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review article

Can leucine supplementation attenuate muscle atrophy? A literature review

A suplementação de leucina pode atenuar a atrofia muscular? Uma revisão da literatura

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Abstract – Currently, there has been new expectations in studying strategies with the potential to mitigate the skeletal muscle atrophy that characterizes conditions such as aging, disuse, cancer, and the use of certain medications. Among them, amino acid leucine has received special attention due to its potential to stimulate specific pathways of protein synthesis in skeletal muscle. Due to the wide spread use of this amino acid by the media, several studies have been aimed at investigating the possible effectiveness of leucine against skeletal muscle atrophy. As a result, this literature review was aimed to analyze recent studies that investigated the effects of leucine supplementation on skeletal muscle atrophy in both humans and animals. Overall, the wide variations in the experimental designs developed, models studied, leucine dose, treatment duration and sample healthiness make it difficult for professionals and researchers to establish guidelines about possible therapeutic effectiveness of this nutritional strategy.

Key words: Dietary supplements; Leucine; Muscular atrophy.

Resumo – *Atualmente, surgem novas expectativas em estudar estratégias com o potencial de atenuar a atrofia muscular esquelética que caracteriza condições como o envelhecimento, o desuso, o câncer, assim como o uso de determinadas medicações. Dentre elas, o aminoácido leucina vem recebendo especial destaque devido ao seu potencial em estimular vias específicas de síntese proteica no músculo esquelético. Devido à grande disseminação do uso deste aminoácido pela mídia, diversas pesquisas vêm sendo desenvolvidas com foco na investigação de possível efetividade da leucina contra a atrofia muscular esquelética. Em virtude disso, essa revisão bibliográfica teve por objetivo analisar os estudos recentes que investigaram os efeitos da suplementação isolada de leucina sobre a atrofia muscular, tanto em humanos quanto em animais. De forma geral, as grandes variações nos desenhos experimentais desenvolvidos, nos modelos estudados, na dose de leucina empregada, na duração do protocolo de suplementação e na saudabilidade da amostra, fazem com que profissionais da área e pesquisadores sofram em poder estabelecer maiores diretrizes acerca da possível eficácia terapêutica desta estratégia nutricional.*

Palavras-chave: *Atrofia muscular; Leucina; Suplementos dietéticos; Uso terapêutico.*

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INTRODUCTION

Skeletal muscle is constantly balancing its anabolism and catabolism state. The balance between these two phenomena is known as protein turnover, i.e. the relationship between degradation level and protein synthesis during a given period[1]. When protein synthesis and degradation level are equivalent, the volume of protein turnover is considered neutral. In contrast, when protein synthesis is higher than the degradation level, the protein turnover can be considered as positive. Finally, the turnover is considered negative when there is a reduction in protein synthesis with or without change in the degradation rate and / or when protein degradation is greater than protein synthesis[2].

Protein turnover in muscle tissue is well characterized in some situations. For example, it is well known that nutrition, specifically, administration of amino acids, induces a sharp and positive protein turnover due to an increased protein synthesis rate[3]. Likewise, it is known that under certain conditions, such as sepsis and acute use of glucocorticoids induce a negative protein turnover[4,5]. Due to the obvious difficulty in performing long-term investigations evaluating changes that occur in protein turnover, it is believed that such changes result in acute chronic adaptations in muscle tissue. Therefore, researchers have speculated that muscle atrophy, which characterizes chronic conditions such as immobilization, cancer, various autoimmune diseases and aging, is the result of acute successive decreases of the protein synthesis rate and / or increased degradation[6,7]. Thus, some strategies aimed to mitigate or even prevent this acute negative protein turnover in such conditions have received special attention in recent years. One of the strategies that have gained prominence is leucine administration[8].

Leucine is an essential amino acid that comprises BCAA (branched-chain amino acids). It is known that it is an important nutrient involved in increasing insulin secretion in the pancreatic beta cells. Furthermore, this amino acid has the capacity to increase activation and expression of the mammalian target of rapamycin (mTOR) in various tissues, especially muscle; mTOR activation, in turn, is a key step in the process of protein synthesis[9] (see Figure 1). Based on the above, it has been consistently demonstrated in literature that leucine is effective to increase protein synthesis and decrease the degradation rate 1) in culture model of animal muscle cells[10]; 2) in muscle tissue samples incubated with this amino acid[11]; 3) via intravenous administration in humans[12-14]; and finally, 4) by means of oral supplementation both in animal models and humans[9,15-17]. Katsanos et al.[18], reported a significant increase in the protein synthesis rate of the skeletal muscle of 0.008% / hour up to 2 hours and half after oral ingestion of an amino acid mixture with high leucine content. When these findings are extrapolated in order to speculate the impact of extended leucine supplementation along with each main meal, this nutritional strategy should theoretically result in an increase in muscle mass of 3.4 kg (from 2.8 to 4.2 kg) over an intervention period of six months, highlighting the ergogenic potential of leucine. Similarly, these results also indicate leucine as

an attractive therapeutic tool in the attenuation of muscle atrophy during catabolic situations. Although results acutely observed have aroused interest from health researchers, it is noteworthy that long-term studies investigating the therapeutic efficacy of this nutritional strategy on maintaining muscle mass are scarce, and their results are controversial.

Thus, due to recent scientific advances investigating possible anti-catabolic effects of leucine, this is an opportune time to review and summarize the main findings in literature about the possible therapeutic effects of chronic leucine supplementation on muscle atrophy. Due to the natural overlap of experimental designs often observed in studies investigating the therapeutic potential of leucine supplementation, it was decided to include in this review only studies that have examined the chronic effect of leucine supplementation on muscle mass and studies that used this amino acid alone instead of the combination with other nutritional strategies. To this end, a search for articles in the following databases: Web of Science, PubMed and Google Scholar, was carried out in 2014. A wide variety of keywords were used to search for information, which included "muscle atrophy", "therapeutic nutrition", "nutritional supplementation", "nutrition and atrophy", "leucine intake" and " leucine supplementation".

EFFECTS OF ISOLATED LEUCINE SUPPLEMENTATION ON MUSCLE ATROPHY

As previously mentioned, recent *in vivo* results from acute studies in humans[16,18] suggest that leucine supplementation is an effective nutritional strategy to increase muscle mass in the elderly, especially when added at each main meal. However, interventions investigating the possible clinical benefits of prolonged leucine supplementation are scarce both in humans (see Table 1) as in animals (see Table 2).

Table 1. Effect of leucine supplementation alone on muscle atrophy in humans.

Study	Sample population	Submitted	Supplementation protocol	Duration	Results
Verhoeven et al.[19]	Healthy men (71 ± 4 years)	Healthy (with no cardiovascular disease or impairment in locomotor system)	2.5g leucine 3x day	3 months	↔ cross-sectional area of the quadriceps / ↔ muscle mass
Leenders et al.[20]	Elderly men (71 ± 1 years)	Type II Diabetes	2.5g leucine 3x day	6 months	↔ muscle mass
Casperson et al.[17]	Healthy men (68 ± 2 years)	Low calorie intake and protein intake	12g/day	2 weeks	Increased protein synthesis / ↔ muscle mass

In a double-blind randomized study, Verhoeven et al.[19] submitted healthy elderly men (71 ± 4 years) with no cardiovascular disease or impairment of the locomotor system to 3 months of leucine supplementation or placebo. Leucine dose used by the authors was 7.5g / day divided into 2.5 g during each main meal (breakfast, lunch and dinner). However, no effects of leucine supplementation on the cross-sectional area of quadriceps or lean mass were observed after 3 months of supplementation[19].

Table 2. Effect of leucine supplementation alone on muscle atrophy in rodents.

Study	Sample Population	Submitted	Supplementation Protocol	Duration	Results
Baptista et al.[30]	Male rats (2 months old)	Unilateral hindlimb immobilization for 7 days	2.7g/kg (gavage)	3 days before immobilization, followed by another 8 days	↑ muscle mass
Peters et al.[32]	CD2F1 Mice (6 to 7 weeks old)	inoculated with C26 adenocarcinoma cells	Standard diet with 8.7g leucine per gram of protein vs. standard diet with 9.6 g leucine per gram protein vs. standard diet with 14.8g leucine per gram of protein	21 days	↑ muscle mass
Zeanandin at al.[22]	Old rats (18 months old)	Healthy	Diet rich in leucine (4.5 %)	6 months	↔ muscle mass
Magne et al.[26]	Old rats (22-24 months old)	Immobilization of unilateral lower limb for 8 days	Control diet + 4.5% leucine	40 days	↔ muscle mass
Faure et al.[24]	Old rats (23 months old)	Dietary restriction	11.2g/kg/day leucine	3 months	↑ muscle mass
Baptista at al.[29]	Male rats (2 months old)	Unilateral hindlimb immobilization for 7 days	2.7g/kg (gavage)	3 days before immobilization, followed by another 8 days	↑ muscle mass
Pedroso et al.[25]	Male rats (2 months old)	Dietary restriction (30% of diet)	71.43g/kg/ day leucine	6 weeks	↑ muscle mass

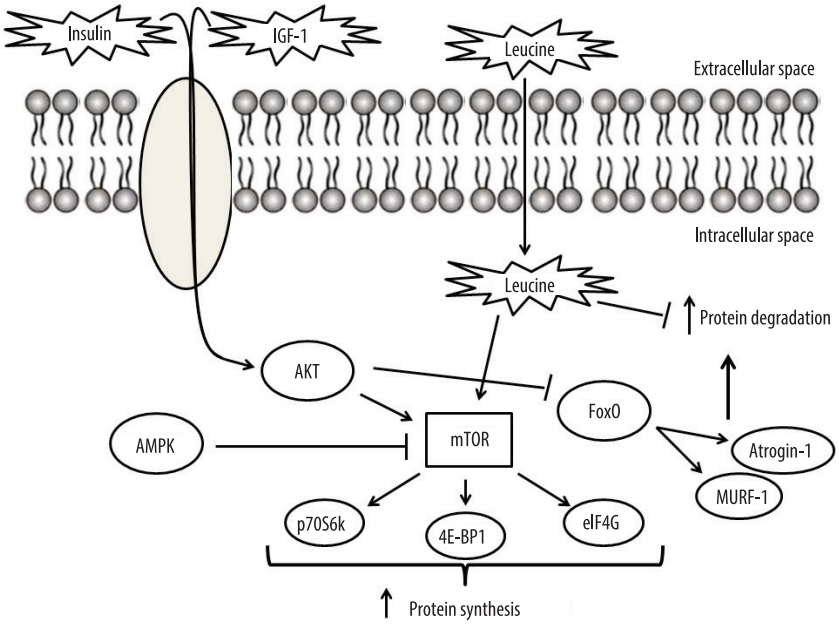

Figure 1. A brief illustration of the degradation processes and muscle protein synthesis and the effects of leucine on such processes. Legend: IGF-1 - Insulin-like Growth Factor 1; AKT - protein kinase B; AMPK - adenosine monophosphate protein kinase; mTOR - mammalian target of rapamycin; p70s6k - ribosomal protein 70 S6 kinase; 4E-BP1 - eukaryotic initiation factor of 4E binding protein; eIF4G - eukaryotic initiation factor 4G); FoxO - forkhead box protein; MURF-1 - Muscle RING-finger protein-1

Obviously, our results contrast with the hypothesis initially established in literature that the acute beneficial positive effects of leucine supplementation on protein synthesis can be translated into chronic benefits on muscle mass. However, it has been speculated that the absence of the expected

positive effects of leucine on muscle mass is related to the relatively short period of intervention or even the specific inclusion of healthy elderly men[19]. In this context, in a subsequent study[20] , the same research group investigated the effects of 6 months of leucine supplementation on muscle mass of older men (71 ± 1 years) with type II diabetes, which is a population known to have a pronounced decrease in muscle mass compared to normoglycemic controls matched for age[21]. Again, in a double-blind, randomized, placebo-controlled study, leucine doses of 7.5g / day subdivided during the three daily main meals were administered. However, as the main outcome, the authors showed that lean body mass did not increase or differ between groups supplemented with leucine and placebo after six months of supplementation.

In a study evaluating the therapeutic efficacy of leucine on muscle mass of human, Casperson et al.[17] submitted healthy elderly individuals with daily protein intake of 0.8 g / kg to 2 weeks of leucine supplementation (12 g / morning). Although the authors observed a significant increase in muscle protein synthesis rate in the post-absorptive and postprandial state after 2 week of leucine supplementation as well as mTOR phosphorylation, there were no significant changes in fat free mass of participants. This study, however, has clear limitations, among them the absence of randomization. Another important limitation, which includes studies previously mentioned with humans, is the inclusion of a limited and healthy cohort (as Verhoeven et al.[19]). Although Leenders et al.[20] have examined the effect of leucine supplementation on muscle mass of elderly type II diabetics, none of the participants was under sarcopenic or "really aggressive" conditions to the skeletal muscle such as chronic use of glucocorticoids, disuse (for example, immobilization) or degenerative or autoimmune diseases (such as cancer).

Some authors have used animal models in an attempt to explain the mechanisms behind the absence of positive results observed in studies with humans. As an example, Zeanandin et al.[22] investigated the effects of 6 months of *ad libitum* administration of a diet rich in leucine (4.5%) on the muscle mass of older mice (18 months old) and on the mTOR signaling pathway in the skeletal muscle of these animals. Similar to the results observed in studies with humans, the authors did not observe any changes in the muscle of animals treated with leucine-rich diet compared to animals treated with control diet. Unlike results previously observed, chronic supplementation with leucine induced a low effect on the activation of the mTOR pathway in skeletal muscle of animals. Thus, it is possible to speculate that the absence of increase in muscle mass with chronic leucine supplementation in studies discussed so far can be explained by a loss of leucine efficiency to stimulate postprandial protein synthesis in the skeletal muscle after a long period compared to acute supplementation. Another possible explanation for the absence of positive results with leucine supplementation is the fact that the effect of leucine is time-dependent, since this amino acid stimulates muscle protein synthesis during a short period of time (approximately 1 hour) after ingestion[23]. This hypothesis gains spe-

cial attention when considering that studies so far discussed in this review offered leucine in meals spaced throughout the day. Thus, in addition to using a high dose, an interesting strategy to observe the chronic effects of leucine on muscle mass would be offering it fractionally throughout the day. However, this hypothesis still needs to be tested in future studies.

Another possible factor explaining the absence of positive results of the effect of leucine supplementation on muscle mass, at least in studies with humans mentioned so far, would be the appropriate protein intake of participants, i.e., within daily recommendations (0.8 - 1.0 g / kg / day). Consequently, moderate or high daily protein intake may provide leucine enough to stimulate an appropriate protein synthesis rate in skeletal muscle by decreasing the "window action" of this amino acid. From this theoretical justification, some authors investigated the effect of leucine supplementation on muscle mass in specific conditions, such as during malnutrition or low daily protein intake. In this sense, Faure et al.[24] evaluated the effects of leucine supplementation on the muscle mass of old Sprague-Dawley rats (23 months old) submitted to three months of dietary restriction. Animals were acclimatized for 2 weeks, with dietary intake (grams of diet) being daily recorded. After acclimatization, a group of animals was submitted to dietary restriction, being deprived of 50% of their daily dietary intake for 3 months; another group was submitted to dietary restriction during the same period, being supplemented with leucine (11.2 g / kg / day) for additional 1 week; and a group kept their *ad libitum* diet during the experimental period. As expected, both groups of animals submitted to dietary restriction had a significant decrease in body weight compared to the group with *ad libitum* diet. However, the mass of the anterior tibialis muscle was significantly higher in the group of animals submitted to dietary restriction and leucine supplementation compared to the group submitted to dietary restriction only. Although without statistically significant difference, the contents of muscle proteins in this group of animals was 71% greater than the group submitted to dietary restriction and only 15% higher than the group with *ad libitum* diet, showing that leucine supplementation proved to be an effective strategy to increase muscle mass and muscle protein content in older rats submitted to dietary restriction. These results are consistent with a recent study published by Pedroso et al.[25], who found a decline of 16.5% in lean mass of young rats deprived of 50% of their daily dietary intake for 6 weeks. However, when rats were submitted to the same procedure but added of leucine supplementation (71.43 g / kg / day), lean mass loss of only 8.3% in relation to the initial weight was observed[25].

In order to examine whether an "aggressive condition" to skeletal muscle could influence the response of muscle mass to leucine supplementation, Magne et al.[26] assessed the effects of supplementation with this amino acid on the muscle mass recovery of older rats submitted to immobilization. To this end, male rats aged 22-24 months were submitted to unilateral leg immobilization for 8 days. Specifically, the paw of the animal was positioned in plant extension to induce maximum atrophy of the gastrocnemius and

soleus muscles[27,28]. For muscle recovery after immobilization, a period of 40 days was allowed, where half of the rats was fed with a control diet + alanine and the other half was fed with control diet + 4.5% of leucine supplementation. In order to increase the possibility of interventions, the authors also performed a pilot study in which another group of animals was submitted to the same immobilization procedure, but half of this new group of animals was supplemented with whey protein, while the other half received a high-protein diet after 8 days of immobilization. In short, the authors demonstrated that 8 days of immobilization induced a significant muscular atrophy of 20% in the gastrocnemius muscle. However, leucine supplementation for 40 days was not an effective strategy to improve the recovery of the gastrocnemius muscle mass compared to control diet + alanine. On the other hand, both high-protein diet and supplementation with whey protein proved to be effective in the recovery of the gastrocnemius muscle mass after immobilization, inducing a gain of approximately 60% of the total lost muscle mass, demonstrating that the administration of such strategies was more effective than leucine supplementation alone on muscle mass recovery after a short immobilization period[26].

Despite the lack of effectiveness of leucine supplementation in this initial study with immobilization, other studies have succeeded in observing positive results with the administration of this nutritional strategy for similar intervention period[29,30]. In fact, in two subsequent studies, male rats aged 8 weeks were submitted to unilateral leg immobilization for 7[29] and 8 days[30] in a very similar way to the study mentioned above[26]. In both studies, leucine was administered via gavage (2.7 g / kg / day) and started three days before immobilization in a group of animals, while the other second group was immobilized but received no nutritional intervention, and a third group was used as control. At the end of interventions, rats were euthanized and the soleus muscle and its mass were evaluated. Similarly to the study by Magne et al.[26], the immobilization protocol adopted in these studies induced a loss of soleus muscle mass of 29 [30] and 40%[29] compared to the respective control groups. However, in both studies, the authors found that leucine supplementation was able to mitigate loss of the soleus muscle mass. Given the similarity between the immobilization protocols of the above studies[29,30] and the study by Magne et al.[26], it is possible that the discrepancy between results is related to the form leucine was administered (gavage vs. diet), that is, ingestion of low fractionated doses vs. bolus. Studies with animal models have shown that acute leucine administration in a single bolus results in a greater acute increase in muscle protein synthesis rate compared to the administration of this nutrient in fractionated doses[31]. Further studies should be conducted to examine whether this significant difference observed between leucine administration protocols could result in long-term differences.

Another example of study aimed at evaluating the therapeutic efficacy of leucine supplementation on the muscle mass loss under conditions considerably "more aggressive" was the study by Peters et al.[32]. The authors evaluated whether there was a dose-response of leucine supplementation on the possible

attenuation of muscle atrophy in CD2F1 male mice aged 6-7 weeks subcutaneously inoculated with C26 adenocarcinoma cells. This animal model is well characterized as a method for the induction of colon cancer, cancer-related anorexia and consequent reduction of muscle and body mass[33,34]. After 1 week of acclimatization, the animals were immediately randomized based on body weight and divided into four groups: 1) control group (animals that were not inoculated with tumor and standard diet containing 8.7% leucine per gram of protein); 2) T group (inoculated with tumor and control diet); 3) T + BL group (control diet containing 9.6% leucine per gram of protein; low dose); and 4) T + AL group (control diet containing 14.8% leucine per gram of protein; high dose). Immediately after randomization, specific groups were inoculated with tumor and 21 days after inoculation, animals were euthanized. As main results, the authors found that the tumor inoculation significantly reduced the size and mass of gastrocnemius, tibialis anterior, extensor digitorum longus and soleus muscles in T, T + BL and T + AL groups compared to the control group. However, a tendency to attenuation of muscle atrophy of the gastrocnemius muscle was observed in T + BL group compared to the T group, while a statistical difference in this parameter was observed in T + AL group compared to the T group. A similar response was observed for the tibialis anterior muscle, with the low dose exhibiting a trend, and the high dose providing a significant attenuation of muscle atrophy. No effects of the low leucine dose were observed on muscular atrophy in the other two muscle groups (extensor digitorum longus and soleus muscles), while the high dose exhibited a tendency to attenuate muscle atrophy in these groups compared to the T group ($p = 0.07$ and 0.09, respectively). The data of this study suggest that under specific conditions and extremely detrimental to skeletal muscle, for example cancer, leucine supplementation appears to be effective in the attenuation of the muscular atrophy, and that this effectiveness can be increased if high doses of this amino acid are used. However, despite a statistically significant effect of this amino acid found in this study, the magnitude of this effect could be considered as low; for example, if we take into account only the mass of the gastrocnemius muscle, low and high doses of leucine induced attenuation of muscle atrophy compared to the inoculated group of approximately 7 and 9%, respectively. In addition, when studies are compared, it was observed that young rats were used for the experimental intervention, and the young age of animals may have been a factor that aided in the muscle mass recovery, since aging is known to be influenced by the anabolic resistance phenomenon[35]. In addition, the lack of muscle function tests in this study makes it doubtful whether the magnitude of the effect observed with leucine supplementation can be considered clinically relevant in terms of muscle mass preservation in cancer.

FINAL COMMENTS

In general, as observed along this review, studies evaluating the therapeutic efficacy of leucine supplementation alone on muscle atrophy do not allow

positive conclusion regarding the clinical efficacy of this amino acid. This is probably due to the great difference among experimental designs, leucine dose, supplementation duration and study models used.

Studies that have examined the effectiveness of this nutritional strategy in healthy sample populations or stable animal models, i.e. in situations where there is no great "stress" or imbalances in the homeostasis of the skeletal muscle did not reveal any beneficial effects of leucine supplementation. On the other hand, the few studies that have investigated the effects of this amino acid on muscle atrophy in situations "more aggressive to the skeletal muscle" such as energy restriction, immobilization and cancer, positive effects of leucine supplementation on the mitigation of muscle mass loss have been reported. Such studies, however, have been conducted in animal models; therefore, caution should be taken when extrapolating these results for the same conditions in humans. In addition, the low supplementation period used in these studies impairs verifying its effectiveness in the long term. Further studies with better internal control and prolonged duration should be carried out to investigate the effectiveness of leucine supplementation in other models also aggressive to the skeletal muscle, and if these results can be applied to humans.

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