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REVIEWS
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Protein food and amino acid supplements in athletes' diet
Hrana proteică şi suplimentele cu aminoacizi în dieta sportivilor

Marius Emil Rusu [1], Daniela-Saveta Popa [2]
[1] *Nutritional Sciences, Faculty of Biology and Geology, Babeş-Bolyai University Cluj-Napoca, Romania*
[2] *Department of Toxicology, Faculty of Pharmacy, "Iuliu Haţieganu" University of Medicine and Pharmacy Cluj-Napoca, Romania*

Abstract
Good nutrition and strenuous exercise are the two pillars of sports performance. Feeding stimulates protein synthesis and, in combination with resistance exercise, induces muscle hypertrophy and strength. A very important macronutrient of this equation is protein. Athletes need high protein doses to stimulate muscle protein synthesis over muscle protein breakdown, and therefore, a positive muscle protein balance. A well-chosen nutrition plan should be based on ingredients made from whole foods and provide the needed protein. But, there are times when athletes choose to use protein supplements. High-quality supplemental protein, such as milk-based protein, whey and casein, help in maintenance or building of muscle mass, and the recovery process after effort. This article will discuss the need for high amounts of protein in enhancing muscle mass and performance, the quality of protein, the most used amino acid supplements, and the security for athletes in using increased quantities of protein and amino acids. The latest information found in scientific journals was analyzed, and the results of this paper will be helpful for athletes and sport specialists regarding optimal protein and amino acid intake in order to enhance sports performance and maintain the athletes' health.
Key words: athletes' diet, protein food, amino acids, protein supplements, creatine, citrulline.

Rezumat
Nutriţia adecvată şi antrenamentele fizice sunt cei doi piloni ai performanţei sportive. Hrana stimulează sinteza proteică şi, în combinaţie cu exerciţiile de rezistenţă, induce creşterea masei musculare şi a forţei musculare. Un macronutrient foarte important este în această ecuaţie proteina. Atleţii au nevoie de un aport crescut de proteine pentru stimularea sintezei proteinelor musculare peste catabolismul proteic şi, astfel, pentru a avea o balanţă pozitivă a proteinelor musculare. Un plan nutriţional bine ales trebuie să cuprindă alimente integrale, care să furnizeze toate proteinele necesare, calitativ şi cantitativ, dependent de particularităţile metabolice ale fiecărui sportiv şi de tipul de sport practicat. În anumite situaţii este necesară şi se recomandă utilizarea de suplimente proteice. Astfel de suplimente de înaltă calitate, cum ar fi proteinele din lapte, zer şi cazeină, ajută la menţinerea sau la construirea masei musculare în perioadele de efort fizic intens şi facilitează recuperarea după efort. În acest articol se va discuta şi sublinia necesitatea unui aport ridicat de proteine pentru creşterea masei musculare şi a performanţei sportive, calitatea proteinelor furnizate, dar şi suplimentele de aminoacizi recomandate, care pot fi utilizate în siguranţă de către sportivi. Acest articol sumarizează cele mai recente informaţii furnizate de articolele ştiinţifice din domeniu şi oferă date importante sportivilor şi specialiştilor din sport cu privire la aportul optim de proteine şi aminoacizi, în vederea creşterii performanţei sportive, asigurând totodată menţinerea sănătăţii sportivilor.
Cuvinte cheie: dieta sportivilor, proteine alimentare, aminoacizi, suplimente proteice, creatina, citrulina.

Introduction

Good nutrition and strenuous exercise are the two pillars of sports performance. The paramount nutritional recommendation for athletes should be to consume a well-balanced diet, based on ingredients made from whole foods. The athletes' body mass and body composition, part of the success in various sports, can be affected by nutrition science. The diet content should be precisely determined and deficiencies of energy, protein and other nutrients should be avoided. Feeding stimulates protein synthesis and, in combination with resistance exercise, induces muscle hypertrophy and strength. A very important macronutrient of this equation is protein. For athletes, nitrogen is balanced (the difference between protein intake and protein degradation) when protein intake reaches 1.2 g/kg body weight/day compared to 0.8 g/kg body weight/day in resting individuals (Poortmans et al., 2012). The latest data recommend ingesting 0.3 g/kg protein after workout and the rest of the amount every 3-5 hours over

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Address for correspondence: Nutritional Sciences, Faculty of Biology and Geology, Babes-Bolyai University Cluj-Napoca, 44 Gheorghe Bilașcu, 400015, Cluj-Napoca, Romania
E-mail: marius.e.rusu@gmail.com
Corresponding author: Marius Emil Rusu marius.e.rusu@gmail.com

several meals (Moore et al., 2009; Phillips, 2014a). A well-chosen nutrition plan should provide the needed protein. However, there are times when athletes choose to use protein supplements. Reasons to use supplements include increasing performance, speeding up recovery, increasing energy, immune support, improving nutrition, and changing the body composition (Braun et al., 2009). Supplements should always augment a balanced nutrition plan; they should not be used separately. Athletes' supplementation should be guided by sport science professionals and dietitians, and not by family, friends or coaches (Braun et al., 2009).

Protein requirements, balanced with the right amino acid supplements, need to be personalized to the individual athlete and the energy availability. An effective eating plan associated with the right supplements can contribute to and positively impact sports performance (Thomas et al., 2016).

This article will discuss the need for high amounts of protein in enhancing muscle mass and performance, the quality of protein, the most used amino acid supplements, and the security for athletes in using increased amounts of protein and amino acids. The latest information found in scientific journals was analyzed, and the results of this paper will be helpful for athletes and sport specialists.

Protein food

Research demonstrates higher protein demands for athletes. The Recommended Dietary Allowance (RDA) for adults is 0.8 g/kg body weight/d, but athletes need to stay well above this recommendation and ingest quality protein food. Slow appearance of amino acids in the portal vein and subsequently in the systemic circulation after protein meals can raise nitrogen retention and diminish urea production (Moore & Soeters, 2015).

The need for protein depends on the sporting event. Endurance athletes seem to necessitate lower doses than weightlifters in search of muscle mass. Nevertheless, all athletes will benefit from a supply of 1.3-1.8 g/kg body weight taken in 3-4 meals daily (Phillips & Van Loon, 2011). Studies suggest that gain in muscle mass and strength is greatest with immediate post-workout supply of protein (Josse et al., 2010). Protein synthesis is optimal by the intake of 0.25-0.3 g/kg body weight of high biological value protein in the early recovery phase, less than 2 hours after exercise (Belen et al., 2010; Phillips, 2012). Post-workout protein diets should have higher leucine and other easily digestible amino acid content, thus optimizing muscle protein synthesis (Murphy et al., 2015; Helms et al., 2014). Research showed even better results for muscle mass and strength if the protein dose taken in the evening before bedtime was slightly higher (Snijders et al., 2015). Gains in muscle mass and strength are positively associated with total protein intake more than the timing (≤ 1 hour) of protein consumption (Schoenfeld et al., 2013).

More and more master athletes participate in endurance events such as long distance running and triathlon. Given the physical costs of endurance training sessions, the recovery methods are of utmost importance. Research proves that after these high energy demanding activities, physical recovery takes longer in master athletes than in younger, similarly fit athletes. Post-exercise protein feeding needed for muscle glycogen and protein resynthesis is constantly recognized. In this age group, dietary protein doses are even higher than 0.25 g/kg/meal, considering 4 meals daily, with attention to the leucine content (Doering et al., 2016).

There are times when athletes need to lose weight. For performance protection, this should be done in the training period, well before competition (Garthe et al., 2011), with hypocaloric diets that maximize loss of body fat and retain lean body mass (Mettler et al., 2010). In a hypocaloric diet, the aim is to lose weight, but not skeletal muscle and strength. Because protein has a thermic effect that exceeds both fat and carbohydrate and has the ability to preserve lean mass, it is recommended to increase the protein intake to 1.8-2.0 g/kg/day (Bosse & Dixon, 2012). There are results suggesting that whey protein may have a greater thermic effect than soy (Rubin et al., 2012). In order to have a negative energy balance the increase in protein consumption should come with a decrease in another macronutrient: fat. Athletes need carbohydrate to achieve performance (Churchward-Venne et al., 2013; Phillips, 2014a).

Protein source seems to be very important. In a study on isonitrogenous and isocaloric diets, two protein sources were evaluated: the first group consumed leucine from whey, while the second group consumed lysine and leucine from wheat. Total caloric intake was divided 16/54/30 between protein/carbohydrate/fat. After 11 weeks of study, the first group had 9.7% body fat, and energy was deposited 30% in adipose tissue, 70% in skeletal muscle. The second group had 12.4%, 55%, 45% body fat, respectively. The results are much more beneficial for the first group, which had leucine from whey (Moulton et al., 2010). Likewise, a research on weightlifters with isocaloric and isonitrogenous diets from two sources – whey and soy, showed the best outcome for the whey diet (Volek et al., 2013).

Two frequent methods to determine the overall quality of protein are net protein utilization (NPU) and protein digestibility corrected amino acid scores (PDCAAS). Based on these methods, the best protein source is whey. It contains a multitude of biologically active proteins and peptides (b-lactoglobulin, a-lactalbumin, glycomacropeptide, lactoferrin, lactoperoxidase, immunoglobulins, IGF/ insulin-like growth factor). Whey protein contains more essential amino acids per weight than other sources, it is a rich source of branched-chain amino acids, and a rich and balanced source of sulphur amino acids (methionine, cysteine - precursors to the potent intracellular antioxidant glutathione). Thus, whey is considered a better protein source than casein, soy, eggs, meat or fish (Smithers, 2008; Devries & Phillips, 2015; McLain et al., 2015). Whey protein alone or a blended protein supplement containing all the essential amino acids, with a longer aminoacidemia period and several digestion rates, clearly enhances muscle protein synthesis with respect to the ingestion of an iso-energetic equivalent carbohydrate or non-whey protein supplement in resistance-training individuals (Reidy et al., 2013; Naclerio & Larumbe-Zabala, 2016).

A higher protein diet could be beneficial for optimal immune function and recovery from intense periods of training. It reduces excessive inflammation and immunosuppression, it helps in oxidative stress, muscle fatigue and muscle injury (Cruzat et al., 2014).

Studies demonstrate that higher protein diets, despite a widespread belief, will not compromise renal function (Tipton, 2011; Phillips, 2014b). Consuming moderately high-protein (1.0 to 1.2 g/kg/day) or even high-protein (>1.2 g/kg/day) diets does not disrupt calcium homeostasis and is not detrimental to skeletal integrity. Protein may help preserve bone mass during weight loss by stimulating insulin-like growth factor and increasing intestinal calcium absorption (Jesudason et al., 2013; Cao et al., 2014; Tang et al., 2014). In another study, male and female weightlifters combined anaerobic exercise and a very high protein diet, 3.4 g/kg/day, which is over four times the recommended dietary allowance. After 4 months there were no side effects, no harmful consequences on blood lipids, hepatic or renal function (Antonio et al., 2015; Antonio et al., 2016).

Amino acid supplements

Protein augmentation can be achieved with the use of dietary protein supplements. This is a convenient way to reach the target level of protein intake when access to quality food is restricted or there is no time for elaborate meals. Protein supplementation pre/post-workout or competition can enhance muscle mass and performance if the training stimulus is consistent and the dietary intake is adequate (Pasiakos et al., 2015). High quality protein or amino acid supplements can be comfortable, time-saving, and an adequate synchronicity between ingestion and competition can bring benefits in many anaerobic or aerobic sport events (Kreider & Campbell, 2009). Because gastrointestinal complaints frequently manifest in young athletes before competitions, when they are advised to avoid high-carbohydrate, high-fat foods at least 2-3 hours before events (Orasan et al., 2014), the importance of adding protein supplements to improve performance is even higher.

Citrulline, a non-essential amino acid found mostly in watermelon, whose name is derived from the Latin word Citrullus, is used as a sports performance and cardiovascular health supplement. Citrulline supplementation can raise energy levels, improve endurance in aerobic and anaerobic exercise, and stimulate muscle protein synthesis (Cynober et al., 2013). In the kidneys, after ingestion, citrulline is converted to arginine, a substrate for NO synthase. Citrulline is better absorbed in the intestines than arginine itself (Schwedhelm et al., 2008). Nitric oxide is a modulator of mitochondrial respiration and muscle energy metabolism during exercise.

Research confirms that both forms used in products, L-citrulline and citrulline malate, provide increases in exercise performance. Supplementation with citrulline malate can be beneficial for moderately trained athletes (Sureda & Pons, 2012), and also for highly trained athletes. Supplementation with 8 g citrulline malate before workout may be beneficial for improving exercise performance in advanced resistance-trained men. Blood pressure, heart rate, and blood lactate were comparable between placebo and citrulline malate groups (Wax et al., 2015). L-citrulline supplementation significantly raised plasma L-arginine levels, improved the athletes' muscle fatigue and concentration immediately after exercise (Suzuki et al., 2016). In a controlled laboratory environment, by consuming a blend of 8 g citrulline malate and 12 g dextrose one hour before test, a group of female athletes aged over 40 improved their aerobic and anaerobic performance (Glenn et al., 2016).

One study showed that 8 g citrulline malate supplementation can increase athletic performance in high-intensity anaerobic exercises with short rest times, and muscle soreness was also relieved (Perez-Guisado & Jakeman, 2010). In another study, trained cyclists were randomly assigned to one of two groups: control or supplemented with 6 g citrulline malate, 2 hours prior to a 137-km cycling stage. Plasma growth hormone concentration, which increases with exercise (Lencu et al., 2016), was higher in the citrulline malate supplemented group, which also showed an increase in the use of amino acids, especially branched-chain amino acids (Sureda et al., 2010).

Adding reduced glutathione (GSH) to L-citrulline may protect against the rapid oxidative destruction of nitric oxide. A small human trial was conducted, where resistance-trained males were randomly assigned to orally ingest a placebo, L-citrulline (2 g/day), GSH (1 g/day), or L-citrulline (2 g/day) + GSH (200 mg/day) for 7 days, followed by a resistance exercise session. Athletes taking L-citrulline along with GSH had significantly higher nitrite and nitric oxide levels compared to placebo or L-citrulline alone (McKinley-Barnard et al., 2015).

Short-term citrulline administration less than 10 g appears to be safe and well-tolerated in humans (Moinard et al., 2008).

Beta-alanine, a modified form of alanine, is a non-proteinogenic amino acid produced endogenously in the liver and found mostly in meat. The mechanism by which beta-alanine supplementation could have an ergogenic effect is still debated; the common view is that beta-alanine supplementation significantly raises muscle carnosine concentration, better than carnosine itself (Derave et al., 2007; Everaert et al., 2013). Carnosine, synthesized in skeletal muscle from beta-alanine, plays a significant role in muscle pH regulation and apparently has anti-aging actions (Hipkiss, 2009). Having muscle buffer capacity, carnosine delays the onset of muscular fatigue. Supplementation with beta-alanine, best done with a sustained-release formulation to avoid urinary spillover and paresthesia symptoms, is used by athletes to improve their high-intensity anaerobic performance, increase resistance training performance and training volume in team sports (Artioli et al., 2010; Harris & Stellingwerff, 2013; Bellinger, 2014). Beta-alanine supplementation with 4 to 6 g daily for at least 2 to 4 weeks had a positive effect on physical performance, muscle relaxation speed, and neuromuscular fatigue (Hannah et al., 2015; Trexler et al., 2015). When beta-alanine supplementation was taken with meals, muscle carnosine increased more (+64%) than when it was ingested between meals (+41%). In this 5-week study, with daily 4.8 g beta-alanine supplementation, slow-release and pure beta-alanine appeared equally effective

(Stegen et al., 2013).

However, extended cellular exposure to beta-alanine may reduce circulating and cardiac levels of taurine, which leads to functional and structural cardiac changes and an increase in oxidative stress in deficient animals (Parildar et al., 2008; Pansani et al., 2012). Hence, until there is sufficient evidence confirming the safety of beta-alanine supplementation and its side effects in humans, caution is advised in using beta-alanine as an ergogenic aid (Quesnele et al., 2014).

Creatine, a nitrogenous organic acid, is produced endogenously from glycine and arginine, mostly in the liver and kidneys, at an amount of about 1 g/day. 95% of the body creatine stores are found in skeletal muscle. It increases phosphocreatine muscle stores, boosting, in higher energy demands, the ability to resynthesize ATP, more and more recognized for its pleiotropic effects (Wallimann et al., 2011).

The antioxidant activity of creatine appears as an additional non-energy related mechanism with valuable effects in a wide number of human degenerative diseases and conditions (Sestili et al., 2011). Also, it can act as a blood antioxidant, protecting cells from oxidative damage, genotoxicity, and can potentially expand their lifespan (Qasim & Mahmood, 2015). Creatine has been found to enhance cognition; it acts as a neuroprotectant and improves mitochondrial efficiency (Rae & Broer, 2015; Rahimi et al., 2015; Cunha et al., 2016).

Supplementation with creatine increased muscle strength in athletes, but did not affect explosive performance (Wang et al., 2016). The results on body composition and muscle strength were superior when taking 5 g creatine immediately post-workout compared to pre-workout (Antonio & Ciccone, 2013). Two creatine monohydrate supplementation protocols demonstrated efficiency: one protocol with supplements in the amount of 0.1 g/kg/day for 28 days; another protocol with supplements of 0.3 g/kg/day for at least 3 days and subsequently, 3-5 g daily for 28 days (Cooper et al., 2012). The sports rehabilitation field could benefit from creatine supplements considering that creatine may be useful in preventing muscle damage from high-intensity exercise and aiding in the recovery process. Further research is needed to find out how creatine does that (Kim et al., 2015). Because creatine has the ability to take water from the bloodstream into skeletal muscle, adequate fluid intake is recommended (Munoz et al., 2015).

Several studies showed that 5 g/day creatine supplementation, together with a normal or high-protein diet, proved to be safe for kidney function either in healthy resistance-trained individuals or in type-2 diabetic patients (Gualano et al., 2011; Lugaresi et al., 2013). However, the cytotoxic effects of creatine supplementation should always be considered on an individual level.

Branched-chain amino acids (BCAAs) - leucine, isoleucine, and valine - are essential amino acids metabolized directly in muscles. BCAAs appear to increase protein synthesis in muscle, positively influence muscle fatigue, and participate in the homeostasis of glucose. As no minimum or maximum limits of BCAAs have been set, in a 2/1/1 ratio of leucine/isoleucine/valine, daily proposal is 40/20/20 mg/kg body weight. To keep a constant ratio of BCAAs in the body, the use of this mixture is advised, rather than individual leucine. No toxicity of BCAAs has been observed even at high doses (Brestensky et al., 2015).

A serum drop in BCAAs appears during exercise, thus BCAAs supplementation might be necessary. A serum decline would normally cause a tryptophan influx into the brain, followed by serotonin production, which causes fatigue. Branched-chain amino acids (BCAA) compete with tryptophan and tyrosine for transporters at the blood-brain barrier. For example, raising blood tyrosine or tryptophan levels raises their uptake into the brain; thus, conversion of tyrosine and tryptophan to catecholamines and serotonin respectively, while raising blood BCAAs levels, raises their uptake into the brain. One study showed that administration of BCAAs with tyrosine, a mixture that brings a drop in serotonin but prevents a fall in dopamine, is an effective way to improve physical performance (Fernstrom, 2013).

A very recent study reported that BCAA supplementation in athletes on a hypocaloric diet can maintain lean mass and preserve skeletal muscle performance while reducing fat mass (Dudgeon, 2016). Dieter et al. (2016) agreed in general with the aforementioned study, but argued that there were small statistical flaws in the study.

Carnitine, an amino acid involved in lipid metabolism, is synthesized in the liver and kidneys from the amino acids lysine and methionine. It transports long-chain acyl groups from fatty acids into the mitochondrial matrix, so they can be broken down through beta-oxidation to acetyl CoA to obtain usable energy via the citric acid cycle. L-carnitine, the biologically active form, has also been found to increase nitric oxide production when combined with aerobic exercise (Bloomer et al., 2009). This relaxes the smooth muscles of the blood vessels, causing them to widen, allowing more blood to flow through. It can further promote muscle endurance through better delivery of nutrients and oxygen to working muscles, along with helping to improve recovery after exercise (Huang & Owen, 2012). Supplementation with 2 g L-carnitine for 3 weeks had a positive effect on muscle tissue damage and soreness after exercise, on free radical formation, and on muscle tissue repair. However, muscle power and strength were not affected by supplementation (Ho et al., 2010). A dose of 1.5 g/day glycine propionyl-L-carnitine supplementation for a 28-day period raised sport performance and anaerobic work capacity more than 3.0 g or 4.5 g doses (Jacobs & Goldstein, 2010).

Increased carnitine content can speed up energy expenditure during low-intensity exercise and prevent an increase in adipose tissue (Stephens et al., 2013). L-carnitine supplementation in conjunction with a carbohydrate beverage, which elevates circulating insulin, can increase retention of carnitine in the body (Stephens et al., 2007). Adding whey protein to a carbohydrate beverage reduced carnitine accumulation; thus a mixture of carbohydrate and protein beverage could inhibit chronic muscle carnitine accretion (Shannon et al., 2016).

It is worth mentioning that two contradictory research outcomes exist. One supports that dietary L-carnitine, abundant in red meat, might contribute to the link between high levels of red meat consumption and cardiovascular disease risk (Koeth et al., 2013). The other one, based on two meta-analyses, concludes that supplementation with L-carnitine is effective in the secondary prevention of cardiovascular disease (DiNicolantonio et al., 2013; Shang et al., 2014). Further studies are needed to determine the safety of carnitine in athletes under all conditions.

Conclusions

1. Athletes need high protein doses to stimulate muscle protein synthesis over muscle protein breakdown, and therefore, a positive muscle protein balance.

2. Optimal doses depend on the end scope or the sport event, can be higher than 1.0-1.2 g/kg/day, and should come from nutritious animal and plant protein sources. High-quality supplemental protein, such as milk, whey and casein-based protein, helps in maintenance or building of muscle mass, and the recovery process after effort. The added convenience may be of interest for athletes.

3. Ideal health and performance can be achieved through optimal variety, quantity, quality, and timing of consumption of nutrients and amino acid supplements.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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