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The role of proteins in sports

Protein-based food supplements are included into Italian law in the categories listed in Annex 1 of Legislative Decree 111/92 (already covered in the article "Definition of dietary supplements and their use in sport" in Sport & Anatomy 2014; 00: 38-42) concerning "special physiological conditions of foods suitable for intense muscular effort especially for sportpeople". The body of sports people, in fact, is mainly anabolic, and this causes a significant physiological increase in overall nutritional requirements to support the plastic requirements and the intense metabolic effort. For this reason, in the labelling of food supplements the following words have been proposed in the list of health claims (EU Regulation 432/2012) "proteins contribute to the maintenance and growth of muscle mass and to the maintenance of normal bones". Proteins, amino acid compounds and their derivatives (such as creatine, beta hydroxy methyl butyrate) are certainly molecules of great importance in the diet of the sportsperson. Physical activity is in fact, first of all, a muscular expression, and although depending on the presence of energy substrates, it requires maximal muscle efficiency (endurance, power and physical strength). An insufficient amount of protein nutrients in the diet can reduce the capacities of defense of the body (the lack of nitrogen compounds, as is known, weakens the immune system). The main role of proteins is therefore to provide the amino acid bases for the synthesis of new cells and tissues. On a chemical level, the proteins are composed of amino acids linked together by peptide bonds, forming polypeptides with high molecular weight, which make the molecular configuration of proteins particularly complex (primary, secondary, tertiary and quaternary). A linear configuration composed of up to 100 amino acids is defined with the term "polypeptide", while longer molecules are referred to as "proteins".

The role of proteins in sport

Physical performance is not highly dependent on protein intake. The known factors that can affect muscle energy metabolism are in fact due to the metabolic energy pathway whose limiting factors are, in brief, made up of availability of energy substrates, efficiency of the cardiovascular and respiratory function, muscle fibers and enzymatic activity. But there is no doubt that the percentage of lean body mass, the functional characteristics of the different types of muscle fibers (slow and fast) used in physical activity, and muscle metabolism prove essential to produce maximum and winning performances. The exact knowledge and correct interpretation of the bioenergetic metabolic pathways followed in different types of sports performances (short maximal efforts, endurance and aerobic activities) allow to assess the extent of use of macronutrients, carbohydrates, fats and proteins. Within the human body, proteins have mainly a plastic function, as they are the main component of lean mass (about 20% of the body component). They are also constituents of enzymes and vitamins. In sports, the most popular proteins are those with the highest intake of essential amino acids. The amino acids, as is known, are classified into essential (the human organism is unable to synthesize them and they must therefore be introduced through nutrition) and non-essential (a definition that must not reduce their importance, but convey the concept that they can be produced, at a metabolic level, from other precursors). The essential amino acids are: isoleucine, leucine, lysine, histidine, methionine, phenylalanine, threonine, tryptophan and valine (in addition to arginine, essential to children, and cysteine and tyrosine in the absence of methionine and phenylalanine).

In sport, glutamine is considered an essential or "conditionally essential" amino acid, given its significant presence in muscle groups. Proteins with high biological value (present in common foods such as eggs, milk, meat and fish) contain all the essential amino acids in optimal quantities to maintain nitrogen balance and enable tissue repair and growth. In special diets linked to lifestyles like the vegan diet, whose only prevailing protein sources are cereals and legumes, qualitative and

quantitative deficiencies may occur in essential amino acids (**1**). In a vegetarian diet, instead, animal by-products (milk and eggs) are included, so the probabilities of a shortage of essential amino acids are very low, provided that the diet is varied and normocaloric. In particular, a "lacto vegetarian" diet doesn't involve any risk of protein deficiencies and is able to guarantee an adequate intake of minerals (calcium and phosphorus) and vitamins (in particular vitamin B12) (Tab. I).

Table I. Main functions of proteins.

Making up hormonal and enzymatic structures (metabolic regulators)
Maintaining the muscle structure and promoting protein anabolism
Preventing inflammations and infections (Antibody immune action)
Neutralizing many toxic substances (detoxicating enzyme systems)
Representing a primary source of organic nitrogen
Performing plastic and adjustment functions
Promoting the growth, maintenance and repair of cells and tissues
Performing energy functions during exercise (glucose-alanine cycle)

Protein Quality Indexes

The methods used for the evaluation of protein requirements can be summed up in two types: nitrogen balance (Protein Efficiency Ratio, PER, Net Protein Utilization, NPU) and factorial method. While the latter determines all the losses of nitrogen compounds after a period of protein-free diet, the analytical methods linked to nitrogen balance determine the minimum amount of food proteins which can maintain the nitrogen balance in a person of medium build (excluding specific metabolic situations such as pregnancy and lactation).

The nitrogen balance can be determined in several ways, such as:

- Protein Efficiency Ratio (PER, efficiency rate of the protein), an indicator used to evaluate the quality of food proteins and representing the ratio between weight gain (in animals) and amount of protein (in grams) administered;
- NPU (net protein utilization), represented by the ratio between the nitrogen retained by the body and the one introduced through the diet multiplied by a factor of 100.

The PDCAAS is instead a method of evaluation of protein digestibility assessed in terms of the amino acid score corrected by an index of protein digestibility. The casein derived from milk, egg white powder, the isolated proteins of soy are all protein compounds characterized by a high index of PDCAAS (1.00), contrary to wheat proteins (gluten) that have a score of 0.25 (2).

Absorption and digestion of proteins

Food proteins are digested and absorbed by the intestine in large daily quantities. They are not all of food origin; some are of endogenous origin (serum albumin, mucoproteins, digestive enzymes), are secreted in the gastrointestinal tract and spilled in the intestine as enteric juices. Considering 100 grams of ingested proteins, about 170 grams is the total amount absorbed and about 10 grams are excreted (fecal loss) **(3)**. Between 50 and 70 g of endogenous proteins are therefore digested daily, which is roughly equivalent to the average amount of protein ingested (**4**). Protein digestion occurs in the stomach, where acid secretion denatures the proteins, exposing them to the attack of pepsins (endopeptidase: trypsin, pepsin, chymotrypsin, elastase), carboxy and aminopeptidase that split the polypeptide chains into fragments of small size (amino acids), which are absorbed by the cells of the intestinal mucosa. The digestion (hydrolysis) of proteins occurs in three phases, gastric, pancreatic and intestinal, through mechanical, chemical and enzymatic disintegration leading to obtain simpler molecules (peptides and amino acids). In the gastric phase, hydrochloric acid contained in the gastric juices of the stomach and specific enzymes (pepsins, trypsin, elastase and chymotrypsins) denature proteins (the process involves only 10-15% of the ingested proteins that are broken down, at this stage, into polypeptides). In the pancreatic phase, which occurs in the duodenum, protein hydrolysis takes place (approximately 50-60%), due to some proteases contained in the pancreatic juice: endopeptidases (trypsin and chymotrypsin), being active on the peptide bonds inside the protein molecule, and exopeptidases (carboxypeptidase), which lead to the release of amino acids. The intestinal phase covers virtually all protein hydrolysis (80-90%) and completes the denaturation of proteins through the action of specific peptidases that release amino acids. This phase leads to the hydrolysis of both the proteins ingested and the endogenous ones (digestive enzymes, desquamated epithelial cells and others).

As for the absorption of proteins, it is important not to neglect the fact that traditional protein foods (meat and fish), once cooked, can present a more or less marked denaturation of protein structures. While on the one hand thermal heating may degrade the quality of the proteins contained in meat, on the other hand some plant foods (cereals, legumes and tubers) can improve the intake as a function of a decrease in anti-nutritional factors. Establishing the actual extent of the positive or negative effect of cooking food on the bioavailability of proteins is a complex task, full of variables, being closely related to the type of food and the heat treatment it undergoes (grilling, frying, boiling, microwaving...).

Branched Chain Amino Acids (BCAA)

Amino acids are complex molecules that differ in their chemical-physical properties (solubility, pH and molecular structure) and metabolic fate (glucogenic and ketogenic). Amino acids are not only the constituents

of proteins, but also play a valuable role as precursors of fundamental biological molecules such as hormones, pigments, purines and co-enzymes. Of all known amino acids, five (leucine, isoleucine, valine, lysine and histidine) cover alone 75% of the needs of human organism. However, these amino acids are present in small concentrations in food (less than 20% in proteins with a high biological value). This factor is of great importance in the athlete's food supplementation, and should lead to reducing the presence of the other amino acids in favor of leucine, isoleucine, valine, lysine and histidine, thus promoting a reduced accumulation of synthesized urea in blood. The definition of "essential" amino acids, as already explained, is not univocal since they can be "essential" not only for the whole body but, in a more selective way, only for some organs (only the liver, for example, has a specific hydroxylase, which is absent in the cells of other organs, and is able to operate the synthesis of phenylalanine into tyrosine) (5). The three branched amino acids (also known as "neutral" or BCAA) L-Leucine, L-Isoleucine and L-Valine, represent the group of molecules being most studied at the level of clinical and sport integration. In terms of performance, the utilization rationale of branched amino acids is due to their oxidation which takes place, preferably, in skeletal muscles rather than in the liver, thus decreasing the time of assimilation. Amino acids allow for the intake of active molecules in muscle construction / reconstruction, without producing metabolic waste and without providing a caloric surplus (Tab.II).

Table II.

The branched amino acids (also referred to by the initials "BCCA": *Branched Chain Amino Acids*) are the three essential amino acids L-isoleucine, L-Leucine and L-Valine
They are especially used in conditions of stress, injury, intense physical exercise
L-Leucine, which is used twice more than the other two branched amino acids (the amino acid being more oxidized during endurance performances), acts as a stimulator of protein synthesis in the phase of plastic recovery, at the end of intense muscular exercises. It is one of the promoters of the release of growth hormone (GH) and insulin
BCAAs are not metabolized in the liver (the liver unlike, the muscle, does not have the specific transaminases needed to obtain the corresponding alpha-keto acids)
The branched amino acids compete with phenylalanine and tryptophan for the same conveyor at the level of the blood brain barrier. As a consequence, during exercise they tend to deplete branched chain amino acids in plasma before the other amino acids, tryptophan and tyrosine are conveyed in the brain with greater efficiency, with beneficial effects on the serotonergic and adrenergic systems controlling sleep, mood and fatigue
They are used as supplements for power and endurance sports and power endurance and in low-calorie diets

Protein requirement

The protein requirement is dependent on several factors such as age, sex, professional and sport activity, and on special physiological conditions (growth, pregnancy, old age). It is usually defined in relation to physiological body weight (in the athlete it is always known as ideal weight), age, sex and workload during training. In order to correctly define the daily protein requirement of an athlete (which must obviously always be equal to nitrogen balance), it is essential to evaluate the weight, the level of hydration and the individual constituents of body mass (lean and fat), and obviously the duration and intensity of the daily physical activity. It is also worth assessing the percentage of essential amino acids ingested with the diet, that should be equal to 36% of the total amino acid intake (**6**) (Tabb. III-IV). In athletes it is therefore particularly important to maintain a sufficient energy intake to support muscle activity. In case of insufficient energy intake in the diet, body proteins are in fact metabolized to make up for the energy deficit. With the reduction of energy reserves in the body (low-calorie diet or fasting), glycogen is less available, and glucose is therefore synthesized from protein compounds and fatty acids (gluconeogenesis). The problem of the correct determination of protein requirement is given by the fact that the response of the nitrogen balance to increasing amounts of proteins of good nutritional quality is not linear.

In case of poor protein intake, the improvement is proportional to the amount of protein introduced with food, but for the amount of protein being capable of maintaining the body balance, the efficiency of protein decreases. Protein requirements, therefore, appear higher than those shown in the case of low protein intakes (Ardent, p. 118) (3).

Protein supplements and production technology

Today, in sports, integrating nutrition with concentrated protein powder from different food sources is common practice. The most widespread protein sources are those of milk, that are produced through technological processes (drying and concentration). While drying occurs typically through the "spray-dried" technique (nebulization in hot air chambers), concentration processes occur through ion exchange (resins which separate proteins according to their electrical charge) or ultrafiltration (through membrane filters).

Dietary protein in the form of supplements (concentrated) are often the result of two or more different protein constituents and produce non-homogeneous nutritional responses according to the resultant of the amino acid profile. Four situations can be defined (8):

1) no complementary effect (in the case of identical amino acids missing or deficient);
2) poor complementary effect (same deficiency of limiting amino acids but in a quantitatively different measure);

Table III. Sports at risk of poor diet.

Criteria	Sport
Low weight Chronically low energy input to maintain weight and muscle definition	Gymnastics, ballet dance, fitness and aerobics
Fast competition weight Rapid and drastic weight loss to access competition categories	Ring sports and tatami
Increase in lean body mass Accentuated muscle definition (drastic loss of fat and body water) lack of liposoluble vit./ cramps	Body building, boxing
Vegetarian diet (strictly vegetarian or vegan athletes)	Endurance and weight lifting

Table IV. Daily protein levels (average requirement expressed in g/kg/day).

Adults	0,8 g
Active people	1,0 g
Endurance athletes	1,0-1,6 g
Team sports (football)	1,4-1,7 g
Strength sports	1,6-2,0 g

Sources: International Society of Sports Nutrition (2) ISSN (7).
Note: the values shown are referred to body weight intended as ideal weight.

3) limited complementary effect (protein sources with common deficiency of an amino acid, where the protein source with the highest intake of the deficient aminoacid prevails);
4) high complementary effect (synergy of the components of the protein mixture where the resulting protein quality exceeds that of each individual component).

Milk proteins (whey and casein)

Serum proteins (also defined by the term "whey protein") are proteins with a high biological value of high quality, soluble in liquids and readily digestible. The protein fraction is composed of albumins (75%) and globulins (15%). Whey proteins are considered as fast proteins (2), contain a high percentage of branched chain amino acids and sulfur amino acids (cystine and methionine). The current trend is to produce delactosed proteins (more compatible with the needs of many consumers with problems of lactose absorption). Casein is instead the main constituent (about 80% of milk proteins). Chemically, it is a phosphoprotein which, together with phosphoric acid and citric acid, binds calcium and favours its assimilation (it is for this reason that milk turns out to be an essential food for the absorption of calcium). Casein is considered as a slow-release protein (2) since it creates a gel in the intestine that slows down the intestinal transit, thus favouring protein absorption.

On the market there are protein compounds from whey proteins and casein in different proportions:

- isolated milk protein: a mixture of whey proteins and casein in a variable ratio, characterized by specific release time and absorption (the formula is often protected by the exclusivity of the manufacturer);
- total milk proteins: they represent the fraction of milk protein as it is (80% conjugated caseins and 20% whey proteins);
- protein concentrate: the protein fraction is made with customized protein ratios by the manufacturer (which may include multiple sources such as, for example, egg white, soya and legumes) according to specific needs of absorption or intolerances to one or more components provided by traditional milk proteins. In addition to proteins derived from milk, other protein sources are produced, such as:
- egg proteins: obtained from egg white (ovalbumin). Despite being characterized by an optimal amino acid profile, they are not particularly pleasing to consumers because of their aroma and flavour which is considered as a bit, or completely, unpleasant;
- soy proteins: mainly required by those who are intolerant to milk protein or do not want to eat proteins derived from animals (vegetarians and vegans). Recently, thanks to the improvement of the processes of extraction and concentration, this type of protein has improved in terms of palatability and this has contributed to a better acceptance;
- hydrolyzed wheat proteins: they are not very common because of their lower biological value (even if, in terms of amino acids, they can bring about 40% of glutamine), but also of a taste poorly appreciated by consumers and of a low solubility in liquids that make them unsuitable for the preparation of drinks with a high protein content;
- proteins from legumes: proteins that can provide a good protein percentage (and a relative share of amino acids); they are suitable in cases of intolerance to milk (lactose) and may provide a basis for a protein mixture (e.g. concentrated proteins from egg whites, soya and peas).

- protein gainers: the “Weight Gainers” represent a type of supplement aimed at increasing body weight and improving the overall calorie intake of the daily diet. They are powder products with a base of carbohydrates and medium release fat(MCT fats), proteins from different sources and their derivatives (creatine, glutamine, branched amino acids), vitamins and minerals depending on the timing of use (before, during or after the workout).

Conclusion

Proteins and their derivatives (BCAA) are a dietary source of great nutritional value also recognized by the Italian legislation governing the production and marketing of food supplements (food suitable for intense muscular effort especially for sportspeople). Like other macronutrients, carbohydrates and fats, proteins require a minimum daily intake (estimated at 0.8 to 1 gram per kilogram of body weight, defined as ideal weight) for people of “medium build” who do not do physical activity. Athletes therefore represent a segment of the population being particularly attentive and sensitive to the increased needs in terms of daily intake. The recommended intake varies in consideration of the body weight and physical activity practised. Short-duration and intense sports closely related to muscle power and strength require a higher protein requirement (up to 2 g / kg / day). Diet represents the basis for guaranteeing a sufficient protein intake (from different protein sources), and, in the sports field, it is often integrated with the use of specially formulated products (food supplements) based on milk proteins and its derivatives (whey and casein), eggs or legumes. Apart from this, protein molecules such as branched amino acids are a significant source of anabolic nutrients to the athlete (increased muscle mass) when adequately combined, of course, to exercise. The athlete and the active person in the end represent a segment of the population with higher protein and energy requirements than sedentary and inactive population.

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