

Leucine: Considerations about the Effects of Supplementation

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Abstract

The main objective of this study is the considerations about the Effects of Leucine Supplementation. Leucine is a branched chain amino acids (BCAA), which is characterized by the ability to be formed from a conformation where an R grouping is non-polar aliphatic. It is noticeable a steady increase in the use of nutritional supplements among physically active, justifying the need of studies to verify the possible benefits and risks that they may lead to health of individuals. In this aspect, this study aimed to highlight the main biochemical and functional characteristics of Leucine. In addition to analyze the possible effects of its supplementation in diets for weight loss on physical performance as well as its benefits to human health, with the largest number of studies that addressed the issue, both in humans and in animals. According to the bibliography found, the Leucine supplementation can influence the protein synthesis, thus contributing to the anabolism, in addition to having an important role in regulating glucose homeostasis aiding weight loss process. Leucine appears as an important supplement for health benefits as an adjunct in the treatment of insulin resistance and may assist in gaining muscle mass in the elderly, as well as contributing to recovery from injuries and wounds in the muscle tissue. It is still necessary more research into the potential health benefits in view that many of the articles analyzed were conducted in animals. It is noteworthy that most of the research conducted tests on animals, highlighting the importance of greater academic literature on this topic. Besides, most relevant studies to conclusions about the effect of intake of leucine in physical performance are also needed, as there are still many disagreements over the results of leucine supplementation on this aspect.

Introduction

The function of the branched chain amino acids (BCAA) are well studied and have played an important role in the central processing body fatigue during prolonged exercise in addition to many other physiological factors, the BCAA is composed of leucine, isoleucine and valine [1].

By the steady increase in the use of nutritional supplements meant for the physically active, it appears necessary to develop studies assessing the possible benefits and disadvantages of psychoactive substances. In humans considered healthy, nine are considered essential amino acids and must be consumed in the diet [2] where the branched chain amino acids are part of this essential group.

Evidence shows the key role of BCAA, especially leucine, in the regulation of anabolic processes involving both synthesis and muscle protein degradation [1,3,4]. Supplementation also leads to therapeutic effects, as reducing the loss of lean muscle mass during programs to reduce body mass, an improvement in muscle protein balance (protein turnover) in elderly subjects, an improvement in the cicatrization process, among other benefits [4-8].

Many controversies still exist about the quantities of consumption and its benefits, so this review study aims at showing the main biochemical and functional characteristics, beyond the mechanisms of action of leucine on muscle anabolism, the effects of supplementation on diets for weight loss, its possible effects on physical performance as well as its benefits to human health, with the largest number of studies that addressed the issue, both in humans and in animals

Biochemical and Functional Characteristics of Leucine

Amino acids are the protein subunits. What characterizes a molecule is that all amino acids have a carboxyl group and an amino group attached to the same carbon atom, and the differences between them are their side chains, which can also be called R group or radical [9].

The leucine is an amino acid which can be formed from a conformation where an R group is non-polar, aliphatic and hydrophobic. Therefore, leucine plays an important role in stabilizing the protein structure due to these hydrophobic interactions inside [9].

The BCAA are leucine, isoleucine and valine. Their concentration may vary according to the type of muscle fiber [3]. Through intake of food, especially protein based, on minimum recommended amounts, it is possible to provide a supply of BCAA, since they are found abundantly in the diet, corresponding to a percentage from 15 to 25% of ingested proteins [8].

BCAAs are the only amino acids which are not degraded in the liver suffering oxidation in muscle, adipose tissue, kidney and brain tissue, and soon after ingestion is already observed increase of their concentrations in blood plasma, muscle tissue and adipose tissue [8]. Of branched chain amino acids, leucine is the amino acid that most suffers oxidation in muscle tissue [2], so in this topic, will be analyzed the metabolic pathways of the leucine.

The amino acid oxidation may occur for three metabolic conditions: in the protein turnover, where amino acids released are rapidly degraded, during fasting periods or severe cases of diabetes mellitus, and finally in hyper protein diets, since free amino acids cannot be stored [9].

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In association with the increase in concentration of BCAA in the blood is also observed an increase in the rate of catabolism of these amino acids into muscle tissue. The degradation of these amino acids can provide carbon for use as a direct energy source, but also provides stimuli for the synthesis of alanine and glutamine that are released by muscles and undergoing enzymatic reactions, can be used as a substrate for gluconeogenesis in the liver [10]. This effect occurs in proportion to intake of BCAA, and ultimately helps control glucose homeostasis, due to association with glucose-alanine cycle [8].

Tayek and Katz [11] concluded in their study that the production of glucose from gluconeogenesis in the liver, act as an important factor in maintaining glucose homeostasis, especially during periods of fasting, because this metabolic process uses mainly amino acids as substrate. Besides, Hong and Layman (1984) in their studies on the effect of leucine supplementation on food restriction in rats concluded that the BCAA have potential to stimulate protein synthesis in skeletal muscles under conditions catabolism. Similar to what was found in the study of Anthony et al. [12] where it was analyzed the ability of leucine to act in muscle recovery after exercise in rats, their conclusions were that the intake of leucine stimulates protein synthesis after exercise and this occurs independently of plasma concentrations of insulin and glucose.

These studies demonstrate the great potential that supplementation with leucine and / or BCAA may carry, in exercise recovery and energy production for cells of the human body, which will be better described below.

Effects of leucine on Anabolism

The physical exercise results in changes in the metabolism of proteins and amino acids, being leucine the most affected amino acid in this process. The leucine can participate in the metabolism of several ways, among them: as a substrate for protein synthesis, such as a metabolic signal, and fuel (LAYMAN, 2002). During exercise, the extraction of BCAA from plasma circulation increases proportionally with exercise intensity [13]. At the beginning of activity, there is an increase in muscle production of alanine and as the intensity increases, the production of alanine stops and starts production of glutamine (Id, Id). In this situation it may be useful the use of leucine, its oxidation have the possibility of resulting in alanine that may be used in the glucose-alanine cycle [8].

The increase in muscle mass is influenced by numerous factors, but is closely related in rates of protein synthesis and degradation (protein turnover). The leucine supplementation has the ability to stimulate muscle synthesis synergistically of the production of insulin [2]. According Kimball and Jefferson [14] Insulin is an anabolic hormone that plays a key role in maintaining muscle protein synthesis, increasing the protein translation system, from this, it appears that insulin alone is not sufficient to stimulate muscle protein synthesis, so is required the ingestion of protein or amino acids for anabolism [3].

From this context, it is apparent that insulin signaling pathway is modulated by the presence of leucine, positively stimulating this pathway, regardless of insulin concentration [15]. Increased intracellular concentration of leucine, promotes the activation of a protein kinase called mTOR (mammalian Target of Rapamycin). The mTOR has the ability to detect changes in intracellular concentrations of leucine, stimulating the protein synthesis mainly through three key regulatory proteins: the ribosomal protein S6 kinase of 70 kDa (p70^{S6k}), Eukaryotic translation initiation factor 4 gamma 1 (4E-BP1), and eukaryotic initiation factor 4G (eIF4G) [16,17].

This increase in ribosomal protein S6 kinase 70 kDa (p70^{S6k}) concentration was confirmed in studies of Karlsson et al. [18], which examined the effects of resistance exercise associated with BCAA supplementation upon the phosphorylation of p70^{S6k} in skeletal muscle after exercise, the authors concluded that the resistance training associated with the intake of BCAA increased in 3.5 times the phosphorylation of p70^{S6k} during recovery, indicating a possible increase in the rate of muscle synthesis.

Koopman et al. [19] sought to determine the influence of the consumption of leucine on protein anabolism after exercise in men, concluding that the group that consumed leucine associated with carbohydrate and protein hydrolyzate have showed a greater increase in the rate of total body muscle synthesis when compared with the group that ingested carbohydrate alone, demonstrating that leucine supplementation can be an effective strategy in promoting protein synthesis after exercise. Similar results were found in the search Koopman et al. [20], which in this case worked with a sample of heterogeneous ages, concluding that the same effect occurs in older individuals.

Anthony et al. [12] studied the effect of leucine supplementation on skeletal muscle recovery after exercise in rats, concluding that leucine stimulates muscle protein synthesis after exercise, regardless of an increase in plasma insulin levels. However, according to Rogero and Tirapegui [3] and Golçalves [2], the stimulatory effects of leucine on muscle protein synthesis occur by mechanisms dependent on insulin and by the activation of mTOR. Thus, it appears that supplementation with leucine stimulates the processes of post-exercise recovery and of protein synthesis, inhibiting the sarcopenia. This occurs through activation of mTOR and also by other pathways such as insulin [2].

Effects of Amino Acid Supplementation in Diets for Weight Loss

According to the study of Layman et al. [8] comparing two hypo caloric diets, one with predominance of protein and another with predominance of carbohydrates, analyzing the loss of fat and lean mass for a period of 10 weeks, it was found that a diet with higher carbohydrate intake guarantee a contribution of approximately 5 g of leucine per day, while the diet with greater protein intake guarantee about 10 g of leucine per day (equivalent to 8% of total protein intake leucine). Both diets had the same caloric intake and proportion of lipids and fiber. The results obtained after the 10 week program were that the two groups had major weight loss, in the group that had more intake of protein where found greater fat loss reduction and greater decrease in levels of triacylglycerol (TAG), when compared to the other group, that had a higher insulin response accompanied a postprandial hypoglycemia.

These results corroborate those found by Layman et al. [21], which used low-calorie diets with the same caloric intake, one with predominance in protein and other with predominance of carbohydrates, as used in previous studies, but in this study was included a program of physical exercise to each test groups, to compare the influence of exercise in reducing body weight. The study concluded that the groups that performed the exercise program showed a greater weight loss, with a greater reduction of body fat and greater preservation of lean body mass, also checking better results in the group that performed the diet with the highest protein intake and exercise [21].

These results suggest that in a hypo caloric diet with high intake

of protein than carbohydrates occur a more expressive reduction in weight and a greater reduction in body fat, sparing the lean mass. The reasons for this greater reduction of weight and fat are still unclear [22], but appear to be related to the synergistic effect that leucine exerts on the regulatory factor of insulin, and its stimulatory effect of protein synthesis [7].

Other factors that may be associated with these results are the interactions of amino acids in carbohydrate metabolism, contributing directly to the glucose production via gluconeogenesis and the supply of substrate via glucose-alanine cycle [23]. With a higher protein intake, and thus BCAA, there is subsequent degradation of leucine via glucose-alanine synthesis pathway, resulting in the formation of alanine and glutamine, as when the plasma concentration of these amino acids increases, occurs also increases in its uptake by liver [24].

The degradation of amino acids can result substrates for other metabolic pathways such as gluconeogenesis, shortly, in diets high in protein and with low amounts of carbohydrates the body is forced to break down amino acid molecules to form substrates to result in a new molecule of glucose [9]. Therefore, the high consumption of carbohydrates in diet reduces the ability to produce glucose via gluconeogenesis using alanine as substrate, verifying a lower rate of blood glucose and higher plasma concentration of alanine, indicating an inhibition to their use as substrates for gluconeogenesis [8].

Most amino acids are degraded in the liver [9], which afforded possible theories that supplementation of amino acids associated with high protein diet might cause problems for the kidneys, liver and pancreas according to the procedures of the urea cycle [9].

However, according to research conducted by Bosch et al. [25] and Brändle et al. [26], protein intake in individuals without health problems, resulted in increased levels of creatinine clearance, indicating that the kidneys have an ability to increase their functional level according to the amount of ingested protein, confirming that the kidneys have a reserve of functional capacity. Bosch et al study [25] also concluded that in subjects with a reduced number of nephrons, or previous kidney problems, the reserve of the renal functional capacity may be reduced or absent, so for these individuals the recommendation of a highly protein diet may be inadvisable.

Effects on Physical Performance in Endurance Exercise

According to Rossi and Tirapegui [27], the ingestion of BCAA can result in changes in sports performance. Assuming that the use of supplements aimed at improving sports performance is increasingly common in athletes and physically active population, many studies have attempted to examine the possible benefits on performance brought by supplementation with BCAA and / or leucine, although many of their results are contradictory. Currently there are few scientific productions that directly examined the relationship between intake of leucine and physical performance, however many articles can be found reporting the influence of BCAA supplementation on performance.

As previously explained, leucine is an amino acid present in the composition of the BCAA, so consider this topic a few articles on the effect of BCAA supplementation on sport performance.

According to the study of Calders et al. [28], about the effect of supplementation with BCAA on endurance performance in rats, concluded that the group that ingested BCAA pre-exercise had a exhaustion time significantly higher than the group that did not ingest. From this study it was also possible to conclude that the glucose consumption associated with BCAA before exercise can eventually

inhibit the effect of BCAA. Very similar results was found in earlier study by Calders et al. [29], where not only the mice who consumed pre-exercise BCAA had a significantly longer time to exhaustion, this group also showed levels of ammonia in the blood much higher than the control group, probably related to the catabolism of amino acids ingested by the process of excretion of ammonia.

Another study that reached similar conclusions was Mittleman et al. [30], which examined the effect of supplementation with BCAA during the practice of endurance exercise in the heat, in men and women. Their conclusions are that the group that consumed BCAA during the exercise presented an exhaustion time longer for both, men and women, which also caused a higher concentration of BCAA in blood plasma, as well as a reduction in the levels of tryptophan, which are related of central fatigue retardation, where an increase of BCAA in blood plasma prevents the elevation of tryptophan levels, because these amino acids are transported by the same carrier system [31].

Knowing that a high concentration of the serotonin neurotransmitter (5-HT), on certain areas of the brain may contribute to the onset of central fatigue during prolonged exercise, and that increase may be triggered by higher concentrations of tryptophan, Hassmén et al. [32] sought to examine whether supplementation with BCAA would have some effect on cognitive performance in several tests performed during a cross-country race of 30 km, since this cognitive performance could be affected by central fatigue. The author concluded that the group that consumed BCAA during exercise had a better performance on cognitive tests, and showed a slight modification of mood changes induced by exercise, which may be associated with a lower central fatigue.

Similar results were found in searches of Strüder et al. [33], concluding that the groups of runners who ingested BCAA showed better results in psychometric tests after physical effort, although none of the authors have founded differences in physical performance among experienced runners. However the study of Blomstrand et al. [34], who analyzed if the consumption of BCAA could increase physical performance during a marathon, it was found in the group of the slower runners an improvement in their times, however this improvement did not occur in the group of experienced runners. This effect on the performance of less experienced runners was also observed in the results of Haraguchi et al. [35], who analyzed the performance of amateur runners in a race of 10 km, concluding that the group that conducted the intake of BCAA before the race showed better times, statistically significant than the control group.

However, some research results show disagreement. In studies of Uchida et al. [36] and Van Hall et al. [37], the authors sought to analyze if the consumption of BCAA could somehow influence the performance of resistance exercise. They concluded that no significant differences were found in time of exhaustion analyzed, which means that the consumption of BCAA did not affect the performance in tests. When the intake of BCAA is associated with carbohydrate intake, also no additional improvements were observed in physical performance, only the improvements provided by carbohydrate intake, indicating that the consumption of BCAA does not entail improvements in performance [38].

Beyond those studies on BCAA supplementation, a study of leucine supplementation, which showed positive results, was also analyzed. The study of Crowe et al. [39], about the effects of leucine supplementation for six weeks in athletes of canoeing, resulted in an apparent increase in strength of the upper body, as well as an increase in time to exhaustion

on a rowing protocol, and even a decrease in RPE (Rating of Perceived Exertion) media was observed when compared with the control group.

Others Benefits to Human Health

During aging is natural that a decline in skeletal muscle mass occurs. This atrophy is associated with a loss of muscle strength, which directly affects the mobility and health of the elderly. It's believed that this occurs due to an imbalance between the rates of synthesis and degradation of proteins, due to a decrease in the ability of muscle to respond appropriately to food ingestion [40].

In his study about the effects of leucine supplementation in rats, Daredevil et al. (2002) concluded that ingestion of leucine was sufficient to restore postprandial stimulation of muscle protein synthesis in aged rats, which means that protein synthesis was similar to that observed in adult rats. This protein synthesis in elderly rats was not observed in the study Rieu et al. [41], who performed the leucine supplementation for a period of 10 days, did not observed this postprandial stimulation of protein synthesis. However, in the research of Rieu et al. [42] performed in humans, it was found that supplementation with leucine did not affect the kinematics of the protein in the body as a whole, but were observed after the ingestion on the diet a large increase in the fractional rate of protein synthesis, thus concluding that leucine supplementation during feeding improves muscle protein synthesis in the elderly. These results were not observed in studies of elderly patients with diabetes mellitus type II [43].

Macotela et al. [6] analyzed the possible benefits of a diet rich in leucine consumption, since it can be used as a modifier of insulin resistance in multiple levels of metabolism. The study was conducted with rats consuming four different types of diet (normal diet, normal diet with leucine supplementation, a high fat diet, and high fat diet supplemented with leucine). The rats that consumed the two high fat diets presented similar increase in body weight and body fat, however, the rats that ingested a high fat diet with supplementation of leucine had a lower increase in subcutaneous fat than the group of rats who ingested a high fat diet without leucine supplementation. Furthermore, the high fat diet with leucine supplementation group did not increase liver weight, beyond reverse many of the metabolic abnormalities that were found only in the rats that consumed the a high fat diet. Were still observed in the group that was leucine supplementation had a clear improvement in glucose tolerance and insulin signaling. These data suggest that supplementation with leucine can act as a support in the treatment of insulin resistance related with obesity and therefore with the increase of cases of type II diabetes mellitus.

Similar results were found by Zhang et al. [44] who concluded that rats who consumed a high fat diet associated with a leucine supplementation showed a reduction in weight gain of 32% and a decrease of 25% in levels of adiposity. There was also a prevention of hypoglycemia caused by the high fat diet, as well as a decrease in total cholesterol and LDL levels (27% and 53% respectively) when compared to the a high fat diet without leucine supplementation.

From this assumption Torres-Leal et al. [45] sought to elucidate the effect of leucine supplementation on glycemic control and insulin resistance, concluding that the administration of leucine may be beneficial, but the period and concentrations to be used are fundamental parameters for the control of glucose homeostasis. Therefore, this approach can be a useful tool for the treatment of obesity and type II diabetes mellitus [46,47].

Another possible benefit that may lead of leucine supplementation

is related to the process of muscle anabolism in skin wounds and muscle injuries, as shown in the article by Zhang et al. [48]. In this article it was concluded that associating leucine supplementation with adequate intake of other amino acids, the anabolic effect may contribute to an improvement of skin lesions in rabbits.

Conclusions

The leucine supplementation could influence the protein synthesis and consequently contribute to anabolism, these stimulatory effects may occur by mechanisms insulin-dependent, or by the activation of mTOR which occurs independently of insulin.

There was evidence that the leucine plays an important role in the metabolic regulation, such as maintenance of glucose homeostasis, and its role as a supplier of substrate for the glucose-alanine cycle regulation and protein synthesis in muscle tissues, which may influence the process of weight loss.

Regarding physical performance, there are still many disagreements about the properties of BCAA contributing to an improvement in performance, where the results vary with the amount ingested, time of treatment, time of ingestion and test performed. Also is needed more studies that associate physical performance, leucine supplementation in isolated form.

Analyzing the possible health benefits that leucine supplementation can generate, were observed in the studies several benefits as: the use for treatment of muscle loss in the elderly; possible adjuvant in the treatment of insulin resistance associated with obesity; besides wound healing and muscle injuries. It should be understood that many of these studies were carried out with animals, being necessary to carry out more research to confirm its applicability in humans.

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