stay, and improve some physiological functions and quality of life. Additional controlled clinical trials involving a larger number of patients are needed to confirm the efficacy of BCAA supplementation for the treatment of cachexia. However, it should be noted that the number of studies on literature evaluating the role of BCAA and HMβ on counteracting muscle atrophy during cancer is still limited. Moreover, studies with cancer patients are also somewhat limited in the use of static and dynamic markers of muscle proteolysis. In view of this, though literature has been providing evidences for the positive effects of such strategies, caution should be exercised when considering its efficacy on muscle protein turnover regulators (Baracos and Mackenzie 2006).

Sarcopenia

The physiological modifications that accompany aging have been studied since the 1970s. The progressive loss of muscle mass, strength, and functionality, also known as sarcopenia, is one of the most frequently reported conditions. Sarcopenia is defined as a chronic and progressive phenomenon in which 3–8% of skeletal muscle is lost per decade beginning at age 30 (Paddon-Jones and Rasmussen 2009). According to Rosenberg (1989), no other progressive loss related to aging is more dramatic or has more impact on function than sarcopenia. Such a debilitating process may result in severe skeletal muscle atrophy, weakness, reduced mobility, reduced autonomy, and increased susceptibility to injuries (Fujita and Volpi 2006). Consequently, sarcopenia increases the rate of morbidity and mortality, and thus increases public health expenses (Sayer et al. 2008). In fact, sarcopenia is estimated to affect 30% of adults over age 60 and more than 50% of people over age 80 (Baumgartner et al. 1998). A variety of factors may be involved in the genesis of sarcopenia. Evidence shows that damage to muscle fibers, decreased neuromuscular function, decreased volitional physical activity, altered endocrine function and cellular apoptosis are major contributors to the initiation of sarcopenia (Doherty 2003; Dreyer and Volpi 2005; Marcell 2003).

Current strategies for attenuating sarcopenia are mostly based on nutritional supplementation and/or strength exercise (Thompson 2007). Some nutritional factors might lead to a reduced anabolic response in skeletal muscle during the human aging process. These factors include lowered protein intake, early satiety, reduced appetite, impaired mastication, altered digestion, altered absorption and peripheral resistance to amino acids (Rémond et al. 2007); (Paddon-Jones et al. 2008; Symons et al. 2007). In the elderly, the anabolic response to nutrients has been shown to be consistently lower than in younger adults, whilst a similar responsiveness of muscle protein synthesis is observed during fasting (i.e., without nutritional stimulation) (Balage et al. 2009). Therefore, anabolic resistance is believed to be the primary cause of muscle atrophy and sarcopenia.

Based on this, some studies have demonstrated that ingestion of adequate amounts of protein and amino acids by the elderly elicits a similar increase in postprandial muscle protein synthesis to that seen in young adults (Paddon-Jones et al. 2004b; Symons et al. 2007). According to data by Symons et al. (2007), ingestion of 113 g of high biological value protein (which can be obtained in a portion of lean meat) can release a sufficient amount of amino acids to increase the protein synthesis rate by 50%. This rate is similar in both young and elderly subjects.

Given that leucine has a well-known role in modulating muscle protein synthesis and degradation, some studies have explored the effects of leucine, combined with other amino acids or by itself, on skeletal muscle plasticity in the elderly. Recently, Verhoeven et al. (2009) did not demonstrate any effect of a 3 month leucine supplementation (7.5 g/kg) on

strength and muscle mass in the elderly. In these experiments, lipid profiles and glucose metabolism (assessed by oral glucose insulin sensitivity index, a homeostasis model assessment of insulin resistance and glycosylated hemoglobin) remained unchanged as well. Interestingly, the authors did not observe any significant increase in plasma leucine concentrations after supplementation, while plasma valine concentrations were lower in the supplemented group than in the placebo group. Leucine-induced imbalance in plasma valine concentration has already been related to body growth deficit (Harper et al. 1984). Thus, the lack of effect of leucine supplementation observed in this study might be attributed to the unchanged plasma leucine levels and to decreased plasma valine concentrations after supplementation protocol. Rieu et al. (2006) evaluated the effects of leucine-enriched meals (0.052 g/kg, twice the postprandial plasma leucine concentration) on protein synthesis and metabolism in the elderly. They showed that supplementation increased the rate of fractional myofibrillar protein synthesis in the treated group when compared to the placebo group. Of note, the diet of the treated group was also enriched with isoleucine (0.0016 g/kg) and valine (0.0068 g/kg) in order to avoid an imbalance in plasma BCAA levels, while the placebo group received an isonitrogenous diet enriched with alanine (0.071 g/kg).

Paddon-Jones et al. (2004b) reported that a single 15 g dose of essential amino acids (EAAs) containing 2.79 g of leucine significantly increased postprandial protein synthesis in both young and elderly subjects. On the other hand, data by Katsanos et al. (2005) indicated that a single dose of EAAs (6.7 g) did not stimulate protein synthesis in the elderly even thought stimulation was observed in young subjects. According to the authors, the most likely explanation for these findings is the low dose of leucine administered (1.72 g of leucine). In a later study, the same group (Katsanos et al. 2006) compared the effects of a single dose of EAAs (6.7 g) with different amounts of leucine (2.8 vs. 1.7 g of leucine) on postprandial protein synthesis in elderly subjects. In this second study, they demonstrated that the group ingesting the higher dose of leucine had a significant increase in protein synthesis, while the group ingesting the lower dose of leucine had no changes in protein synthesis, supporting the findings of the earlier study (Katsanos et al. 2005). These data show that leucine is a potential anti-atrophic stimulant for elderly subjects, and suggest that there is a minimal dose that exerts these effects.

Regarding skeletal muscle protein degradation Attaix et al. (2005) and Combaret et al. (2009) reported that leucine supplementation can return the postprandial inhibition of proteasome-dependent proteolysis, which is defective in aged muscle, to its normal rate. In the study by Combaret et al. (2005) with elderly rats, the ingestion of a single meal supplemented with 5% of leucine resulted in a complete restoration of the defective postprandial inhibition of proteasome-dependent proteolysis. In this same study, the authors also showed that overall in vitro muscle proteolysis was inhibited in the postprandial state in old animals chronically fed (10 days) with 5% leucine-enriched diet. Thus, studies in animal models provide evidences supporting that leucine can represent an interesting strategy against skeletal muscle proteolysis in aging.

In light of this evidence, one can conclude that leucine supplementation promotes a significant increase in muscle protein synthesis. Leucine supplementation is effective in the elderly, despite the increased resistance of skeletal muscle and decreased response to nutritional stimulation. There are no studies looking at the dose–response of leucine supplementation. Information from such studies would be helpful in determining the optimal dose that maximizes the anti-atrophic effects of leucine. Further studies should also determine the best combination of leucine and other amino acids in order to prevent amino acid imbalance. Finally, it is important to mention that recent evidence from animal studies suggest that the effects of leucine on skeletal muscle plasticity may be optimized if the oxidative and/or the inflammatory state that occurs with aging is attenuated or reversed. This suggests that a combination of leucine and anti-oxidant supplementation may have beneficial therapeutic effects in elderly people (Marzani et al. 2008). This, however, is yet to be investigated.

Adverse effects and tolerance to supplementation

Although there are many benefits to leucine supplementation, there is evidence that leucine can also have some adverse effects such as reduced food intake (Blouet et al. 2009) and antagonism (imbalance) of the other BCAA (isoleucine and valine) (Allen and Baker 1972). These effects are strongly related to the dose given as well as the time of consumption. Therefore, even in atrophic conditions where leucine has possible therapeutic benefits, adverse effects can also be expected depending on the protocol of supplementation.

Since the Michaelis constant (K_m) for amino acid uptake in the brain is low (Harper et al. 1984), the appetite suppressive effect is achieved by reducing the brain uptake of neutral amino acids and reducing production of dopamine and serotonin (Fernstrom 2005). Thus, the possible therapeutic effects of leucine in reducing muscle atrophy could be attenuated or even inhibited if supraphysiological agents are used, since there would be a reduction in food intake and consequently in the substrates required for skeletal muscle protein synthesis. There is also evidence that

excessive intake of a specific BCAA has antagonistic effects on others, possibly resulting in a reduction in plasma and muscle levels of some amino acids. However, unlike what is observed when an excess of isoleucine or valine is ingested, only leucine seems to have an effect regardless of diet composition (Harper et al. 1984). Harper et al. (1955) showed that leucine in excess had deleterious effects on the growth of young rats that were fed a low-protein diet, while an excess of isoleucine and valine only resulted in mild growth depression. Only when there were changes in dietary patterns did leucine prove to be a limiting factor. Thus, the excess of leucine requires higher amounts of isoleucine and valine in the diet. Since all of the evidences above discussed were found in studies with animals and because the dose of leucine necessary to elicit muscle responses in humans is quite smaller than the dose given to rats, caution should be exercised when extrapolating those findings to humans. In fact, in the study of Nguema et al. (2007), leucine supplementation has promoted no changes in food intake. Therefore, it is unlike that leucine supplementation promotes reduction in food intake because the dose able to increase plasma leucine concentration protein turnover is not supraphysiological and it normally varies around 1.5 times the amount of leucine consumed with diet.

One of the mechanisms by which excess leucine could promote antagonism of these two amino acids would be their use for protein synthesis. Leucine could prevent the use of isoleucine and valine for protein synthesis. However, Clark et al. (1968) compared changes in the human plasma amino acid pool after administration of leucine and found that concentrations of isoleucine and valine declined to a greater than would be expected if they were only being used for protein synthesis. Leucine as an insulin secretagogue, through leucine and α-ketoisocaproate, could exert modulatory effects on the plasma concentration of these amino acids. However, Pozefsky et al. (1969) demonstrated that intravenous infusion of 5.9 g of leucine in humans promoted a 25% increase in plasma insulin which is not enough to influence tissue exchange of amino acids. The transport of neutral amino acids also does not appear to be the major mechanism since it has been already demonstrated that rat tissue and plasma amino acid pools change similarly during leucine infusion (Harper et al. 1984). Therefore, the most likely mechanism responsible for this imbalance in isoleucine and valine plasma and tissue concentrations after the consumption of high doses of leucine is the stimulation of oxidation. Oxidation activates the enzyme branched-chain amino acid dehydrogenase (BCKDH) that is responsible for the oxidation of ketoacids produced by BCAA transamination through catalysis by the branched-chain amino acid transaminase (Block and Harper 1984; May et al. 1991).

Given these undesirable effects, it is important to discuss the tolerance of leucine supplementation. According to Baker (2005), most of available data on BCAA tolerance levels were obtained from animal studies while evidence in humans is yet to be established. While animal studies have yielded some important findings, changes observed in rodents due to BCAA supplementation may not reflect changes occurring in humans. For example in rats, isoleucine and valine requirements are higher than leucine requirements, while for humans, EAR (estimated average requirement) values for isoleucine and valine are much lower than leucine (Baker 2005). Also, the standard dietary guidelines, such as EAR (estimated average requirement) and RDA (recommended dietary allowance) might underestimate BCAA requirements because of some methodological issues [for details see (Baker 2005)]. A study by Riazi et al. (2003) demonstrated that BCAA requirements may be underestimated. This study showed, through an indicator of oxidation (L-[1-^{13}C]phenylalanine) that daily BCAA maintenance requirements for adults might be underestimated by approximately 110% in relation to EAR. Thus, it is not possible to assume that studies described above are using high doses of leucine since recommendations are underestimated.

Some human studies have reported no adverse effects of BCAA supplementation in both pathological (Brennan et al. 1986; Marchesini et al. 1990) and healthy conditions (Blomstrand et al. 1991; De Lorenzo et al. 2003). However, since there are few human studies exploring the tolerance to high doses of leucine intake, it is important to review the data obtained from animal models. Consumption of high doses of leucine or isoleucine (isolated), but not valine, causes growth depression in young rats (Allen and Baker 1972). This effect is potentiated by low-protein diets, especially in the case of supplementation with leucine, and attenuated or even annulled by adequate protein intake (Sauberlich 1961). Furthermore, diet composition plays a direct role in leucine tolerance as rats fed BCAA-rich diets had lower growth deficit (Tsubuku et al. 2004). It is tempting to assume that skeletal muscle mass would be subject to this same deleterious effect especially during atrophic states. However, growth deficit might be a consequence of BCAA-rich diets since the reduction in muscle concentration of isoleucine and valine may impair protein synthesis. Hence, even though high levels of BCAA and leucine alone are well tolerated, they must be accompanied by optimal levels of protein and other BCAA.

There are studies that have also described leucine as an insulin secretagogue (da Silva et al. 2009; Filiputti et al. 2010). Therefore, leucine supplementation could have significant impact on blood glucose and insulin concentrations. Some recent reports have characterized this leucine-induced modulation in glucose homeostasis in muscle cells

(Iwanaka et al. 2010; Tremblay et al. 2005). In humans, Tremblay et al. (2005) studied the short-term effect of low and high dose amino acids infusion on glucose homeostasis in the presence of low and postprandial-like peripheral hyperinsulinemia in skeletal muscle biopsies. After 360 min of high amino acids infusion, plasma BCAA concentration was significantly raised (~3-fold for leucine, ~2.5-fold for isoleucine, and ~2-fold for valine) while the same effect was not observed after low dose infusion. Plasma insulin was significantly raised in both groups and no differences were found between groups. At low peripheral hyperinsulinemia, amino acids infusion did not change endogenous glucose production; however, amino acid infusion increased the inhibitory insulin receptor substrate-1 phosphorylation (IRS-$1^{Ser312\ and\ Ser636/639}$) and decreased insulin-induced PI3k (phosphoinositide 3-kinase) activity and, consequently, muscle glucose uptake. Another study with humans demonstrated that elevation of plasma amino acids to postprandial concentrations (~4.6 mmol/l; 259 μmol/l of leucine) through an infusion protocol reduced the whole-body glucose disposal by 25% and the rate of muscle glycogen synthesis by 64%. Thus, the authors concluded that the elevation of plasma amino acids modulates peripheral insulin sensitivity in order to promote insulin resistance by direct inhibition of muscle glucose transport with subsequent reduction in rates of glycogen synthesis (Krebs et al. 2002).

An important consideration about these studies is that the route of administration was intravenous infusion. Thus, factors that are present after orally consumption such as bioavailability, digestion, and absorption rate do not play a pivotal role in this methodology. Nonetheless, since leucine has been considered an insulin secretagogue, its use in conditions such as diabetes and glucocorticoid treatment might be carefully examined.

Finally, it is important to consider that we still do not know when high doses of BCAA are ingested, how much of each individual BCAA (i.e., leucine, isoleucine, and valine) is absorbed in the intestine versus how much is converted to their respective ketoacids that are produced by transamination in the stomach. It is important to think of how efficiently these ketoacids can be used in humans (Baker 2005). Recent data indicate that enterocytes isolated from pigs' small intestines substantially degraded BCAA with extensive transamination and oxidation (Chen et al. 2009). Interestingly, in milk protein fed piglets, 32% of leucine is extracted by the portal-drained viscera in first-pass metabolism, with 21% of the extracted leucine being utilized for intestinal mucosal protein synthesis (Stoll et al. 1998). These results agree with another study that determined that 44% of total BCAA was extracted in first-pass splanchnic metabolism (Elango et al. 2002). Since BCAA catabolism in the liver appears limited, it is very likely that the small intestine plays an important role in regulating plasma BCAA concentrations in organisms (Harper et al. 1984).

Conclusions and perspectives

In summary, based on the data presented, leucine supplementation presents a safe and efficacious method for improving muscle remodeling in the atrophic states described above. Regarding safety, although leucine is a possible insulin secretagogue, this effect does not seem to be deleterious in conditions where there are no disturbances in glucose metabolism. On the other hand, in conditions such as insulin resistance, the use of leucine could play a negative role in glucose metabolism. Of note, the adverse effects of supplementation might be related to the specific use of leucine that. In some cases, supplementation may not induce a negative effect, but instead attenuate or inhibit a positive effect by causing plasma oxidation of other amino acids involved in protein synthesis.

The characterization of a possible dose–response effect in humans should be explored. Data describing and comparing the kinetics of leucine supplementation in atrophic states to the kinetics in healthy states are scarce and should be an important focus of new research. Still, the lack of control of some variables, such as protein-caloric consumption, intake of antioxidants (vitamins and minerals), combined with poorly characterized exercise protocols complicate the interpretation of such results. Thus, future studies in humans should control such variables so they can assess the possible effects of leucine supplementation alone and increase the external validity of the results.

Finally, although this review did not focus on elucidating the cellular mechanisms by which leucine modulates muscle protein turnover, it is important to point out that the "basic science" studies have shown significant results pointing to possible mechanisms. The characterization of genes and proteins modulated and expressed as direct or indirect targets of leucine supplementation in different atrophic states has aided in the identification of important cellular targets that could be the therapeutic focus for devising new treatments.

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