PORTO SUPERIOR SCHOOL OF HEALTH
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SUPPLEMENTATION WITH WHEY ISOLATE AND ITS INFLUENCE IN MUSCLE HYPERTROPHY AND STRENGTH ON AMATEUR ATHLETES

A RANDOMIZED TRIAL

Dissertation submitted to the Porto Superior School of Health within the scope of the Master's Degree in Pharmacy, carried out under orientation of PhD. Agostinho Cruz and co-orientation of Professor Graça Cruz and MSc. Diogo Silva.

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Content

Abbreviations

ATP – Adenosine thriphosphate

ADP – Adenosine diphosphate

MS- muscular strength

NPPHENS – National Program for Promotion of Healthy Eating and Nutrition in Sports

DC – *Dietitians of Canada*

AND – *Academy of Nutrition and Dietitians*

ACSM – *American College of Sports Medicine*

TEN – Total Energy Needs

IOC - *International Olympic Comittee*

estAE – estimated avaiable energy

FFM – Free Fat Mass

ISSN – *International Society of Sports Nutrition*

MH- Muscle Hypertrophy

EAA – Essential Aminoacids

MPS – Muscle Protein Synthesis

EFSA – *European Food and Safety Authority*

HIIT - *High Intensity Interval Training*

CSA – Cross Sectional Area

Akt – Protein Kinase B

mTOR – mechanistic Target of Rapamycin

IGF-1 – Insulin-like Growth Factor 1

p70S6K - S6 Kinase 1 protein

AMPK – Activated Kinase AMP protein

SRF – Serum Release Factor

IL-4 – Interleukin 4

IL-6 – Interleukin 6

nNOS – Nitros Oxide Sintetase

MVC – Maximal Voluntary Contraction

1RM – 1- Repetition Maximum

β-LG - β-lactoglobulin

α-LA- α-lactoalbumin

BCAA – Branched Chain Amino Acids

MPB – Muscle Protein Breakdown

GIP – Glucose-dependent Insulinotropic Polypeptide

CHAPTER I

This chapter consists of a classic bibliography review. The aim of this first chapter is to provide a background to the reader about the thesis theme.

1.1 General

1.1.1 – Related to the musculoskeletal system

Muscular contraction

Skeletal muscle tissue can generate work from the interaction of different components. These components are responsible for the typical striated appearance of this tissue, formed by bands that repeat consecutively. Skeletal muscles are made up of cylindrical muscle fibers that extend throughout the muscle. In fact these muscle fibers are multinucleated muscle cells that contain inside them myofibrils formed by numerous proteins, of which the most important are actin and myosin. All muscle fibers are surrounded by dense connective tissue which is continuous with tendons (Figure 1). In this way, any tension generated by the muscular fibers is transmitted by these connective tissues to the tendon and consequently to the bones, which allows them to move (Baechle, 2008).

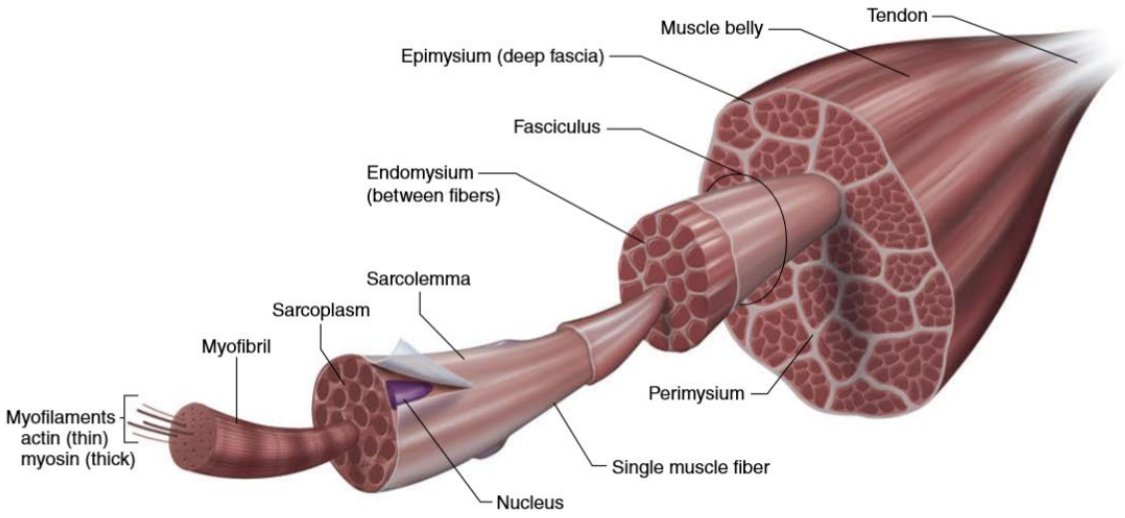

Figure 1 - llustrative diagram showing the structure of the skeletal muscle and connective tissue surrounding the muscle fibers From (Baechle, 2008)

The mechanism of muscular contraction, well known and described at the molecular level, consists of a set of interactions of different proteins that constitute the functional unit of the muscle, the sarcomere. The sarcomere contains contractile fibers, actin (thin filaments) and myosin (thick filaments) as well as accessory proteins, tropomyosin, troponin, titin and nebulin. The titin and nebulin fibers bind to myosin and actin respectively, attaching them

to the Z-line of the sarcomere (line corresponding to the anchoring of the actin fibers). Tropomyosin and troponin form a protein complex that blocks the binding site between actin and myosin. This complex modifies its conformational state when in presence of large amounts of calcium ions (Ca^{2+}), released after neuronal stimulation at the eminence of the neuromotor plate, thus allowing the interaction between the fine and thick filaments. In addition to the presence of Ca^{2+}, muscle contraction is dependent on the presence of Adenosine Triphosphate (ATP). The thick filament, constituted by myosin, contains ATP bound to the "heads" of the myosin molecule. These myosin structures, responsible for actin binding, still interact with actin, but weakly, which explains the ever-present muscle tone that prevents the total relaxation of muscles and consequently the body's necessary resistance to gravity. From the Ca^{2+} stimulation, ATP is hydrolyzed (mediated by the enzyme myosin ATPase) to adenosine diphosphate (ADP) and movement of the myosin head to a position that allows a stronger bond with actin occurs, in what is called a cross-bridge. It is the number of cross-bridges occuring simultaneously in the muscle that determines the production of muscular strength (MS). Then the shortening of the sarcomere with the myosin heads pulling the fine actin filaments to the center of the sarcomere results. The energy for this is provided by the ATP hydrolysis (Figure 2). This process is only interrupted, returning all fibers to their initial position when new ATP molecules bind to the myosin heads. As long as sufficient ATP and Ca^{2+} is present, the cycle is repeated (Baechle, 2008), (Månsson, Rassier, Tsiavaliaris, 2015).

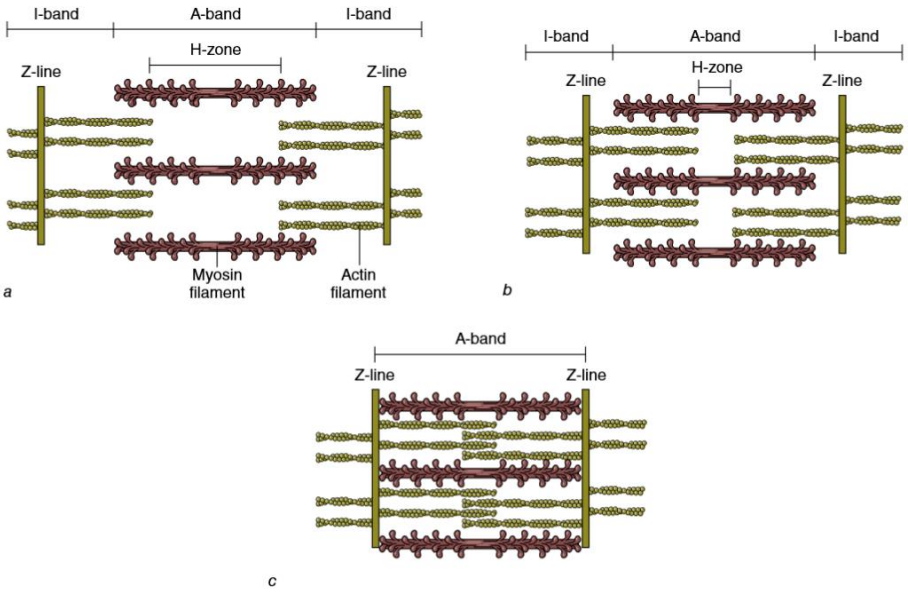

Figure 2 - Contraction of a myofibril. (a) The relaxed sarcomer, the elongated H and I zones. (b) and (c) formation of cross-bridges and shortening of the sarcomere producing contraction and generating muscular strength. From (Baechle, 2008).

Muscle Fibers and Muscular Architecture

The muscle fibers are activated by electrical stimuli from motor neurons, however the response of the muscles is different. This different answer relates to the fact that there are different types of fibers. Classically the muscle fibers are classified according to the heavy chain of the myosin protein. Muscle fibers are classified into fatigue resistant slow contraction fibers (I - oxidative fibers), fast - twitch fibers (IIx - glycolytic fibers), and fast - twitch hybrid fibers that exhibit an oxidative metabolic profile. Type I fibers are efficient and used under aerobic conditions, but their potential for anaerobic effort is limited, i.e. their ability to generate force quickly is low as evidenced by their weak myosin ATPase activity. In contrast, the type II fibers are very inefficient and easily fatigable, but have high activity of myosin ATPase which allows them to respond quickly generating a great amount of force (Rivera-Brown, Frontera, 2012).

The types of fibers referred to exist in different proportions in different muscles. It is expected that in postural muscles, such as the soleus muscle, type I fibers prevail whereas in larger muscle groups that allow locomotion, as for example the quadriceps muscle, there is no prevalence of any type of fiber, observing instead mixture of different muscle fibers. Recruitment of muscle fibers also occurs in different patterns depending on the muscles requested and the strength to be made. If sustained effort, such as *endurance* exercises is required, slow-twitch motor units (type I fibers) are activated. If a *sprint* is necessary at the end of a race, for example, quick-twitch motor units are activated. In other types of exercises, both type I and type II fibers can be requested, each contributing in different proportions depending on the type of effort (Baechle, 2008).

Body movements and their relationship with skeletal muscles

The body movements performed by the muscles depend on the range of motion provided by the joints, i.e. the structures that bind the bones. The force that the muscles exert allows the rotation of limbs and body structures that manifest themselves as Torque. Torque is a magnitude vector perpendicular to the axis of rotation of a force applied to the pivot point that moves an object, in most cases a limb. The higher the Torque, the greater the tendency for the applied force to rotate the limb or body part around one or more axes in a joint (Baechle, 2008) (Rivera-Brown, Frontera, 2012).

Body movements performed in different sports require varied actions by the muscular system. The muscles exhibit three major types of actions:

Isometric actions - in this type of action the muscles generate force without shortening or lengthening and without occurring movement around the axis (s) of rotation of the joint. This type of action is considered static action because force is being generated but not Work (Work = force * displacement), since there is no displacement. There is energy expenditure, but no movement occurs because the external resistance equals the force generated by the muscles.

Isotonic actions - can be of two types, concentric or eccentric. In concentric actions, muscles exert enough force to overcome external resistance, thus shortening their length, moving the joint and producing displacement resulting in Work generated. In eccentric actions also occurs movement of the joints and production of displacement and consequently Work but the length of the muscles increases. The concentric actions are also called "Positive work" and the eccentric actions are called "Negative work". A good example of a concentric exercise is the muscle contraction that occurs in the muscle groups of the legs to run while the eccentric actions are typical of resistance training exercises such as when an athlete resists the downward movement of a bar of weights (Rivera-Brown, Frontera, 2012).

Although it is instinctively considered that the contraction of a particular muscle or more concretely the movement of flexion and therefore concentric, is the movement that generates more force, this does not happen. In fact, as the angular velocity in a joint increase, the torque decreases in the case of concentric exercises. In the eccentric actions up to a 90º joint angle, the Torque increases. This means that the greater amount of force produced comes from eccentric actions when resisting an external force (Baechle, 2008).

1.1.2 – Related to athelets diet

Proper nutrition, along with regular practice of physical exercise, are the two variables that most contribute to maintaining a healthy body. In the particular context of the sportsman, whether amateur or professional and according to the guidelines of the National Program for the Promotion of Healthy Eating and Nutrition in Sport (NPPHENS), *"Food significantly influences sports performance"* (Sousa, Teixeira, Graça, 2016, p. 13). Some of the world's most recognized nutrition organizations such as *Dietitians of Canada* (DC), *Academy of Nutrition and Dietetics* (AND) and *American College of Sports Medicine* (ACSM) agree that sports nutrition is an area of science of major importance so the intake of foods that provide

sufficient energy *"... are the mainstay of an athlete's diet since they support optimal body function ... and assist in the manipulation of body composition. "* (Dietitians of Canada, the Academy of Nutrition and Dietetics and the American College of Sports Medicine, 2016, p. 7) .

The goal of the athlete's diet is to provide the right energy for its physical activity. In order to achieve this goal, it is necessary to consider not only the type of nutrients ingested and their composition, but also other aspects such as the type of training performed, the schedule of competitions (in the case of professional athletes), mealtime, trips to places of training and competition as well as modifications of the corporal composition, necessary in certain sports like boxing or wrestling (Sousa, Teixeira, Graça, 2016), (Potgieter, 2013).

The athlete's energy need, despite the lack of consensus among the different world organizations, can be estimated by simple equations. The accuracy of these estimates will depend very much on " *the quality with which physical activity is reported* ... " according to NPPHENS guidelines. This is due to the fact that the calculation of total energy needs (TEN) is based on equations such as *Cunningham* or *Harris-Benedict* that introduce a level of physical activity factor, difficult to estimate in most cases. In any case, the use of these equations is recommended by ACSM. Other organizations such as the *International Olympic Committee* (IOC) emphasize the calculation of another variable, the "estimated available energy (estAE)" in addition to the TEN, calculated from the intake of energy subtracted from the energy expended in the exercise and expressed in Kcal/kg free fat mass (FFM)/day. This recommendation is associated with a set of evidences in several studies that prove that a low estAE value is associated with the alteration of physiological processes. It has been demonstrated that values lower than 30 Kcal/kg FFM of est estAE affect the bone density and the menstrual cycle of female athletes. It is concluded that calculating TEN may not be sufficient in the case of athlete's needs (Sousa, Teixeira, Graça, 2016), (Potgieter, 2013).

Carbohydrate intake

Today, large world nutrition and sports organizations recommend that athletes macronutrient needs be expressed as grams per kg (g/kg) of body weight instead of traditional percentages by NET. This allows to adjust and individualize the amounts of macronutrients ingested according to the goals and training of the athletes. Regarding the

daily requirements of carbohydrates, the body's primary source of energy, the *International Society of Sports Nutrition* (ISSN) the IOC a DC and AND choose to recommend values between 3 and 12g/kg body weight of hydrates of carbon depending on the athlete's physical activity that can vary from low intensity physical exercise to high intensity physical exercise. More important than the daily needs of athletes are the needs related to the goals of the training and competitions as well as the type of sport that takes place and the period of the day when the carbohydrates are ingested. The recommendation of carbohydrate intake by these organizations focuses mainly on strategies that can be adopted to manipulate the amount of fuel available for training or sporting events. Thus, if the goal is to stimulate adaptive physiological processes, the athlete can train without ingesting carbohydrates, fasting or within a few hours following a training session, which leads to increased expression of glycogen binding sites in the muscle cells, increased availability of fatty acids and increased catecholamine concentrations. In other cases, it may be interesting for the athlete to carry out *carbohydrate loading*, for example before a sporting event that will allow greater resistance to fatigue and better sports *performance, such* as an *endurance* race (Potgieter, 2013), (Dietitians of Canada, the Academy of Nutrition and Dietetics and the American College of Sports Medicine, 2016). In the period before the exercise it is intended to prevent hunger and restore glycogen stores, especially if the training is in the morning. Intake of 1-4g of carbohydrates/kg body weight 1 to 4 hours before exercise is recommended. Athletes should opt for low-fat, fiber, and protein foods since these foods aid gastric emptying and are less likely to cause gastrointestinal disorders (Dietitians of Canada, the Academy of Nutrition and Dietetics and the American College of Sports Medicine, 2016). Intake of carbohydrates during exercise increases sports performance, prevents hypoglycemia, delays muscle glycogen depletion, and saves hepatic glycogen. This supplementation should always be accompanied by adequate fluid intake (Sousa, Teixeira, Graça, 2016). More curious seems to be the increased *performance* of athletes when they ingest small amounts of carbohydrate during exercise. Although insufficient to restore glycogen stores, it seems to suggest receptors in the oral cavity that activate specific areas of the brain, improving fatigue and physical performance (Dietitians of Canada, the Academy of Nutrition and Dietetics and the American College of Sports Medicine, 2016). Finally, in the post-exercise period the IOC, ACSM and the ISSN agree that ingestion should be performed up to 30 minutes after the end of exercise to maximize glycogen synthesis and replenishment. The amounts vary between 1 and 1.5g/kg body weight/hr until the daily energy requirements of carbohydrates are reached. The maximum limit for DC and AND

guidelines is 1.2g/kg body weight/h (Potgieter, 2013), (Dietitians of Canada, the Academy of Nutrition and Dietetics and the American College of Sports Medicine, 2016). As for the recovery time between exercises being reduced, for example between two training sessions on the same day, then the athlete should repeat this intake every 2 hours for 4 to 6 hours (Potgieter, 2013).

Protein Intake

The recommendations for protein intake in the case of athletes is remarkably superior to that of the general population. In a review published recently by Brendan Egan it is possible to verify the tendency to emphasize the volume and load of training, specific goals such as muscle hypertrophy (MH) or reduction of fat mass in contrast to the focus on the amounts of protein ingested daily. In this same work the sources of proteins, protein dose per meal and the *timing* of the intake are also discussed. Higher quality proteins, which provide more essential amino acids (EAA) such as leucine, are considered as these proteins are responsible for the molecular signaling that induces muscle protein synthesis (MPS). The sources containing the highest amounts of these EAA are of animal origin and within these the dairy products are the ones with the highest amounts. As for the dose per meal, a practical range of 20 to 40g of protein is indicated, depending on the athlete's sport and weight (Egan, 2016). This range is consistent with other studies that established beneficial effects with doses of 40g of protein (Lindsay, Macnaughton, Sophie, 2016). Considering that athletes and active individuals perform about 5 meals a day, it easily reaches the daily amount of 100g/day or more of protein intake. In fact, this daily amount of protein for athletes is consonant with the ranges suggested by most sports organizations' recommendations. The ISSN recommends ingestion of proteins ranging from 1.0g/kg/ day to 2.0g/kg/day depending on the level of exercise intensity. ACSM recommends values ranging from 1.2g/kg/day to 1.7g/kg/day. All these recommendations contrast with the *European Food Safety Authority* (EFSA) reference values for protein intake for the general population, which is set at 0.83g/kg/day of protein (EFSA, 2012). This EFSA value is increasingly contested as it is found to be inadequate in certain population groups such as the elderly suffering from lean mass loss (Egan, 2016). In the case of protein intake within 30 minutes of exercise, there is a consensus between ACSM ISSN and IOC. All these organizations recognize that ingesting 20g of protein after exercise (up to 30 minutes later) helps the athlete's physical recovery and saves lean mass (Potgieter, 2013). It is concluded that the values nowadays recommended for the ingestion of proteins are increasing which raises a historical concern, the adverse effect of the proteins in the renal

system. Despite this, there are no studies to prove the deleterious effect of a "rich" protein diet on the kidneys. Thus, for all intents and purposes, there is no strong evidence that increased dietary protein, even for values above 2.0g/kg/day, is harmful to the body (Egan, 2016). In addition, protein intake before exercise seems to maximize MPS since a highest amount of EAA is present in the acute phase after exercise in the bloodstream (Sousa, Teixeira, Graça, 2016).

Lipid intake

Athletes' lipid needs do not differ much from the needs of non-athletes. Being a macronutrient that aims to provide energy to the body, ACSM recommendations suggest that lipid intake is between 20% and 35% of TEN. Values below 20% of TEN are not recommended since lipids are essential for the absorption of fat-soluble vitamins and essential fatty acids, as well as being essential for the proper functioning of the immune system. The ISSN indicates values of 30% of NETs and the IOC recommends that the values should never be less than 15-20% of NETs. All organizations agree that diets rich in "fats" (> 30% of NETs) are detrimental to athletes and impair athletic performance. Ingestion should consist primarily of foods high in unsaturated fats (Potgieter, 2013).

Fluid intake

Regarding fluid intake the focus is on the loss of 2% of body weight through sweat. Several studies have shown that losses of this magnitude or greater significantly decrease physical *performance*. This loss of fluids is easily determined by weighing the athlete before and after exercise. This loss of fluids varies according to the athlete, which can release more sweat compared to others and depending on the environmental conditions being more intense in hot and / or humid environments. Another aspect to take into consideration is the loss of sodium that accompanies the release of sweat. It is recommended that during and after exercise the athlete consume drinks with electrolytes namely containing sodium, essential electrolyte in the maintenance of water balance and homeostatic parameters such as blood pressure, thus allowing to achieve euhydration more quickly. Although less frequent, athletes should not ingest too much fluid during practice/competition. The state of overhydration leads to dilution of serum sodium and hyponatremia whose most immediate symptoms are headache, swollen extremities, fatigue, disorientation, confusion, and wheezing (Potgieter, 2013), (Sousa, Teixeira, Graça, 2016).

1.1.3 – Related to physical exercise

The practice of physical exercise is critical for maintaining a healthy body. The various sports require the practitioner a myriad of body movements that lead to the use of different energy pathways of the body. These energy pathways also determine the muscle fibers that are recruited. In general, exercise can be divided into *endurance* exercise and resistance exercise. The *endurance* exercise can also be divided into *high intensity interval training* (HIIT), characterized by short and intermittent "bursts" of vigorous movements interspersed with low intensity movements for recovery, and the traditional *endurance* characterized by continuous submaximal muscle contractions to increase the body's oxidative capacity. The HIIT exercise translates into advantages over traditional *endurance* when the goal is to obtain oxidative (aerobic) conditioning while developing some MS and explosive power. Resistance training on the other hand, aims only at gaining MS, MH and explosive power and is characterized by maximum muscle contractions (Pim Knuiman, Maria Hopman, Marco Mensink, 2015). It is a type of exercise that primarily resorts to the anaerobic energy production pathways, such as the lactic (glycolytic) pathway and the alpathic pathway (from creatine phosphate). Depending on the type of sport practiced, the body may perform more aerobic exercise (long distance runners), anaerobic (weightlifting, bodybuilding) or resort to both concurrently (soccer, volleyball, basketball) (Baechle, 2008).

Endurance exercise is vital in all sports because it allows the athlete's conditioning. *Endurance* training consists of a large number of repetitions of movements with submaximal muscle contractions, which results in the practice of low intensity and high-volume training (Pim Knuiman, Maria Hopman, Marco Mensink, 2015). This means that aerobic exercise induces a series of essential cardiovascular adaptations in any sport, even if it is not subsequently requested to do that sport. Some of the adaptations that aerobic exercise allows, consist of an increase in cardiac output due to an increase in cardiac volume, with the heart rate reducing at rest. It is not uncommon in highly conditioned athletes a heart rate between 40 and 60 beats per minute, also known has induced bradycardia. In addition, there is an increase in capillary density in muscle fibers that supports a more efficient transport of O_2 and removal of CO_2 (Baechle, 2008). Mitochondrial biogenesis is another important adaptation of this type of exercise (Pim Knuiman, Maria Hopman, Marco Mensink, 2015). In the case of skeletal muscles, the most recruited fibers are type I fibers, since they have a lower activation threshold than type II fibers, so that the practitioner develops the oxidative

capacity on a larger scale. There is also a selective hypertrophy of these fibers, although this hypertrophy is not as evident as that of type II in resistance training (Baechle, 2008).

In resistance exercise, whose primary route of energy is the anaerobic route, the exercises consist of sessions of high intensity and low or moderate volume. Athletes perform movements, usually weight lifting or body weight, in which maximum contractions occur. This type of training, focused on the generation of MS, induces a set of neuronal adaptations that start in the cerebral cortex and are expressed later in changes in the corticospinal tracts of the spinal cord and ultimately in the motor units. The final result of these neuronal adaptations is a greater amount of action potentials with activation of a greater number of motor units and greater recruitment of muscle fibers. Muscle fibers have different recruitment thresholds, and type II (rapid contraction), responsible for the generation of greater strength and explosive power, have higher thresholds. As in these exercises more action potentials are generated, these have an additive effect which is expressed by recruitment of these higher threshold fibers and consequently more contractile force is generated. For the same reason, intense resistance training increases the size of muscle fibers (MH) and their responsiveness in a later effort, since all or almost all muscle fibers are recruited in order to generate force for lifting heavy loads in contrast with the *endurance* exercise (Baechle, 2008). In summary, resistance training has the ability to induce MH, detectable by increasing cross-sectional area (CSA) and MS by increasing myofibrillar volume, predominantly in Type II fibers (Pim Knuiman, Maria Hopman, Marco Mensink, 2015).

1.2 Muscular Hypertrophy and Muscular Strength

In the last two decades, it has been possible to determine with precision and accuracy the development of musculoskeletal tissue size and strength. Imaging methods such as magnetic resonance imaging, computerized tomography, and dual energy X-ray densitometry are considered the *gold standard* in measuring variables such as muscle CSA. Other techniques such as the radioactive tracer screening technology in amino acids allows the evaluation of protein *turnover* in skeletal muscle. In conjunction, these techniques and methods have expanded knowledge of muscle cell and molecular biology and paved the way for determining the underlying mechanisms of muscle accretion and adaptation associated with physical training and nutrition (Rennie, Wackerhage, Spangenburg, Booth, 2004). In this chapter, the signaling pathways associated with increased muscle mass, the influence of nutrition and exercise on MH, the MS concept and aspects influencing it will be addressed.

1.2.1 – Muscle growth - Signaling pathways

Muscle growth cell signaling pathways contain numerous proteins with various functions. Some of these proteins are hormones, others are transcription factors and others are enzymes. The most well-studied signaling pathways are the Protein Kinase B pathway / mechanistic target of rapamycin (Akt / mTOR), which acts as a positive signal for muscle accretion and the myostatin-Smad3 pathway that acts as a negative signal, ie of muscle degradation. The Akt / mTOR pathway is particularly involved in adult MH in response to resistance exercise. The constant overload induced by resistance training seems to frequently activate this pathway that results in MPS. For this path converge others that are activated by different stimuli. One is for example the presence of amino acids in sufficient amounts, which activate the mTOR pathway directly. Some of the complex pathways that regulate muscle growth are described in Figure 3 (Schiaffino, Dyar, Stefano Ciciliot et al. 2013).

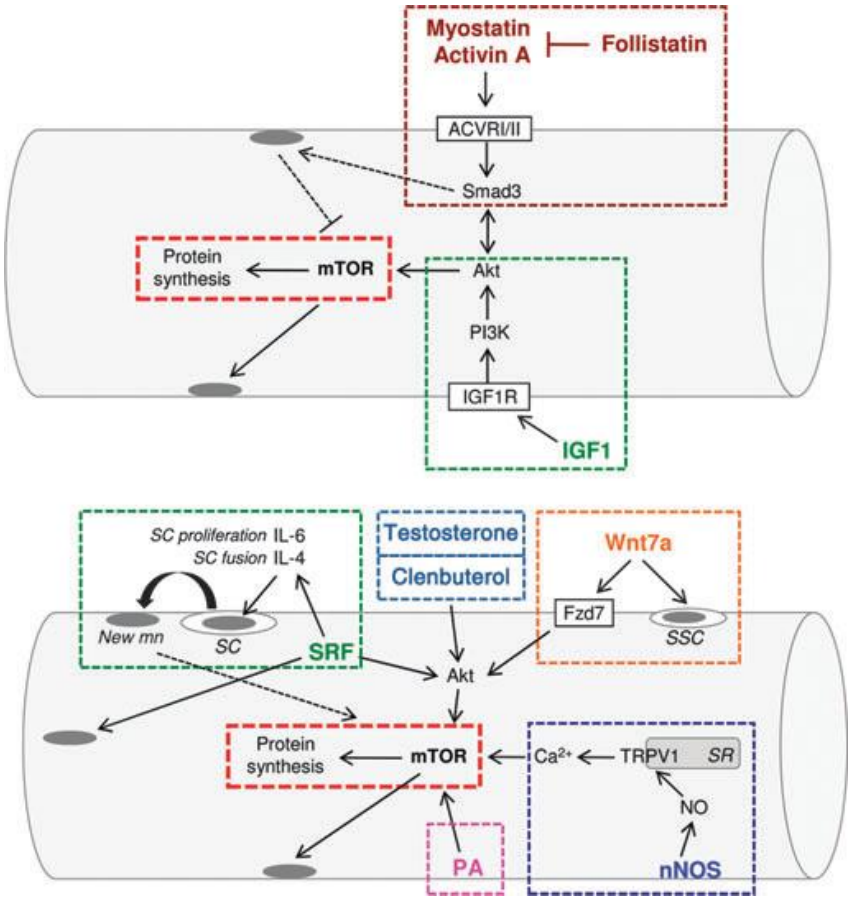

Figure 3 - Signaling pathways that regulate muscle growth in the adult during regeneration or mechanical overload. From (Schiaffino, Dyar, Stefano Ciciliot et al. 2013).

Insulin-like growth factor 1 (IGF-1) is one of the most important proteins in MH. Produced mostly in the liver in response to hormonal signals such as growth hormone, insulin and testosterone, it induces the activation of the PI3K-Akt-mTOR pathway in skeletal muscle. The regulation of IGF-1 production is dependent on regulation of gene transcription, and it is known that increased expression of this protein in rats diminishes age-associated sarcopenia (Rennie, Wackerhage, Spangenburg, Booth, 2004). Activation of mTOR leads to the interaction of this protein with others resulting in the formation of two complexes, mTORC1 and mTORC2. Only the first complex, mTORC1 plays a major role in MPS. MTORC 1 appears to activate a translation factor, the S6 Kinase 1 protein (p70S6K) without which MPS does not occur. This translation factor binds to the S6 ribosome by phosphorylating it and activating it. From there and if there are available amino acids, MPS can occur. This positive effect is counter-regulated by activated kinase AMP (AMPK). Signaling in p70S6K after a muscle training session after 6 to 24 hours and the occurrence of MPS after 12 to 24 hours were reported in rats. In contrast, when there is an increasing

demand for energy AMPK pathway is activated, resulting in the decrease of this process (Schiaffino, Dyar, Stefano Ciciliot et al. 2013), (Rennie, Wackerhage, Spangenburg, Booth, 2004).

In figure 3 it is possible to identify another important pathway in the regulation of muscle growth, the myostatin pathway. This protein, belonging to the superfamily of growth factors β (TGF β), is produced in the skeletal muscle and its action is mediated by transcription factors such as the Smad3 factor. The exact mechanisms of inhibition of MPS by this route are not known, but it is believed to interfere with the Akt-mTOR pathway.

Other pathways elucidated in the image may also contribute greatly to muscle growth. It is the case of the serum response factor (SRF) whose function is the activation of genes involved for example in the expression of cytoskeletal and sarcomere proteins. It is therefore a transcription factor that, in addition to the activation of these genes involved in muscle structure, also appears to exhibit an effect on the proliferation and fusion of satellite cells via release of interleukin 4 and 6 (IL-4 and IL-6 (Schiaffino, Dyar, Stefano Ciciliot et al. 2013). Satellite cells are cells present in skeletal muscle that have been given much relevance in the study of MH since they are thought to be responsible for the increase in the number of myonuclei observed in muscle fibers, essential during MH, and may potentially be responsible by a more controversial process, muscle fiber hyperplasia (Rennie, Wackerhage, Spangenburg, Booth, 2004). The androgens and β2 agonists are known to be potent stimulators of Akt / mTOR pathway and nitric oxide synthase (nNOS), responsible for nitrogen monoxide production, which vasodilator effect is well known, increases the release of Ca^{2+} that activates the mTOR pathway in response to mechanical overload (Schiaffino, Dyar, Stefano Ciciliot et al. 2013).

MH appears to be related to the activity of so-called satellite cells. As noted earlier, satellite cells, located at the periphery of multinucleated muscle fibers, are responsible for increasing the number of myonuclei of muscle fibers as they grow. They are undifferentiated, mononucleated cell populations with great mitogenic capacity and are in a quiescent state. At the time of extensive muscle damage or postnatal development, satellite cells are very active, proliferating and fusing to form new myonuclei (Rennie, Wackerhage, Spangenburg, Booth, 2004). Studies in mice have clearly demonstrated the function of these cells and their role in MH, however, there is much controversy as to the ability of muscle to generate new

muscle fibers from these cells (hyperplasia) as well as their absolute need for MH to occur. The hypertrophic effect of clenbuterol does not involve the activation of these cells and the late phases of postnatal muscle development also do not resort to satellite cells. In contrast, the use of testoesterone and other anabolics stimulate the proliferation and fusion of these cells. The usual and gradual load on the muscles does not induce cell proliferation, however the use of intense eccentric exercises that provoke cellular damage or the ablation of synergic muscles induce an acute mechanical overload that immediately stimulates the proliferation and fusion of satellite cells. The evidence from the different studies thus suggests that there are two modes of MH. One involves the response to an acute stimulus with muscle damage involved, such as intense eccentric exercises and ablation of synergistic muscles that activate satellite cells, and another way in which slower adaptations are present such as the more gradual overload exercise that does not involve muscle damage and consequently satellite cells. Thus, even in the absence of satellite cells, the occurrence of MH is possible, via MPS alone, which demonstrates the adaptive capacity of skeletal muscle tissue (Schiaffino, Dyar, Stefano Ciciliot et al. 2013).

1.2.2 – Physical exercise and its influence on MH

In a study in 2006 by Seynnes, Boer, and Narici, published in the academic journal, *Journal of Applied Physiology*, they reinforce the growing evidence that physical resistance exercise has an anabolic effect and contributes to MH from an early age. This study elicited seven active young adults to participate in a resistance training program for 35 days in which they performed concentric and eccentric movements of the femoral quadriceps. Variables such as CSA (magnetic resonance imaging), maximal voluntary contraction (MVC) using an isokinetic dynamometer, electromyographic activity of some muscles of the muscle group under study, and the muscular architecture of the *vastus lateralis* muscle *in vivo* through ultrasound were measured. In all measured variables there were modifications that suggest the occurrence of muscular accretion in only 5 weeks of resistance training. The participants CSA increased as well as neuronal activity, evidenced by electromyographic activity. MVC increased substantially (corresponding to increased muscle strength) but there is no linear correlation between MVC increase and electromyographic activity, which means that although much of the increase in muscle strength is due to neuronal adaptations (more motor

fiber units activity with more muscle fiber recruitment) part of this increase must be explained by the effect of MH that occurred during the study. Another interesting aspect of the study is the observation of architectural modifications of the muscle. The remodeling of muscle fibers is evidenced by the analysis of the *vastus lateralis* which demonstrates a greater pennation angle and greater length of the fascicula, both associated with adding more sarcomeres both in series and in parallel to the muscle fibers (Seynnes, Boer, Narici, 2006). Through this study and others similar, it is now well established that systematic resistance training induces increased strength and muscle size very early, from 5 weeks of training. Other studies attempt to understand how this hypertrophic response occurs in individuals of different ages. In 2016 a study was published by Finnish researchers in the journal of the *American Aging Association* which demonstrates the effect of this covariate on muscle accretion in a training program of 24 weeks (6 months) in ages from 19 to 78 years. Another objective of the study was to quantify individuals who respond above average to resistance training, as well as those who respond below average, thus attempting to elucidate which phenotypic characteristics may influence the resistance training response. They used a control group who did not perform the training program, continued their daily activities normally and performed measurements of the same variables. The study revealed that there is great variability regarding gains in muscle strength, however, there are no significant differences between men and women. Many of the individuals gained muscle strength when compared to the control group who did not perform the training program. Again, this study corroborates previous findings demonstrating that muscle strength derives primarily from neural adaptations at the beginning of resistance training, since it exists a small correlation between increased muscle size and muscle strength in the first few months of training. As for the hypertrophic effect, the study concludes that there are similar responses between groups of different ages and sex. Regarding the training response, some individuals did not respond with great force gains (about 7%) and muscle mass (about 30%), but only 9 out of 283 individuals did not respond with gains in strength and muscle mass concomitantly. These findings allowed researchers to state that all age groups benefit from endurance training and that the hypertrophic response is similar between genders and ages. The heterogeneity of the response is also similar among the different groups, so the resistance training should be customized according to the response of each athlete (Ahtiainen, Walker , Peltonen, 2016).

Resistance training and its anabolic effect is well established, however there is no consensus as to when the MH occurs. The studies indicate varied periods, but it is accepted that the hypertrophic effect has a relatively long latency time. Some studies dispute this assertion as is the case of the works already quoted from Seynnes et. al. In contrast, a recent study conducted by Felipe Damas of the School of Sports and Physical Education of São Paulo, Brazil and published in 2015 in the Journal *of Applied Physiology,* suggests that increased muscle CSA in the first weeks of systematic resistance training in young adults may be related to muscle edema caused by muscle damage associated with resistance training. They developed a 10-week endurance training experiment of the lower limbs and evaluated variables, in this case CSA, muscle edema via ultrasound technique, MVC, and muscle injury markers such has IL-6 and myoglobin, through the collection of blood samples. The researchers determined that there is a marked increase in the first three weeks of muscle CSA, but also a very significant increase in muscle edema as well as muscle damage markers, IL-6 and myoglobin. This allows to conclude that in fact part of the increase in muscle size, at least in the first weeks the training are "... *attributable to induced muscle edema, probably resulting from muscle damage ."* and any increase in muscle size should not be "labeled" MH without simultaneously measuring muscle edema (Damas, Phillips, Lixandrão, 2015).

Endurance exercise, as opposed to resistance exercise, seems to interfere with MH. Over the last 30 years it has become axiomatic that performing "aerobic" exercise along with resistance training interferes with the hypertrophic adaptations of resistance training. This type of training, called "concomitant training" in which the athlete performs endurance training and *endurance* training in the same session seems to obtain smaller hypertrophic results than only resistance training alone. In a study conducted by Greek and Danish researchers in moderately trained young women, the interference of the low intensity run on power training was evaluated. Participants were randomly allocated into two groups, one performing only power training and the other performing power training followed by 30-minute low-intensity running at about 60-70% of their maximum heart rate. Measured variables were 1-maximal repetition (1RM), jumping performance, isometric strength and strength development ratio, estimated aerobic capacity, and speed of conduction of muscle fibers. It was concluded from this study that in fact the power gains are lower in the group that performed concomitant training with lower values of 1RM, isometric strength and strength development ratio, and the group that performed concomitant training increased their aerobic capacity, that is, resistance training does not seem to interfere with the gains produced by the training of *endurance* (Terzis, Spengos, Methenitis, 2016). The researchers suggest that this interference effect may result from activation of AMPK signaling pathway, which inhibits mTOR pathway, induced by low levels of glycogen and therefore inhibits MH (Terzis, Spengos, Methenitis, 2016) (Wilson, Marin, Rhea, 2012).

In contrast, many other studies seem to challenge the general acceptance of an interference effect from *endurance* exercise. In fact, most studies showing an interference effect by *endurance* training on the hypertrophic adaptations of resistance training have limited designs (animal vs. human models, unrealistic training programs, etc.). In addition, any interference effect found at the cellular level is usually acute (minutes or hours) which may not translate into the usual training responses that take weeks or months. The body of evidence in favor of concomitant training has grown in recent years and these studies increasingly emphasize the need to perform this type of training program at different times and in low volumes so as not to fatigue the muscle groups involved (Murach, Bagley, 2016).

1.2.3 – Physical exercise and its influence on MS

The term "muscle strength" has been used colloquially throughout this work, however the term should be used according to its scientific definition in order to avoid ambiguity. Thus, we can define MS as the ability to exert mechanical tension at a given velocity, ie, MS is the product of the mass of a body and its acceleration (*a*) being acceleration defined as the change of velocity over time, also known as Force ($F = ma$). When we measure power we are in reality measuring Force, although in the case of power what is reflected is the ability to exert Force at a given speed in a given time (*t*) being the power defined as the product of the Work (*W*) performed and the elapsed time ($P = Wt$). Work is the product of the Force produced and the displacement (*s*) of an object ($W = Fs$). Next, the biomechanical factors involved in MS manifestation are discussed (Baechle, 2008).

One of the factors that have more weight in the development of MS, especially in the first weeks of resistance training are the neuronal adaptations. The term "recruitment" defines the body's ability to recruit muscle fibers for a particular effort and this recruitment is dependent upon the activation of motor units by the nervous system. As the training program progresses, especially in the first few weeks of training, a substantial gain in MS usually occurs, and it becomes more attenuating later on. This abrupt gain in MS at the beginning is due to the fact that the brain “remembers” and activates more motor units via a greater number of efferent signals sent to the muscles. Several studies already cited here demonstrate this principle as it is the case of the works of Seynnes et al. or Ahtiainen et al. (Seynnes, Boer, Narici, 2006) (Ahtiainen, Walker , Peltonen, 2016).

The CSA is another factor related to the capacity to generate MS. The CSA reflects the number of myofibrils and consequently the number of parallel sarcomeres available in the muscle. The more sarcomeres in parallel the greater the contractile force generated. Thus, the maximum contractile capacity of a given muscle is proportional to its CSA. In a study of 2001, Aagaard and colleagues studied the correlation between different aspects of muscle architecture such as the pennation angle or the anatomical CSA and the MS exercised, demonstrating precisely that there is a strong correlation (pennation angle and CSA increases also increase MS) adding that resistance training increases all these variables (Aagaard, Andersen, Dyhre-Poulsen, 2001).

The way the muscle fiber is organized also influences the ability to generate force from the muscle. The pennation angle corresponds to the angle between the muscle fibers and an imaginary line between the origin and the insertion of the muscle. Pennation angle is one of the most important aspects since the higher the pennation angle of the muscle the more muscle fibers are in the muscle and consequently more sarcomeres in parallel are present. This factor can vary with training and depends on hereditary factors which explains that many individuals with the same size of muscle have different speeds and forces. Muscle CSA also allows the determination of the capacity to generate force (Baechle, 2008).

1.3 Sports Supplements and *Whey* Protein

1.3.1 – Food supplements - legal framework

Food supplements in general are considered safe products for human consumption and are defined as *", food genre intended to supplement and/or supplement the normal diet and constitute concentrated sources of certain nutrients or other with nutritional or physical effects, alone or in combination, marketed in dose form, such as capsules, pills, tablets, and other similar forms, powder sachets, liquid ampoules, bottles with dropper and other similar forms of liquids or powders which are intended to be taken in units of reduced quantity*.. (Decreto Lei 136/2003 de 28 Junho). These products only need to be properly labeled according to what is defined in the law and be notified to the competent authority when it is marketed, in this case to the Food and Veterinary Office, under the supervision of the Ministry of Agriculture. The Portuguese law defines maximum limits only for micronutrients, vitamins and minerals, which means the manufacturer or importer of the food supplement can market its product with the amount of macronutrients that it considers appropriate. Despite all these apparent "facilities" in the marketing of food supplements, manufacturers or importers cannot advocate prophylactic or curative properties of human diseases to products. (Decreto Lei 136/2003 de 28 Junho) Despite this, dietary supplements may contain health claims. This is the case with most sports food supplements containing claims of ergogenic effects. In 2010 it is issued at the request of the European Commission, the scientific opinion of the panel of dietetic products, nutrition and allergies of the EFSA on the various health claims associated with *whey* protein. In all claims, which include growth and maintenance of muscle mass, increase of lean mass during calorie restriction and resistance training , reduction of fat mass during caloric restriction and resistance training , increased MS, repair of skeletal muscle tissue and rapid recovery of muscle fatigue after exercise, the EFSA panel concluded that there is insufficient evidence to state that there is a causal relationship between *whey* protein and the claims, however it assumes that the studies performed demonstrated a positive influence in all these respects (EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), 2010).

1.3.2 – Market trends of protein supplements

Sports supplements and more specifically protein supplements have been gaining more and more supporters, which is reflects in market growth. Once sought only by professional athletes, foods containing high amounts of protein in general and protein supplements in particular, are now being used by a growing number of different age groups, most notably the younger groups between the ages of 20 and 40 years. At a seminar held in April 2014 (Protein Trends & Technologies Seminar) in the United States, the analyst at *Euromonitor International*, Chris Schmidt, a specialist in consumer health, presented a set of statistics and trends that accurately reflect this market growth. According to the data presented, protein supplements have grown steadily between 2008 and 2013 in the United States and around the world, with a new record for values in 2018. In this report it is also interesting to note that manufacturers and brands begin to change the image of these products in order to reach other " *targets* " in the market and not only the athlete. Protein supplements are increasingly associated with an image of "health benefits." The ability of protein supplements to increase satiety is directed primarily to women who wish to lose weight, or to more advanced age groups, because of the maintenance effect of muscle mass, avoiding sarcopenia and even the cosmetic effects associated with " *Anti-aging"* properties. These new marketing strategies by brands and manufacturers are related to data also presented at this seminar that demonstrate an aging population that is becoming more and more concerned about their health (Chris Schmidt, 2014).

1.3.3 – *Whey* protein - processing and composition

Products containing *whey* protein are known not only by professional athletes but also by the population that regularly practices sport and desire a healthy lifestyle. It is naturally more sought after by the younger age groups whose goal is to increase muscle mass, maintain lean mass, feel satiated, or decrease fat mass. The *whey* is a byproduct of cheese production. During milk coagulation two by-products are obtained, the curd used to produce the cheese and the serum, the liquid fraction of the process. This serum contains the *whey*, whose final destination was the feeding of livestock, fertilization of land for agriculture or simply discarded. The commercial potential of *whey* was recently discovered, mainly due to new processing techniques. From the 1970s onwards with the introduction of new processing

techniques such as ultrafiltration, nanofiltration, electrodialysis or reverse osmosis, powdered *whey* products were available which could now be used for a wide variety of purposes. The first products were obtained with ultrafiltration techniques whose product is termed " *whey* protein concentrate ". This concentrate may contain from 20 to 89% proteins, the remainder being lactose, mineral salts and fatty acids. This product can be used in human food to add a source of protein to processed products, however due to its huge variability in the amount of protein is not used in sports supplements. With other additional techniques such as electrodialysis or reverse osmosis for example, it was possible to remove the lactose and demineralize the *whey* protein concentrates by obtaining the so-called " *whey* protein isolates ". These isolates, whose minimum amount of protein is 90%, due to their purity, ability to solubilize easily in water and lack of taste or smell is suitable for use in sports drinks and supplements (Onwalate, Huth, 2008).

Whey protein is curiously not a protein, but a mixture of several proteins. Bovine milk (Figure 4), from which *whey* containing products are produced, consists of about 50% of the protein, β-lactoglobulin (β-LG), rich in the amino acid cysteine. The other abundant protein present in bovine milk is α-lactalbumin (α-LA), which binds with calcium and is rich in EAA's, namely branched chain amino acids (BCAA) such as leucine, isoleucine and valine, responsible for the molecular signaling involved in the MPS. This is also the protein most present in human milk, and it is used in infant formulas.

Component	Concentration (g/L)
Water	873
Fat	37
Lactose	48
Ash (minerals and salts)	7
Casein	28
Whey proteins	6.0
β-Lactoglobulin	3.2
α-Lactalbumin	1.2
Bovine serum albumin	0.4
Immunoglobulins	0.8
Lactoferrin	0.2
Lactoperoxidase	0.03
Enzymes	0.03

Figure 4 – Composition of bovine milk. From (Onwalate, Huth, 2008).

In addition to these two proteins, it is found that bovine whey contains many other proteins with the most varied physiological effects. Many of these proteins interact with the immune system and in athletes who train intensively there is an immunosuppressive response. It is theorized that many of the protein components of *whey* may be involved in the recovery of the immune response. In a literature review of the *Journal of Nutritional Biochemistry* conducted by Ha and Zemel, several examples of the bioactivity of these proteins are pointed out. Lactoferrin, for example, appears to have an antimicrobial role, or the amino acid glutamine present in *whey* is associated with the rapid cellular division that occurs in situations of metabolic stress, caused by disease or strenuous exercise. Other beneficial effects are pointed to *whey* components such as improved bowel function and antioxidant effects (Onwalate, Huth, 2008) (Ha, Zemel, 2003).

1.3.4 – Supplementation with *whey* protein

Since the beginning of the century, numerous studies have been developed in the field of sports supplementation, namely protein supplementation. These studies allowed the establishment of some concepts considered key to athletes and coaches. Some of these concepts such as the leucine threshold or the "anabolic window of opportunity" allow to customize protein intake according to the goals of the athletes.

MPS is recognized as one of the dominant factors in the remodeling of skeletal muscle tissue and consequent increase in muscle mass. Other phenotypic adaptations such as repair of damaged muscle proteins also contribute to the increase of muscle mass, however MPS is considered the key factor of the whole process. In an untrained and healthy individual, MPS is in balance with muscle protein breakdown (MPB). The individual has a balanced nitrogen balance and consequently preserves muscle mass. For an accretion of muscle mass, the nitrogen balance must be positive which means that MPS should increase and MPB should remain equal or decrease. The two major stimuli of MPS are physical exercise and diet, rich in proteins. Physical exercise increases MPS, but also MPB. MPB is also increased in other circumstances, such as hypocaloric and protein-poor diets and cases of incipient sarcopenia associated with advancing age (Devries, Phillips, 2015). The high protein diet increases MPS both at rest periods and following a physical exercise session in the recovery period. Thus, protein supply is a key stimulus in preserving muscle mass during rest periods and increasing muscle mass in training periods. This MPS stimulus is closely linked to the availability of amino acids at the intracellular and extracellular levels. This bioavailability is

in turn influenced by the source, amount (at a single dose) and time of protein intake as well as the pattern of intake of other macronutrients (Witard, Wardle, Macnaughton et al. 2016).

Whey, Casein and Soy

The most common comparison that is performed in studies of intact proteins is between *whey* and casein, another milk protein. Casein is practically insoluble in water and is therefore subject to alkaline treatments, which alters its solubility, however, whether in the micellar form or in the form of a more soluble salt, casein is a more difficult protein to digest which alters its bioavailability. The *whey* is a "protein" with acid character which allows greater digestibility and consequent rapid exit from the stomach into the intestines, resulting in faster plasma amino acid concentrations. In addition to this feature it is noted that *whey* contains a high content of BCAA's, namely leucine, which has already mentioned, is considered crucial in the signaling of MPS pathways. Thus, the concept of "protein quality" which refers precisely to these parameters: digestibility, BCAA's, EAA's and bioavailability. *Whey* is considered a high quality protein compared to casein because it is more palatable, it has a EAA's (particularly BCAAs) largest and fastest plasma peak (crucial in post-exercise) and has greater bioavailability, being dubbed has "fast absorption protein " while casein is dubbed" slow acting protein. " It should be noted that, *whey* is obtained, as already mentioned, from dairy products and therefore is an animal protein. *Whey* is often compared with soy protein, a vegetable protein. Vegetable proteins are generally considered to be of "inferior quality" and not complete since they do not have all the EAA's, however soy protein is a complete protein having all of the EAA's *whey* and casein possess. Despite this, in studies comparing *whey* and soy protein supplements, the latter does not stimulate MPS to the same level of *whey* since its content of EAA's in general and of leucine in particular are lower. In addition, the bioavailability of soy protein is lower than that of *whey* and many amino acids are directed to catabolic processes of urea synthesis and oxidation processes (Devries, Phillips, 2015). These aspects are especially important for vegetarian athletes who should review the amount of protein to be eaten since the general recommendations of international organizations may not be sufficient for this group of individuals.

The leucine threshold and the role of *whey*

A concept that also emerged in the previous decade and is now established is the "leucine threshold". Several studies have determined that in order to have a significant MPS stimulus, plasma levels of leucine must reach a certain value. This value varies according to the level of physical activity and age in a population. It can be manipulated mainly by physical activity reaching a minimum value in well-trained individuals and a maximum value in individuals who do not perform constant physical exercise. Age also tends to increase this leucine threshold (Devries, Phillips, 2015) (Witard, Wardle, Macnaughton et al. 2016). Studies conducted to determine the leucine threshold don't agree, however, it was possible to establish that in the majority of young individuals a similar MPS stimulus is obtained with 25g of isolated *whey* and of 6.25g of isolated *whey* added of 5g of leucine (Churchward-Venne, Breen, Donato, et al. 2014).

Amount of protein and MPS stimulation

About the amount of protein consumed, it is well established that in young adults the intake of 30g of protein (corresponding to a standard portion of beef steak) stimulates postprandial MPS at the same level as 90g of protein (corresponding to a larger portion of beef steak). This suggests that there is a dose of protein saturation ingested for MPS stimulation. The data presented are valid for individuals at rest. For individuals who exercise, this value is not well defined despite the studies showing that there is no statistically significant difference in the stimulation of MPS in the intake of 20g vs 40g of proteins in the post exercise period. Despite this, the ingestion of 40g of proteins induced a 10% increase in mean values of MPS stimulation. The relevance of these data to the increase in lean mass is unknown and requires further research (Witard, Wardle, Macnaughton et al. 2016). Besides this, another more recent study by Macnaughton and colleagues revealed a significant increase in MPS after ingesting 40g vs. 20g of isolated *whey* protein following resistance training (Lindsay, Macnaughton, Sophie, 2016).

Pattern and *timing* of protein intake

Intuitively it would be expected that the intake of proteins before or during physical exercise would result in an increase in bioavailability of amino acids, since during physical stress an increase of MPB and consequent negative balance of nitrogen occurs. Ingestion

during this period, at least theoretically would be beneficial and would also benefit from the fact that there is an increase in blood flow in the skeletal muscle, which would allow a greater availability of amino acids in the muscle. It has been established that in the post-exercise MPB continues to occur for at least some time and the physiological stimulus for MPS is subsequently initiated. It is thought that this period of " *lag* "MPB lasts approximately 1 hour, so it is during this post-exercise period that the muscle is anabolically more sensitive, losing this sensitivity over time (although MPS occurs within 48 hours of a training session). This period of about onehour post-exercise was termed the "anabolic window of opportunity" because in theory the ingestion of a protein/amino acid source at this stage maximizes MPS. Despite this theoretical justification, studies that focus on acute metabolic responses do not always support this notion (Witard, Wardle, Macnaughton et al. 2016). One study demonstrated for example that the MPS response to the intake of EAA's at 1h, 2h, and 3h following resistance training in untrained young men did not show significant differences (Rasmussen, Tipton, Miller et al. 2000). From these data it can be concluded that the so-called "anabolic window of opportunity" may extend beyond 1 hour in the post-exercise. Regarding protein intake before and after exercise, the different studies performed demonstrate a similar MPS response. Of note, a longitudinal study did not detect differences in lean mass gain after 12 weeks of resistance training in groups of older men who did supplementation before or after training (Candow Chilibeck Facci et al. 2006). In an acute metabolic assessment study, Tipton and colleagues evaluated nitrogen balance in a group of healthy subjects divided into two groups, one with *whey* intake before one training session and one with *whey* intake after one training session. They concluded that there were no significant differences between the groups with regard to post-exercise nitrogen balance (Tipton, Elliot, Cree et al. 2007). Thus, skeletal muscle is considered to respond identically whether the protein is administered before or after exercise.

Insulinotropic effect of BCAA's and *Whey*

W*hey* protein has also shown a marked insulin response. This hyperinsulinemia observed after *whey* intake is important since insulin is the anabolic hormone per excellence, influencing the metabolism of carbohydrates. The high BCAA content of *whey* appears to be responsible for this anabolic response. Mikael Nilsson and colleagues compared the insulinotropic effect of different beverages containing different amino acid blends, a *whey*-containing beverage, and as a control beverage they used pure glucose. The results demonstrate a considerable increase in the production and release of insulin in the

individuals with beverages containing BCAA's, however the drink with greater insulinotropic effect was the drink containing *whey*. In addition, the *whey* beverage stimulated the release of glucose-dependent insulinotropic polypeptide (GIP). It is concluded from this work that there are key amino acids contained in *whey* responsible for an insulinotropic effect that in addition to being important for athletes wishing to increase muscle mass may also explain why *whey* is recommended to change body composition, namely lowering fat mass (Nilsson, Holst, Björck, 2007). These results are also interesting for diabetic patients, suggesting that the intake of proteins, particularly rich in BCAA's may aid in disease control.

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CHAPTER II

This chapter is the systematic review conducted for support to the field study of the next chapter. This review has been submitted for publication on 28 of February 2018 to the Journal of Dietary Supplements (JDS) and is written in accordance with the Journal's guidelines.

Adaptations provided by whey on muscle hypertrophy and strength in adult population with or without resistance training. A systematic review.

Abstract

This review aims to analyze the effects of whey protein supplements in hypertrophy and strength metrics of adult population engaged or not in resistance training (RT) compared with other protein sources or isocaloric placebos. Using a systematic approach, studies were searched through PubMed and Web of Science using the terms “whey”, “muscle”, “resistance” and “strength”, in different combinations as keywords. After exclusion of articles a quality assessment was executed resorting to an adapted Risk of Bias tool by Cochrane, converted to the Agency of Health Care Research and Quality standards. Heterogeneity of trial designs demonstrate a wide variety of findings, however most of the studies reviewed report positive differences in hypertrophy and/or strength metrics. Increased duration of trials and higher leucine content of whey result in lean mass, strength and muscle size increase. Other aspects such as increased total protein intake from diet and tailored training programs greatly influence the effects of whey supplementation resulting in greater gains. Whey supplementation in the context of RT can enhance long term phenotypic adaptations, provided some key factors are controlled such as duration, intensity and frequency of training programs, sufficient protein dietary intake and content of supplement in Branched Chain Amino Acids namely leucine.

Keywords: dietary supplementation; exercise program; skeletal muscle; body composition; strength training.

Introduction

Recreational and professional athletes engage in resistance training (RT) to gain muscle mass and strength. It is well established that RT is a powerful stimulus for muscle hypertrophy although the influence of RT depends on volume, frequency and intensity of workouts (Wernbom, Augustsson, & Thome, 2007). Skeletal muscle size is dependent on muscle protein turnover which in turn is controlled by cellular mechanisms comprised of gene activation, transcription and enzymatic pathways. The amount of myofibrillar synthesis results from muscle protein synthesis (MPS) and muscle protein breakdown (MPB), two physiological processes that control the amino acid pool in the muscle. In fed state and other stimulus being absent, MPS and MPB are in balance resulting in no net growth or loss (Rasmussen & Phillips, 2003). It follows from previous studies that one of the main contributors for MPS is the mammalian target (mTOR) intracellular pathway. Activation of this pathway by insulin, RT or amino acid uptake results in mRNA translation and ribosomal upregulation favoring myofibrillar synthesis and consequently muscle hypertrophy (Bolster, Jefferson, & Kimball, 2004) (J. J. Hulmi et al., 2009). Muscle growth and strength gain are some of the most important objectives for athletes and MPS is required for this accretion process. Acute metabolic studies have shown that combining resistance exercise with protein ingestion further stimulates MPS when compared with resistance exercise only (Tipton et al., 2012) (Kakigi et al., 2014). Other acute studies compared the effects of different protein sources combined with resistance exercise on MPS and net protein balance (NPB). Wilkinson, et al. reported a more sustainable raise in blood amino acid availability for milk protein compared with soy protein indicating a greater rate of MPS in regards to milk protein(Wilkinson et al., 2007). Analyzing two different milk proteins, casein and whey, Reitelseder, et al. concluded that both resulted on an equal MPS response after a bout of resistance exercise, although insulinotropic effects and branched chain amino acids (BCAA's) blood availability were higher in the whey group (Reitelseder et al., 2011).

Findings in acute settings may not reflect long term adaptations of skeletal muscle, albeit acute studies clearly established resistance exercise and sufficient protein intake has hallmarks of muscle mass accretion and strength development (Phillips, 2014). More importantly, a growing body of evidence

demonstrates that timing of ingestion of protein is as determinant as the daily amount ingested. Cribb and Hayes conducted a single-blind randomized trial, where two groups consumed the same supplement (mixture of protein, creatine and glucose) but at different periods and reported a significantly greater increase in strength, muscle cross-sectional area (mCSA) and lean body mass in the group consuming the supplement immediately before and after the RT as opposed to the other group ingesting the supplement in the morning and in the evening (Cribb & Hayes, 2006). Previously, Andersen, et al. found a difference in muscle hypertrophy and strength in a double-blind study setting, between a supplemented protein group and a carbohydrate group. All supplements were ingested immediately before and after training and on non-training days in the morning. Fiber CSA only increased in the protein supplement group compared to baseline. Isokinetic peak torque increased, in both groups whereas the effect was more pronounced in the protein group (Andersen et al., 2005). These results highlight the need for a feeding strategy, having practical implications on athlete's nutritional plans.

Regarding the composition of different proteins, it's suggested that BCAA's play an important role in mTOR pathway and MPS, namely the amount of leucine (Churchward-Venne et al., 2012), (Rieu et al., 2007). These findings led to the "leucine threshold" hypothesis, stating that a certain amount of leucine will optimally trigger MPS (Phillips, 2014). The leucine threshold seems to fluctuate depending on aspects such as aging, (Katsanos, 2006) which increases this threshold, and physical activity (Glover et al., 2008), that lowers it.

Whey protein is commercially available as whey protein concentrate (WPC), whey protein isolate (WPI) or whey protein hydrolysate (WPH). WPC may contain between 29% to 89% total protein (Juha J Hulmi, Lockwood, & Stout, 2010), meaning that WPI and WPH may be viewed as improvements to the protein concentration. Whey protein is considered a high quality protein because of its essential amino acids (EAA) and BCAA content (Ha & Zemel, 2003) . Amino acid availability is influenced by absorption in the gut and in this regard whey exhibits a rapid absorption rate hence whey is considered a "fast" protein, allowing a rapid transient hyper aminoacidemia resulting in greater MPS than casein, a "slow" protein (Boirie et al., 1997). In recent parallel study protocols where whey, casein and/or soy are compared, whey promotes a higher MPS response, differences probably related with the rate of digestion,

absorption and leucine content (Tang, Moore, Kujbida, Tarnopolsky, & Phillips, 2009), (Burd et al., 2012), (Yang et al., 2012).

The purpose of this systematic review was to address the most recent clinical studies evaluating metrics of strength and hypertrophy on active adults (already engaged or not in RT) taking a whey supplement and included in an RT program.

Methods

We have conducted a database search on PubMed and Web of Science between September and October of 2017, using the terms "whey", "muscle", "resistance" and "strength", in different combinations as keywords. Search was refined using filters for "english, portuguese and spanish language", "randomized control trials", "last 10 years" "humans" and "adults". After duplicates were removed, studies were screened by title and abstract, and relevant articles were fully revised.

Articles were excluded with the following reasons; Did not had any direct measure of muscle hypertrophy, strength or both (Agergaard, Bulow, & Jensen, 2014), (Dideriksen et al., 2011), (Farnfield, Carey, Gran, Trenerry, & Cameron-Smith, 2009), (P. Reidy et al., 2012) ; Design did not include at least an 8 week uninterrupted RT program (Agergaard et al., 2014), (Dideriksen et al., 2011), (Farnfield et al., 2009), (Boone, Stout, Beyer, Fukuda, & Hoffman, 2015), (Hwang et al., 2017), (P. Reidy et al., 2012) ; Intervention adherence below 80% (Chalé et al., 2013), Design did not consist of a randomized trial (Juha J Hulmi et al., 2010), (Hayes & Cribb, 2008); Statistical analysis did not provided a comparison between groups on the outcomes of interest (Naclerio et al., 2017) ; Article was a conference meeting (Oates et al., 2007) .

Thirteen papers were included in the review and reported findings on strength and/or hypertrophy metrics, with whey protein (as intervention or comparator) in combination with a prolonged RT program (>8wk).

Articles containing combinations of whey protein and carbohydrate or other possible ergogenic supplements were also included in the final analysis, provided the comparator(s) had the same supplement (Fig.1).

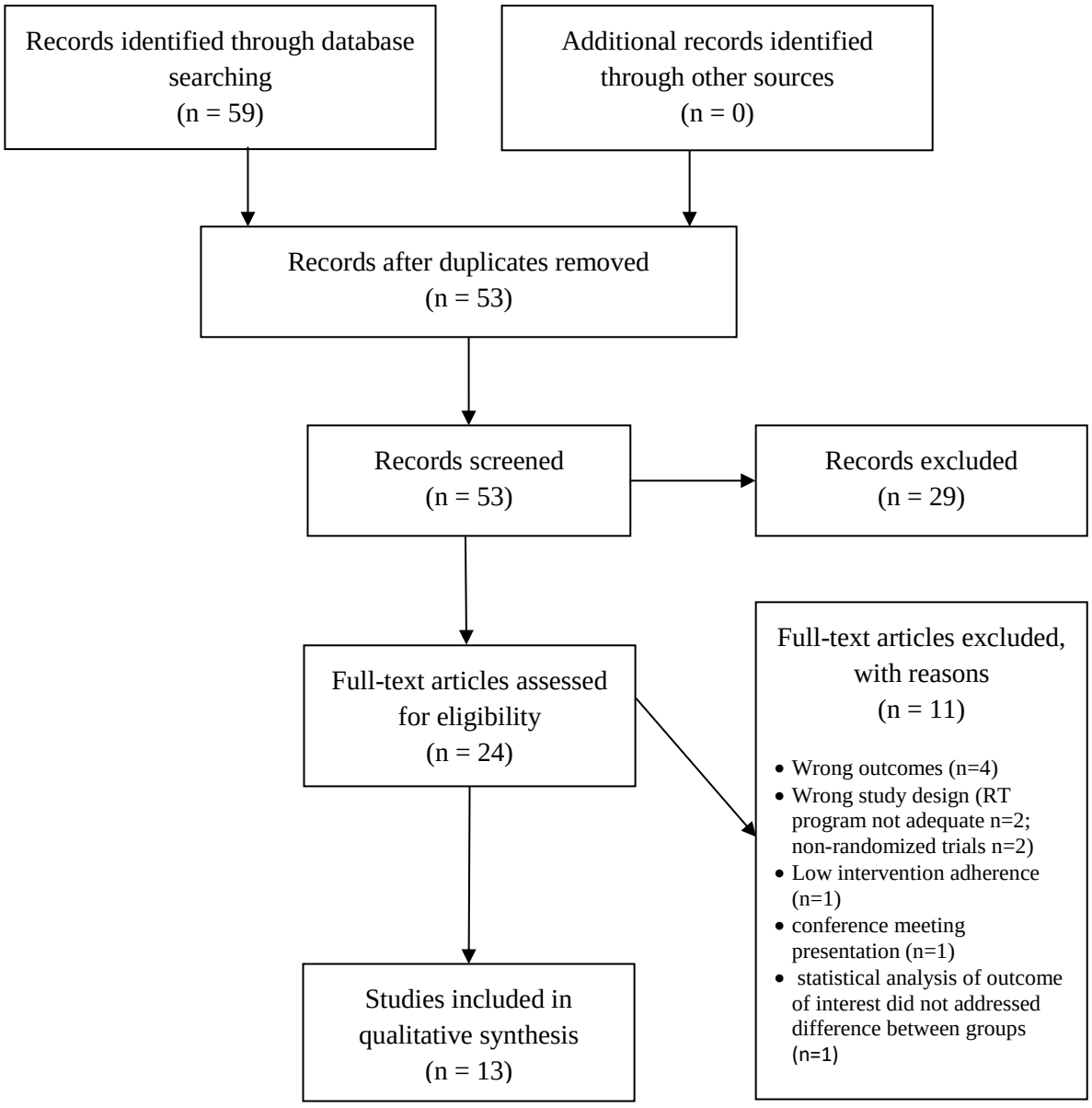

Figure 1 - Flow diagram of articles selected, screened and reviewed

Results

Included studies were evaluated using an adapted Risk of Bias (RoB) tool from Cochrane for Randomized Clinical Trials (RCT's) and converted to the Agency of Health Care Research and Quality (AHRQ) standards. Most studies were conducted in young untrained males, two in males and females and two included older adults. Nine of the studies included outcomes of strength and direct hypertrophy metrics, considering that lean body mass (LBM) is an indirect measure of muscle hypertrophy. Heterogeneity in training protocols is common in these studies, however all of them except for one whose protocol was not described in the article, are designed as progressive, increasing intensity throughout the weeks and usually adjusted between 60 to 80% of 1Repetition Maximum (1RM). Most participants did a twelve-week program, two did an eight-week program and some studies were as long as twenty-one weeks. Diet control was present in nine studies and supplement intake although variable was not inferior to twenty grams of protein. Results are displayed in table 1.

Regarding muscle thickness and strength as outcomes, Babault et al.(Babault et al., 2015) compared pea protein to whey and a placebo and concluded that there is no significant statistical difference between the groups for muscle mass and strength metrics. Although there was no diet control, the amount of whey ingested greatly exceeds normal recommendations and most likely compensate protein intake below recommended dietary allowance (RDA). The study also demonstrates, by a sensitivity analysis on the weakest participants, a significant interaction group x time but when a post-hoc test was applied, this effect was not related to whey. Interestingly, after 6 weeks of training, only the whey and the other protein group had an increase in muscle thickness outcome. In contrast, a group x time effect was observed for strength and contractile protein content in a study by Cribb et al.(Cribb, Williams, Stathis, Carey, & Hayes, 2007) for whey protein and although not statistically significant, a trend emerged in favor of whey, relative to CSA increases in fiber type, IIa and IIx. An indirect measure of muscle mass is LBM, assessed by Dual-X-Ray Absorptiometry (DEXA), but there were no differences for whey group in this study. Herda et al.(Herda et al., 2013) reported no differences between groups for strength, thigh mCSA and body composition despite using a bio enhanced whey protein (containing additional leucine), albeit one of the groups, which had 55 to 59% less training volume achieved similar strength

and mCSA results when compared to other groups. Another study with a design that included younger and older untrained subjects, strength metrics was tested using two different methodologies and strength was different for eccentric torque in the young group taking WPI (Farnfield, Breen, Carey, Garnham, & Cameron-Smith, 2012). Similarly, strength was augmented by whey combined with RT in trials conducted both in young(Juha J. Hulmi et al., 2009) and elderly(Karelis, Messier, Suppère, & Briand, 2015) as well as hypertrophy of some(Juha J. Hulmi et al., 2009) or all quadriceps muscles(Farup et al., 2014) regardless of contraction mode(Rahbek et al., 2014). Other studies found only a time effect regarding strength(Juha J Hulmi et al., 2015),(Brooks Mobley et al., 2017),(Volek et al., 2013), muscle hypertrophy(Brooks Mobley et al., 2017),(P. T. Reidy et al., 2016) and LBM(Karelis et al., 2015),(P. T. Reidy et al., 2016),(Weisgarber, Candow, & M. Vogt, 2012). Most studies recruited adult active men, but two of them were conducted also in older subjects, some of them women. Examining the results of Karelis et al. there was a significant difference between groups for muscle strength normalized by body weight and LBM but not for total muscle strength. Overall the authors seem to indicate whey was capable of augmenting strength without changing body composition(Karelis et al., 2015). In contrast, Farnfield et al. using a sample of young and older men only detected a difference for eccentric strength in young subjects taking whey. Older subjects didn't show any differences although authors acknowledge that measures of strength may not have been sensitive enough to detect small but meaningful differences in this outcome (Farnfield et al., 2012). The initial conditions of the subjects, namely their training status may be a confounding aspect since it is well established that neural adaptations from RT, greatly influence muscle strength (Aagaard, Simonsen, Andersen, Magnusson, & Dyhre-Poulsen, 2002),(Higbie, Cureton, Iii, Prior, & Warren Iii, 1996). As observed by Hulmi et al. maximal voluntary contraction and 1RM were increased with no supplement influence, in a sample of seventy eight untrained men, who undergone a four week preparation RT period to standardize training status (Juha J Hulmi et al., 2015). Despite the RT preparation period, subjects trained two to three times a week in a total of twenty-eight sessions, which might not be enough to detect an effect by supplementation. Also, Mobley et al. reported no differences in supplementation groups on strength or hypertrophy outcomes in a four-arm trial comparing a leucine, soy, CHO, and two whey supplements (isolate and hydrolysate), but surprisingly, satellite cell count was higher for whey concentrate group

when compared to CHO group(Brooks Mobley et al., 2017). Satellite cells are thought to be responsible for the increase of myonuclei in muscle fibers and can even play an important role in a controversial process, muscle fiber hyperplasia(Rennie, Wackerhage, Spangenburg, & Booth, 2004). Examining thigh CSA, Rahbek et al. detected a significant increase in this outcome for whey group, independent of contraction mode .The training protocol involved only knee extension exercise (CON and ECC)(Rahbek et al., 2014). In contrast some studies with whole body training protocols, don't report significant differences between groups(Cribb et al., 2007; Juha J Hulmi et al., 2015), (Weisgarber et al., 2012). Despite the aforementioned, other well designed whole body training studies determined a whey effect on variables like LBM(Volek et al., 2013) and isokinetic knee extensor peak torque (P. T. Reidy et al., 2016)

Table 1 – Study characteristics included in the systematic review

Article	Population	Training program		Intervention		Diet control	Outcomes	Comments	Quality assessment (AHRQ)
		Duration (Weeks)	Protocol	Suplemment intake (g)	Leucine content (g)				
Babault et al. 2015	161 adult males	12	2-5 sets/15-5RM for 3 arm exercises	50 WPC - AD 50 OP - AD 90 PLA- AD	>3 >3	No	WPC=PLA for muscle thickness WPC=PLA for arm circumference WPC=MVT (CON/ECC/ISO)	1RM updated bi-weekly	Fair
Cribb et al. 2007	33 adult males	11	Standard protocol (Max-OT AST Sports Science)	1,5g.Kg^{-1}.$day^{-1}$of supplement - AD	Not reported	Yes	WPI=CHO for CSA fiber type I/IIa/IIx WPI>CHO for contractile protein content WPI>CHO for 1RM exercises	1RM used to adjust intensity (periodicity not reported)	Good
Farnfield et al. 2012	31 adult males (16 young males and 15 older males)	12	2 sets@50% to 80% 1RM for bench press; leg press; leg extension	26,6 WPI – TD No report for PLA - TD	>3	Yes	WPI=PLA for strength (CON) WPI >PLA for strength (ECC) in young group	50% 1RM in the 1st week increasing to 80% 1RM at 6th week	Fair
Farup et al. 2014	22 adult males	12	leg extension with large number of sets and reps	39 WHD - TD 39 PLA - TD	2,77	No	WHD>PLA for quadriceps mid-level CSA and ∑CSA of quadriceps muscles WHD=CHO for MVC	Progressive training. Within subject design for CON and ECC movements	Good
Herda et al. 2013	106 adult males	8	1-3 sets for LV and 3-5 sets for MV at 80% 1RM for bench press and leg press	40 WPC/BWP - TD 20 WPC/BWP - NTD 27 PLA – TD 54 PLA - NTD	WPC-not reported BWP>3	Yes	WPC=BWP=PLA for thigh mCSA and strength	Recreationally trained sample. BWP was added additional leucine.	Good
Hulmi et al. 2009	31adult males	21	From 2-3 to 3-5 sets and from 15-20 to 5-6 reps throughout 21 weeks	30 WPI – TD 30 PLA - TD	>3	Yes	WPI=PLA for quadriceps CSA WPI>PLA for V.Lateralis CSA WPI>PLA for ISO leg press strength WPI=PLA for strength in other exercises	Whole body training (emphasis on leg press and bench press)	Good

Hulmi et al. 2015	78 adult males	12	Until concentric failure (HS training) or Maximal concentric speed (PS training)	37 WPC - TD 34,5 CHO - TD 37+34,5 WPC+CHO – TD	Not reported	Yes	WPC>WPC+CHO and CHO for FFM.kg^{-1} body weight WPC=WPC+CHO and CHO for leg extensor muscle CSA, MVC and 1RM	Complex training protocol - variation in week blocks	Good
Karelis et al. 2015	67 elderly non frail adult males and females	19	Whole body training 3 sets of 10 reps at 80% 1RM	20 WPI - AD 20 OP - AD	Not reported	No	WPI=OP for all LBM variables WPI>OP for strength normalized by BW and LBM	Effect size on strength metrics higher for WPI group	Fair
Mobley et al. 2017	75 adult males	12	Free weight barbell exercises – 4 sets x 10 reps/6 sets x 4 reps/5 sets x 6 reps in different days of the week	52,6 WPC - AD 50,8 WPH- AD 78,4 OP - AD 4,6 LEU - AD 88,8 PLA - AD	>3	Yes	WPC/WPH/OP= PLA/LEU for TBMM, V. Lateralis thickness, CSA fibers (type I and II), 3RM strength and IMTP WPC>PLA for satellite cell count	A rating of perceived exertion was used to adjust training progress	Good
Rahbek et al. 2014	22 adult males	12	Knee extension (CON and ECC) Progressive 6-12 sets x 10-15 reps	19,5+19,5 WPH+CHO – TD 39 CHO- TD	2-3	No	WPH+CHO>CHO for thigh mid-level CSA (regardless of contraction mode)	Dropouts regarding 1st and 2nd study phases. Recruitment of additional subjects for 3rd phase	Fair
Reidy et al 2016.	58 adult males	12	Whole body resistance training (sets and reps not reported)	22 WPI and PB - AD 44 PLA - AD	2,31 WPI 2 PB	Yes	WPI=PB=PLA for muscle thickness and mCSA and LBM metrics WPI>PLA for isokinetic knee extensor torque	Training program not described in article but in supplemental methods	Good
Volek et al. 2013	63 adult males and females	32	Whole body resistance training (3-5sets;3-15reps)	21,6 WP - AD 20,0 OP - AD 45,2 CHO - AD	2-3 WP 1-2 OP	Yes	WP>OP and CHO for LBM WP=OP=CHO for 1RM bench press and squat	LBM response was similar in men and women	Good
Weisgarber et al. 2012	17 adult males and females	8	Whole body resistance training (3 sets;6-10 reps)	0,3g.Kg^{-1}-WPI - TD 0,3g.Kg^{-1} PLA - TD	2-3	Yes	WPI=PLA for muscle thickness, chest-press strength and lean tissue mass	Drink was taken between exercises; no train or supplement supervision	Poor

RM repitition maximum, *WPC* whey protein concentrate, *OP* other protein, *PLA* placebo, *AD* all days, *MVT* maximal voluntary contraction, *CON* concentric, *ECC* eccentric, *ISO* isometric, *WPI* whey protein isolate, *CHO* carbohydrate, *CSA* muscle cross-sectional área, *TD* training days, *WHD* whey hydrolisate , *LV* low volume, *MV* moderate volume, *BWP* bioenhanced whey protein, *NTD* non-training days, *HS* hypertrophy and strength, *PS* power and strength, *FFM* free fat mass, *LBM* lean body mass, *BW* body weight, *LEU* leucine, *TBMM* total body muscle mass, *IMTP* isometric mid-thigh pull, *PB* protein blend, *WP* whey protein

Discussion

Supplementation with whey as gained popularity over the years, mainly because acute metabolic studies provided a scientific basis which demonstrates that whey is capable of inducing MPS(Witard et al., 2014). These positive effects are related to mTOR signaling pathway activation by whey(J. J. Hulmi et al., 2009) as well as by other stimulus, such as protein dose, quality and time of intake, RT, training status and fed state(Morton, McGlory, & Phillips, 2015). Findings of acute metabolic studies allows extrapolation of whey effects regarding muscle mass and strength for long term adaptations. In this context it is expected that phenotypic modifications will be found with chronic intake of whey protein in combination with RT(Coburn et al., 2006). The papers reviewed approach the problem in similar way, providing two interventions, an RT protocol and whey supplementation, while controlling for confounding factors such has diet and training status very differently. Instruments for determining outcomes are also similar, albeit methods applied might be divergent. Taking this into account, it's demonstrable that there is a lot of heterogeneity, which makes comparison among studies very challenging. Considering this we proceed to discuss some key aspects which may influence the outcomes.

Population: Studies usually use adult males as the population of choice, which might influence some of the outcomes evaluated. Antonio et al. examined the effects of RT and amino-acid supplementation exclusively in a women population, finding no modifications on LBM or other variables of body composition as well as modification on strength(Antonio et al., 2000). In contrast, Volek et al. detected differences in LBM in a population that included men and women, and when analyzing data from women only, LBM increased in a similar way in the whey intervention(Volek et al., 2013). These two studies differ significantly in duration of interventions, since the first one was six weeks while the second one lasted for nine months. It follows that intervention duration is a determinant factor for adaptations to RT(Wernbom et al., 2007) and consequently whey to become apparent. Training status can also influence outcomes, mainly because untrained subjects have an intensive response to RT which may mask the effects of supplementation. As stated in a recent review, MPS responses are longer lived and peak later in untrained state, resulting in greater overall

MPS and hence greater net protein accretion(Damas, Phillips, Vechin, & Ugrinowitsch, 2015). Except for one paper in this review, all the subjects recruited were either untrained or recreationally active, meaning they didn't engage in training protocols with the intent of gaining muscle mass. Interestingly, in the only study of this review were the sample is composed of trained subjects (bodybuilders) it was found a group x time interaction for whey in contractile protein content, 1RM strength for all exercises, and a trend for whey regarding muscle CSA fibers type IIa and IIx, despite a small sample size (5-7 subjects) for each arm of the trial(Cribb et al., 2007).It is noteworthy that this population is in principle less responsive to hypertrophic stimulus, since many of these elements might have achieved a plateau in measured outcomes.

Training Protocol: Another aspect of concern is training programs applied to subjects. Most of the papers reviewed include a whole body training program and while there is superiority for whey group in outcomes like strength metrics(Cribb et al., 2007; Juha J. Hulmi et al., 2009)·(Karelis et al., 2015) or LBM(Juha J Hulmi et al., 2015; Karelis et al., 2015; Volek et al., 2013), most do not found differences for direct hypertrophy metrics like muscle CSA or muscle thickness. Other studies rely on isolated exercises, mainly for arm or knee joints, emphasizing muscle groups such as the muscles of the quadriceps or biceps and triceps. Results from these training programs show increased muscle CSA of quadriceps muscles[38,39] for whey group and increased strength(Farnfield et al., 2012). As expected, gains in muscle size is more evident when following a training program which targets a specific muscle group. In addition, Hulmi et al. also reported Vastus lateralis CSA and isometric leg press strength increase in whey group despite the whole-body training protocol but with emphasis on leg press and bench press. Others didn't found any differences, although hypertrophy metrics relied on muscle thickness of biceps, a smaller muscle with no diet control through the study(Babault et al., 2015), and small duration of trial(Herda et al., 2013). Volume and intensity of protocols also greatly affects gains in size and strength. Since most participants in the studies are novice to hypertrophy and or strength training, most protocols apply a progressive training consisting of 6 to 12 repetitions with workloads starting at 60% of 1RM and 3 to 5 sets in each exercise. The number of repetitions tend to decrease while the number of sets tend to increase, and workloads reach as high

as 80% of 1 RM. These protocols are in line with what is recommended for this type of subjects according to the position stand of the American College of Sports Medicine("American College of Sports Medicine position stand. Progression models in resistance training for healthy adults.," 2009). Some studies refer a frequency of 2 times a week of training and authors promptly admit that this frequency may explain why no difference with whey is detected(Juha J Hulmi et al., 2015; Weisgarber et al., 2012). In summary it appears to be easier to detect effects of whey supplementation when training programs rely on isolated exercises, working specific muscles, namely large muscles such as quadriceps. Additionally, training frequency, volume, and loading should be tailored to the studied population since variability in these training characteristics may affect the capacity to detect small but clear differences between whey and placebo.

Diet control: Caloric intake and macronutrient report must be controlled if a study wants to isolate the effects of whey supplementation. The validity of findings can only be supported if this confounding factors are properly addressed(Bird, Tarpenning, & Marino, 2006), which usually takes the form of diet diaries or questionnaires. All of this methods are inaccurate, biased with a tendency to underreport macronutrient and caloric intake however with proper education of subjects and an intensive follow-up by qualified personnel, reliable date can be obtained(Rodriguez, Di Marco, & Langley, 2009). Of studies reviewed, four recommended that subjects continued with their diet habits without using food logs to control for caloric and macronutrient intake(Babault et al., 2015)·(Farup et al., 2014)·(Karelis et al., 2015; Rahbek et al., 2014) and all of them found no differences for body composition or hypertrophy outcomes, except Rahbek et al. which included an within-participant design that allowed for a control of this covariate(Rahbek et al., 2014). Other studies reported diet control through the methods mentioned above, although no values of caloric or macronutrient intake are presented. Another aspect worthy of note is protein intake, which is essential for understanding the effects of whey supplementation and vary considerably from the recommended daily intake (RDI) of 0,8g.kg^{-1} per day to 2,0g.kg^{-1} per day and above. Since it is well known a higher response is obtained regarding muscle size and strength, by subjects with a lower protein intake, it is expected to observe a greater effect of whey on these participants(Morton et al., 2015)·(Egan, 2016).

Conversely, Farnfield et al. only detected a greater strength increase in the young group taking whey albeit the two groups (young and old) started the study with protein intake ranging from ~ 0,84g.kg^{-1} per day to 1,07g.kg^{-1} per day(Farnfield et al., 2012). Herda et al. started with relatively high levels of protein intake (from 1,3 to 1,6g.kg^{-1} per day) in all arms of the trial and 8 weeks of training detected no difference between groups(Herda et al., 2013). Also, Hulmi et al. on a 12 week trial and similar protein intake only reported differences between supplement groups in FFM..kg^{-1} boy weight and fat mass(Juha J Hulmi et al., 2015). In contrast, it was found a greater increase in CSA Vastus lateralis and isometric strength in favor of whey with similar intake of protein as reported by Hulmi et al. only this trial was 23 weeks in length(Juha J. Hulmi et al., 2009). In one study a trend was found in favor of whey although it didn't reach statistical significance. Protein intake was higher that other studies since subjects were trained bodybuilders. These results demonstrate trained subjects might benefit from protein doses well above the RDI and recommendations for athletes. All data suggest protein intake is a decisive factor for whey supplementation effect to be noted and values closer to RDI result in a more pronounced effect of whey, although duration of training, as stated above, interacts significantly with this factor. Briefly, it's possible that with longer training periods, the effects of whey become more apparent regardless of protein intake. In addition, higher protein intakes seem to favor subjects already well trained in strength and hypertrophy.

Whey composition and timing: Whey protein is considered high quality protein due to its digestibility, EAA (especially BCCA) content and bioavailability(Devries & Phillips, 2015; Egan, 2016). Its acid-soluble characteristics, makes it readily digested and absorbed, promoting a transient but quick hyper aminoacidemia and consequently triggering an MPS response via mTOR pathway(Devries & Phillips, 2015). Leucine content is believed to be one of the key aspects of MPS response, since there is evidence that leucine muscle intracellular concentration need to reach a given level for MPS to be optimal, giving rise to the concept of a leucine threshold(Tang et al., 2009; Katsanos, 2006). The exact range of concentration is not fully established and may vary with other factors such as age (aging makes the threshold higher)(Katsanos, 2006) or training status (trained status makes the threshold lower)(Devries & Phillips, 2015). A leucine content as low as 1,7g is thought to represent

the absolute minimum required to stimulate MPS, according to some studies, considering the subjects testes were resistance trained young men (Witard et al., 2014)·(Moore et al., 2015). Studies included in the review were mostly done in untrained subjects which probably needed a leucine content greater than 1,7g and in all studies, excluding the ones that did not report leucine content, leucine was usually in the range of 2 to 3g. Interestingly in studies were leucine content reached values very close or above 3g it was found greater increase of strength(Cribb et al., 2007; Farnfield et al., 2012; Juha J. Hulmi et al., 2009) and hypertrophy(Cribb et al., 2007; Farup et al., 2014; Juha J. Hulmi et al., 2009) metrics although others closer to 2g had found results also favoring whey (Rahbek et al., 2014; P. Reidy et al., 2012). It is noteworthy that Cribb et al. did not reported the leucine content of their protein supplement but given the amount ingested by the subjects it is fair to assume it reached or surpassed 3g. Babault et al. was the only study with leucine content above 3g that did not found any significant difference between groups. Nevertheless, the study didn't control for subject's diet(Babault et al., 2015). Results from Weisgarber et al. also found no differences for measured outcomes of strength and hypertrophy with the authors recognizing the training effects might have been maximized by whey if the sample was larger and the methods used to detect differences were more accurate. In addition, subjects were not supervised in training protocol and no control was made of supplement intake. This is visible, since protein and energy levels lowered from the first to the last week of training(Weisgarber et al., 2012). Considering this, it is possible to suggest a leucine range from 2-3g for optimizing muscle growth and strength gaining, with values closer to 3g possibly being more important for untrained population.

Timing of protein intake is another crucial factor for enhancing adaptation from RE(Cribb & Hayes, 2006). Some studies form this review instructed their participants to take the supplement all days (including non-training days) while others only on training days, but all of them had to ingest the product near the training session, immediately before or after it. One study distributed the intake after each exercise. In this regard, timing of whey ingestion doesn't suggest influence on outcomes. This is evident when comparing timing of whey ingestion between Farup et al. which found no interaction between training and whey(Farup et al., 2014), and Hulmi et al. which applied a similar timing of

ingestion and found differences on outcomes of hypertrophy and strength(Juha J. Hulmi et al., 2009). These findings are in line with a recent metanalysis which concluded, after adjustment for all covariates, that total protein intake is a strong predictor of muscle hypertrophy, not timing of intake(Schoenfeld, Wilson, Lowery, & Krieger, 2016).

Conclusion

The aim of this review was to analyze the evidence regarding the effects on muscle size and strength, as well as body composition changes from whey supplementation. Studies are very heterogenous in objectives, design, types of intervention, duration and methods applied. There is overwhelming evidence from acute metabolic studies that whey protein, due to BCAA's content, namely leucine and physicochemical characteristics, possesses an ergogenic effect especially when combined with RT These findings are not always consistent with results from long term studies, although most of them exhibit superiority of whey in some outcomes of hypertrophy or strength metrics, suggesting an ergogenic effect in these conditions. In addition, many covariates influence the results of studies, like subject's diet, training status, population recruited, composition of whey used or leucine content. It follows that more research is needed to fully determine if whey protein is capable of reliably maximize long term phenotypic adaptations to RT using more standardized study designs.

Practical implications

- Gains in muscle mass and strength are more visible when training programs are combined with enough protein intake.

- Supplementation with whey protein demonstrates a tendency to maximize hypertrophy and strength.

- Leucine content is crucial for differences in strength and body composition.

- Individuals training status influence gains provided by whey and resistance training since response in untrained individuals appears to be higher, at least initially.

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Conflicts of interest

The authors wish to declare there are no conflict of interests.

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CHAPTER III

This chapter is the field study conducted to examine the effects of whey supplementation in combination with resistance training in muscle hypertrophy, strength and body composition in amateur athletes. It consists of a pilot study and is written in accordance with the Journal of Science and Medicine in Sport (JSAMS) guidelines.

Intake of whey isolate supplement and muscle mass gains in young healthy adults when combined with resistance training – a blinded randomized clinical trial (Pilot study)

Abstract

Whey protein is consumed worldwide by professional and amateur athletes due to its alleged benefits on muscle mass and strength. Because of its rich branched chain amino acids content, namely leucine, whey appears to favor muscle protein synthesis through the mTOR pathway in combination with resistance training, when taken after exercise in sufficient amounts.

In the present study resistance trained (≥ 3 months) participants (men and women) between the age of 20 and 30 years old were randomized in a blinded fashion to whey protein isolate (n = 4) and an isocaloric placebo (n = 4) groups. Both groups were subjected to a 12-week RT protocol designed to increase muscle mass and strength. Muscle thickness of the biceps brachii (BB) at 67% of its length and quadriceps muscles, Vastus Lateralis (V.L.); Vastus Intermidius (V.I.) and Rectus Femoris (R.F.) at 30% and 50% of its length were assessed using ultrasound technique. Muscle strength was assessed using an isokinetic protocol at angular velocities of $60^{o}.s^{-1}$ (5 repetitions) and $180^{o}.s^{-1}$ (10 repetitions) with a range of motion of 0^{o} to 100 on a dynamometer to determine peak torque (PT). Lean body mass (LBM) and body fat percentage (%BF) were assessed using a body composition analyzer through segmental multi-frequency bioelectrical impedance method. All variables were assessed before and after interventions.

Results show an increase in muscle thickness of all muscles from RT except for V.L. and R.F. at 30% (p > 0,05) with an increase in V.I. at 50% (p = 0,045) and a trend in V.I. at 30% (p = 0,075) related to whey protein intake. PT increased with RT for all knee flexors/extensors (p < 0,05) and for elbow flexors/extensors at 60^{o} extension and 180^{o} flexion (p < 0,05) with no effect from whey. LBM increased with RT (p = 0,015) and %BF was maintained during the trial (p > 0,05). No interactions were found between training and supplementation.

Supplementation with whey protein, combined with RT can increase muscle mass with no effects on muscle strength. Whey protein supplementation may alter body composition in favor of additional fat free mass with no significant changes in body fat.

Keywords: Protein; Supplement; Hypertrophy; Resistance exercise; Strength; Muscle size.

Introduction

Several benefits may be obtained with resistance training (RT) such has increased strength and muscle mass (McCall, Byrnes, Dickinson, Pattany, & Fleck, 1996; Staron et al., 1994), as well positive changes in risk factors associated with osteoporosis, cardiovascular disease, diabetes, lower back pain and obesity (Winett & Carpinelli, 2001). Evidence provided by some studies (Farup et al., 2014; Hulmi et al., 2009), but not all (Reidy et al., 2016), support the concept of an additive effect between RT and protein rich diets on muscle and strength gain . Less controversial is the positive effect of RT and increased ingestion of proteins in older adults to mitigate sarcopenia and other complications associated with aging such as injuries resulting from falls and bone wasting (Breen & Phillips, 2013). Another aspect addressed in recent research was the possible negative effects of very high protein diets on body composition, namely on body fat. Despite these fears, studies demonstrated that even with more than 5 times the recommended daily doses of protein, for trained individuals there were no increases in body fat (Antonio, Peacock, Ellerbroek, Fromhoff, & Silver, 2014). Conversely, when training regimens are intensified percentage body fat decreases in hypercaloric protein diet individuals with no deleterious effect detected (Antonio et al., 2015). Similarly, a growing body of evidence reinforces the safety of high protein diets, demonstrating in healthy individuals with no background of renal disease, that occurring glomerular pressure and renal hyperfiltration consequence of hyperaminoacidemia do not lead to decreased renal function (Martin, Armstrong, & Rodriguez, 2005). The signaling pathways which translates external stimuli to processes such as gene transcription and mRNA translation associated with muscle growth are vast and complex. However, one of the most common and best-known cascades of enzymes involves the mammalian target of rapamicyn (mTOR) pathway. When activated, mTOR leads to phosphorylation of downstream proteins, resulting in increased translation of mRNA and cell growth (Bolster, Jefferson, & Kimball, 2004). The activation of this pathway is promoted by the presence of amino acids, namely branched chained amino acids (BCAA's), such as leucine, valine and isoleucine, resulting in greater muscle protein synthesis (MPS) in fed state, especially when combined with RT (Churchward-Venne et al., 2014). Since muscle accretion only occurs when there is a positive net protein balance, the transient but significant MPS increase, surpasses muscle protein breakdown (MPB) thus leading to muscle hypertrophy (Morton, McGlory, & Phillips, 2015). In this context, many protein sources can be used by athletes to take advantage of their possible ergogenic effects. Whey protein source is considered one of the best sources of protein (Tang, Moore, Kujbida, Tarnopolsky, & Phillips, 2009), and is one of the most popular sports supplement. Whey is extracted from milk by different physical processes such as ultrafiltration or ion exchange. The final product is named whey isolate because of its content on whey that may yield up to 90% protein or more (Jäger et al., 2017). It is considered the ideal protein source since it has a greater leucine content is rapidly digested and promptly absorbed resulting in an intense MPS response has mentioned above

(Jäger et al., 2017; Morton et al., 2015; Volek et al., 2013). Whey protein dose and timing are two factors considered by athletes. In acute metabolic studies a plateau was identified demonstrating that ingesting more than 20g of whey after RT protein has no advantages (Witard et al., 2014) although this has been disputed, especially when considering whole body RT, in trained males (Macnaughton et al., 2016). Time of intake is an important consideration and is recommended that whey or other sources of protein are ingested before and/or immediately after RT in the period termed "anabolic window" (Bird, Tarpenning, & Marino, 2006; Hulmi et al., 2009). Some studies have recently disputed this concept of "anabolic window" lending support to the theory that it's more important the total protein intake during the day, thus allowing constant available amino acids for muscle tissue to grow (Areta et al., 2013; Schoenfeld et al., 2017).

The aim of this investigation was to examine the long-term adaptations obtained when combining RT and whey protein in high doses. We hypothesized that ingesting high doses of whey during a 12-week RT protocol would increase muscle mass as well as strength in trained adults. Secondarily we evaluated the effects of a high protein diet on fat mass (FM) and lean body mass (LBM).

Methods

Subjects

Recruitment enlisted 22 resistances trained, healthy adults for the study. To be eligible, participants needed to be between 18 and 30 years old, engaged for at least three months in RT and training consistently (at least 3-5 d.week^{-1}), not taking any sports supplements for at least 12 week before the start of supplementation, had a body mass index between 19 and 26, had a body fat less than or equal to 30% (females) or 25% (males) body weight, no history or ongoing kidney disease, no neuro-muscular-skeletal injuries or muscle and/or joint chronic pain. Women that were pregnant, breastfeeding or considering this were also excluded. All participants agreed not to take any other nutritional supplements or nonprescription drugs that might enhance their physical performance during the study. A total of 11 subjects (three women and eight men) started the study with four withdrawals before initial baseline measurements. Two participants were injured during the trial and one participant dropped out by his own initiative. Eight participants completed the trial (Fig. 1). Before commencing all participants were informed of the purpose and potential risks of the investigation and gave their written informed consent. The study was conducted in accordance with the Declaration of Helsinki, and ethical approval of procedures was granted by the Ethics Committee of Porto School of Health - Polytechnic Institute of Porto.

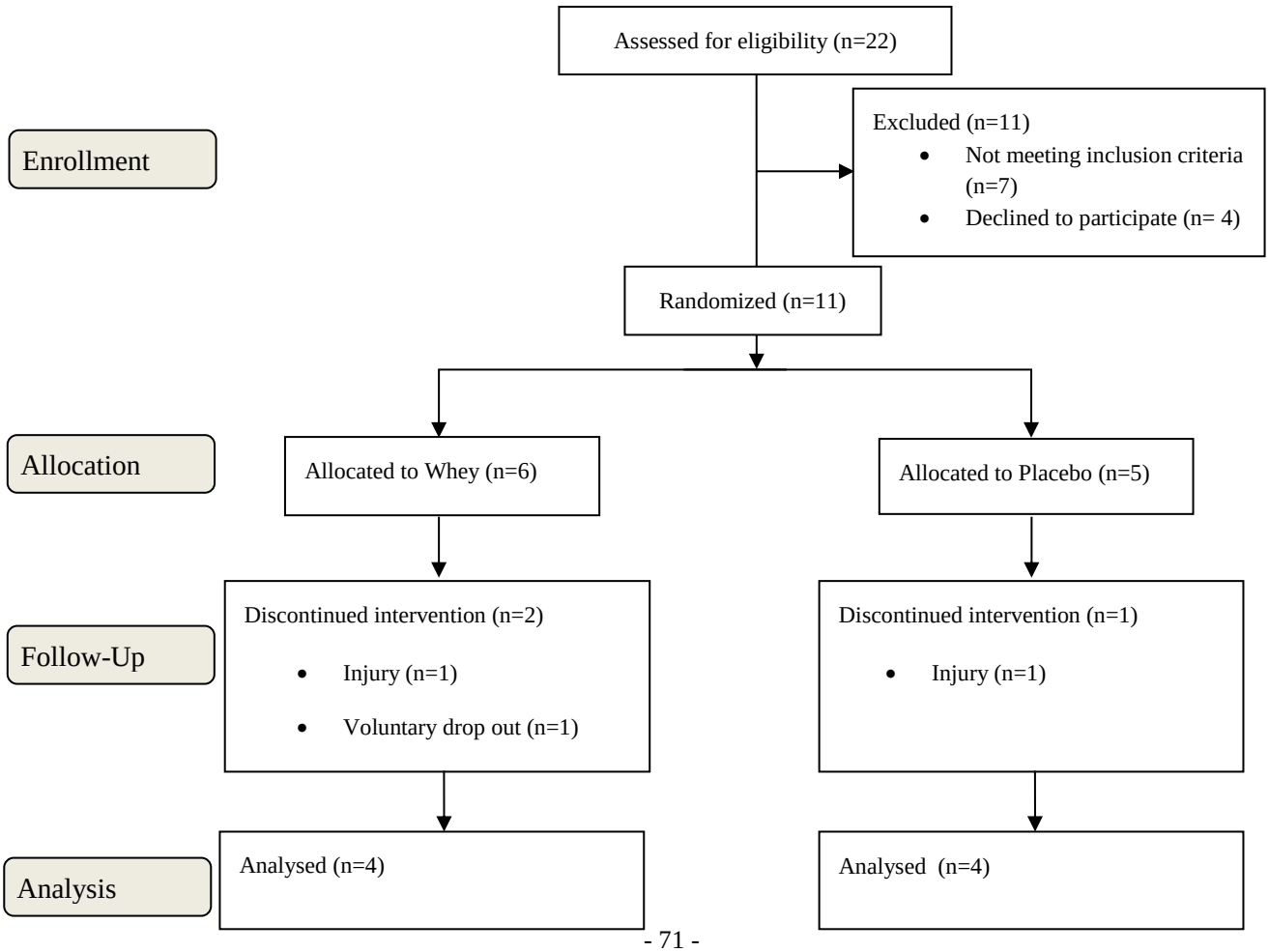

Figure 1 - Study flow diagram.

Design

This trial was conducted in a blinded fashion in relation to supplementation. Following inclusion, participants were matched for sex, body fat and randomly assigned to either a whey isolate protein group (WPI, n = 6) or a isoenergetic placebo group (PLA, n = 5) to ensure equal distribution of men and women of similar body composition in each group. All participants engaged in a RT protocol specifically intended to maximize strength and muscle mass for 12-week while taking the provided supplement daily. Participants were instructed to maintain their recreational activities such as group sports, walking, swimming or cycling and required to fill three days diet diary's in the first week of intervention and again on the last week of intervention. Baseline measurements were taken one week before the RT protocol and supplementation. After the 12-week intervention, in the week immediately after, subjects returned for a second assessment. All assessments were made at the same hour in the morning on both moments. Participants were required not to drink, take supplementation or engage in any kind of exercise 48h prior, to minimize confounding. The main dependent variables measured were thickness and strength of biceps brachii (BB) muscle, vastus lateralis (VL), vastus intermedius (VI) and rectus femoris (RF) muscle. Secondary variables measured were total body fat (BF), and lean body mass (LBM).

Resistance exercise program

The RT protocol used for this study consisted of a 10 to 15 minute warm up and 40 to 60-minute weight training, 3 days/week for 12 weeks. The warm up entailed light to moderate aerobic endurance exercise like bicycle or treadmill for the lower limbs and cycle ergometer or rowing for the upper limbs. The warm-up, classified as "mild" and "moderate" complies with the recommendations of the American College of Sports Medicine (ACSM). It uses the percentage of maximum heart rate (%HRmax) since it is an easy method to apply. The mild to moderate warm-up was carried out between 57-76% HRmax (American College of Sports Medicine, 2010). Resistance training was standardized and consisted of four compulsory exercises required for all training sessions, performed at 60-80% of one repetition maximum (1RM). Participants performed 3 sets of 8-12 reps to fatigue with 2 minutes rest between sets. The 1RM was adjusted every two weeks in the first session of the week (adjusted at the 3rd week; 5th week; 7th week, 9th week, 11th week) for compulsory exercises using the Brzycki formula. The four compulsory exercises, muscle groups targeted, sets and reps variation along the 12 week intervention are listed in table 1 and follow the general recommendations for healthy adults on intermediate resistance training according to the ACSM (American College of Sports Medicine, 2009). The focus of this RT protocol to ensure progressive increases in muscular volume and strength, while accounting for neuromuscular adaptations. Qualified trainers supervised the participants in all training sessions, calculating 1RM, adjusting the loads accordingly and keeping session logs. It was established that data collected from participants which not attended at least 70%

of training sessions, had to be excluded from final analysis.

Table 1 – RT Protocol

Exercises	Muscle group	Weeks	Sets, Reps and RM %
Low pulley	biceps brachii	1 - 2	3 sets
High pulley	Triceps brachii		
Leg curl	Hamstrings		12 reps at 60% RM
Leg extension	Quads		
Low pulley	biceps brachii	3 – 4	3sets
High pulley	Triceps brachii		
Leg curl	Hamstrings		10 reps at 70% RM
Leg extension	Quads		
Low pulley	biceps brachii	5 – 12	3 sets
High pulley	Triceps brachii		
Leg curl	Hamstrings		8 reps at 80% RM
Leg extension	Quads		

Reps, repetitions; RM, Repetition Maximum.

Supplementation

During the 12-week trial, participants were instructed to ingest the given supplement (WPI or PLA) according to their group assignment and following instructions communicated verbally in baseline assessment and using, if necessary, video instructions published in the study website. The supplements were provided in 2Kg containers of powder chocolate flavored with a measuring spoon to be prepared has a shake, mixing one spoon of supplement in approximately 0,2 L of water. Participants had to self-administer the supplement on training days, up to 1 hour after RT and on non-training days after a meal. Participants were given a 6-week supply and a refill took place mid trial. All the containers were weighted before and after intervention to ensure compliance. It was established that data from participants that didn't ingest at least 75% of the given supplement had to be excluded. A dose of WPI supplement powder contained approximately 34 g of product and 30 g of whey, with a leucine content of 2,91 g which is concordant with the "leucine threshold" believed to be a critical BCAA in MPS maximal stimulation (Crozier, Kimball, Emmert, Anthony, & Jefferson, 2005; Moore et al., 2009). A serving of approximately 35g of PLA was composed of a mixture 30% cocoa and 70% amylopectin. Both drinks were identical in appearance, texture, flavor and PLA was isocaloric to WPI (table 2). The supplements were supplied by Econutracêuticos Lda.

Table 2 - Macronutrients of drinks for 100g of powder

	Supplement	
	Whey (n=4)	Placebo (n=4)
Energy (Kcal)	373	400
Proteins (g)	87	2,1
Carbohydrates (g)	1,9	92,2
Lipids (g)	1,2	1,95
Fibers (g)	0,86	2,7

Dietary assessment

One week before baseline, participants received dietary recommendations from a certified nutritionist to standardize caloric and macronutrient intake. The objective was to obtain a protein/body weight ratio between 1,1 and 1,3 g/Kg/day. Dietary intake was assessed by 3-day food diaries, over 2-week days and 1 weekend day, on 2 occasions, the first and last week of the trial. In each occasion, participants received detailed instructions on how to fill the diary and a form to log their meals. The forms of the first week were collected at mid trial and the forms of the last week were collected in the week after the trial finished on second assessment. The data from food diaries was loaded into "Nutrium®" software to determine macronutrient and energy intake.

Muscle thickness

Muscle thickness was determined at baseline and after the 12-week intervention, using a B-mode ultrasound (Toshiba Viamo SSA-640A) with a high frequency linear probe at 7,5-MHz. This is a direct method of assessing muscle mass accretion (Brooks Mobley et al., 2017; Weisgarber, Candow, & M. Vogt, 2012). Participants were lying in supine position with arms and legs completely relaxed. In the upper limb positioned at approximately a 20º angle with respect to the trunk, the transducer was placed at 67% the distance between the acromion process of the scapula and the cubital fossa to determine BB muscle thickness. In the lower limb, the transducer was placed at 30% and 50% of the distance between the great trochanter and epicondyle of the femur to assess VL muscle thickness. In both upper and lower limbs, a marker was used on the skin to mark the precise point of measurement, but in the lower limb a line was marked along the lateral point to the anterior aspect of the leg to determine RF and V.I. thickness at 30% and 50%. The transducer was coated with a water-soluble transmission gel to ensure acoustic contact and equal pressure on the skin. Thickness was determined by the distance between the superficial and deep aponeurosis measured in 3 different equidistant points of the ultrasound image. The average of these measurements was calculated and considered the value of outcome. To avoid measurement biases, the procedure was always executed by the same operator and reliability was assessed by calculating intraclass correlation coefficient (ICC) in a mixed model with absolute agreement. ICC values were 0,954 for BB muscle, 0,945 for VL muscle at 30% and 0,980 at 50%, 0,987 for VI muscle at 30% and 0,915 at 50% and 0,847 for RF muscle at 30% and 0,950 at 50%.

Muscle strength

To determine muscle strength of the upper and lower dominant limb, participants initially performed a 10-min warm up on a friction-braked lower-limb and upper-limb cycle ergometry (824E cycle ergometer and 881E rehab trainer, Monark, Varberg, Sweden) (with 2% and 0,5% of the body weight, respectively), followed by assessment of isokinetic peak torque (PT) of the elbow flexors/extensors and knee flexors/extensors (Biodex_System 4;Biodex Medical Systems, Inc. 20

Ramsey Road, Shirley, NY, USA) (Brown, Bowser, & Simpson, 2012). Briefly, for the lower limb, participants were seated upright with a 90º hip flexion, lever arm of the dynamometer aligned with the lateral femoral condyle and restraining straps across the torso and abdomen to prevent compensatory movements. Resistance of the dynamometer was applied at the distal third of the leg, 3 cm above the malleolus (Fig 2.) (Teixeira, Carvalho, Moreira, & Santos, 2014). In the upper limb the arm was flexed at the shoulder and elbow with the forearm in neutral position and hand holding the dynamometer lever. A restrain strap was applied in the arm to prevent compensatory movements (Fig 3.) (Colebatch, Gandevia, & Spira, 1986). Limbs were weighed to enable gravity correction for measured torque. Angular velocities used in both limbs were $60^{o}.s^{-1}$ (5 repetitions) and $180^{o}.s^{-1}$ (10 repetitions) with a range of motion of 0º to 100º. Participants had a rest period of 1 minute between velocity measurements. Participants were familiarized with the equipment through sub-maximal workout at the defined velocities (Teixeira et al., 2014). After familiarization, participants were instructed to exert full strength and received verbal encouragement. All measurements occurred with participants fully relaxed and after a 48h period after the last exercise session.

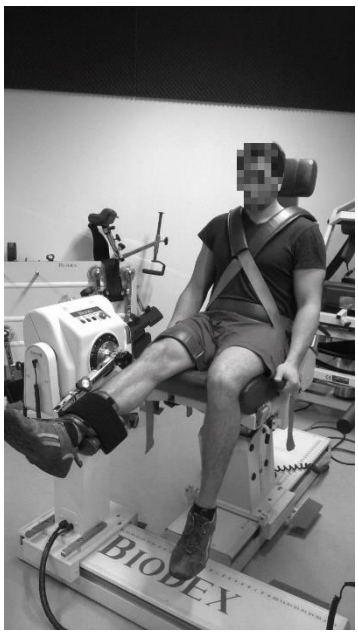

Figure 2 - Position for Knee extensors/flexors PT assessment.

Figure 3 - Position for elbow extensors/flexors PT assessment.

Body composition

Body fat and LBM were assessed using a body composition analyzer (Tanita InnerScan® BC-545) through segmental multi-frequency bioelectrical impedance method (Ling et al., 2011; Salmi, 2003). Participants were weighed in the upright position wearing shorts and shirt, with bare feet on the electrodes of the machine, holding the electrode handles as described by the researchers. Briefly, small electrical current passes through all the body at various frequencies and the equipment uses the conductive resistance of the body to estimate %BF and LBM.

Statistical analysis

Data was evaluated using a general linear model analysis of variance (ANOVA) repeated measures with group (supplement) has between subject's factor and time (training) as within subject's factor. Dependent variables were tested for normality with Shapiro-Wilk test and sphericity of matrix variances-covariances. Variables that were not normally distributed were log transformed (muscle thickness of rectus femoris at 50% and PT extension of elbow at 60º) for statistical tests. For between subject's effects, homogeneity of variances was tested with Levene's test. When homogeneity of variances was not verified a Welch's, test was conducted to evaluate between subject's effects. All values are presented has means and standard deviation (SD). p value <0,05 was taken at the level of statistical significance and p value of < 0,09 was considered a trend. All data was analyzed using SPSS for Windows version 16.0 (SPSS Inc., Chicago, IL, USA).

Results

Participants characteristics

All participants eligible for analysis ingested at least 75% of the supplement and attended at least at 70% of planned training sessions. Descriptive characteristics for participants at baseline are shown in table 3. A total of 8 participants completed the study and no differences were found between groups for any variable (p>0,05).

Table 3 - Participants characteristics at baseline

	Treatment	
	WPI (n=4)	PLA (n=4)
Age (years)	25 ± 5,2	21 ± 2,7
Weight (Kg)	69,7 ± 10,3	69,2 ± 14,2
Height (cm)	171,0 ± 10,7	174,5 ± 2,9
BF (%)	19,2 ± 10,0	17,8 ± 7,6
LBM (Kg)	54,1 ± 13,3	54,0 ± 4,4

Values are means ± SD. WPI, whey protein isolate; PLA, placebo; BF, body fat; LBM, lean body mass.

Muscle thickness

In table 4 is shown muscle thickness means and standard deviation (SD) for WPI and PLA groups before and after the training protocol. Participants increased muscle thickness following 12 weeks of resistance training on all dependent variables (time, $p < 0,05$) except for V.L. at 30% ($p = 0,073$) and R.F. at 30% ($p = 0,116$). A trend was detected for V.I. at 30% in favor of whey. No interaction between training and supplement was detected (time*group, $p > 0,05$). Muscle thickness on V.I. at 50% was significantly higher in WPI group (F (1,6) = 6,398, $p = 0,045$, Power = 0,563). Effect size is considerable ($\eta^2_p = 0,516$), and it can be expected an average increase of muscle thickness in V.I. at 50% between 0,1 and 7,9 mm (] 0,132; 7,943 [; 95% CI) on participants taking WPI.

Table 4 – Muscle thickness

Variable	WPI (n=4)	PLA (n=4)
Biceps Brachii 67% (mm)		
Pre	22,7 ± 7,2	21,5 ± 2,7
Post	25,2 ± 9,8*	23,1 ± 2,1*
Vastus Lateralis 30% (mm)		
Pre	24,5 ± 5,5	22,7 ± 2,4
Post	25,1 ± 4,8	23,2 ± 2,4
Rectus Femoris 30% (mm)		
Pre	21,8 ± 4,3	23,5 ± 2,2
Post	22,9 ± 3,0	25,9 ± 0,9
Vastus Intermedius 30% (mm)		
Pre	19,6 ± 2,3	14,7 ± 3,1
Post	22,9 ± 4,0*↓	17,7 ± 4,3*
Vastus Lateralis 50% (mm)		
Pre	24,8 ± 6,1	24,1 ± 1,2
Post	26,9 ± 5,4*	27,0 ± 2,1*
Rectus Femoris 50% (mm)		
Pre	18,1 ± 3,5	19,3 ± 3,1
Post	20,6 ± 3,0*	21,2 ± 2,5*
Vastus Intermedius 50% (mm)		
Pre	16,8 ± 2,3	12,3 ± 1,7
Post	19,1 ± 3,1*,#	15,6 ± 2,4*

Values are means ± SD. WPI, whey protein isolate; PLA, placebo. Rectus Femoris 50% was log transformed for statistical analysis, and data is presented non-transformed. * Training effect within participants (p<0,05); # Supplement effect on muscle thickness between groups (p<0,05). ↓ Trend towards significance ($p < 0,09$).

Peak Torque

Figure 4 and 5 show the findings for PT. Strength increased for elbow extensors and flexors at 60º extension and 180º flexion angular velocities. A trend was detected for elbow extensors/flexors at 180º extension angular velocity (p = 0,094). No effects were detected (p > 0,05) for elbow extensors/flexors at 60º flexion angular velocity. Strength increased with training (time, p < 0,05) on post measurements for all knee extensors and flexors. Intake of supplement did not interact with training and no group differences were detected (p > 0,05).

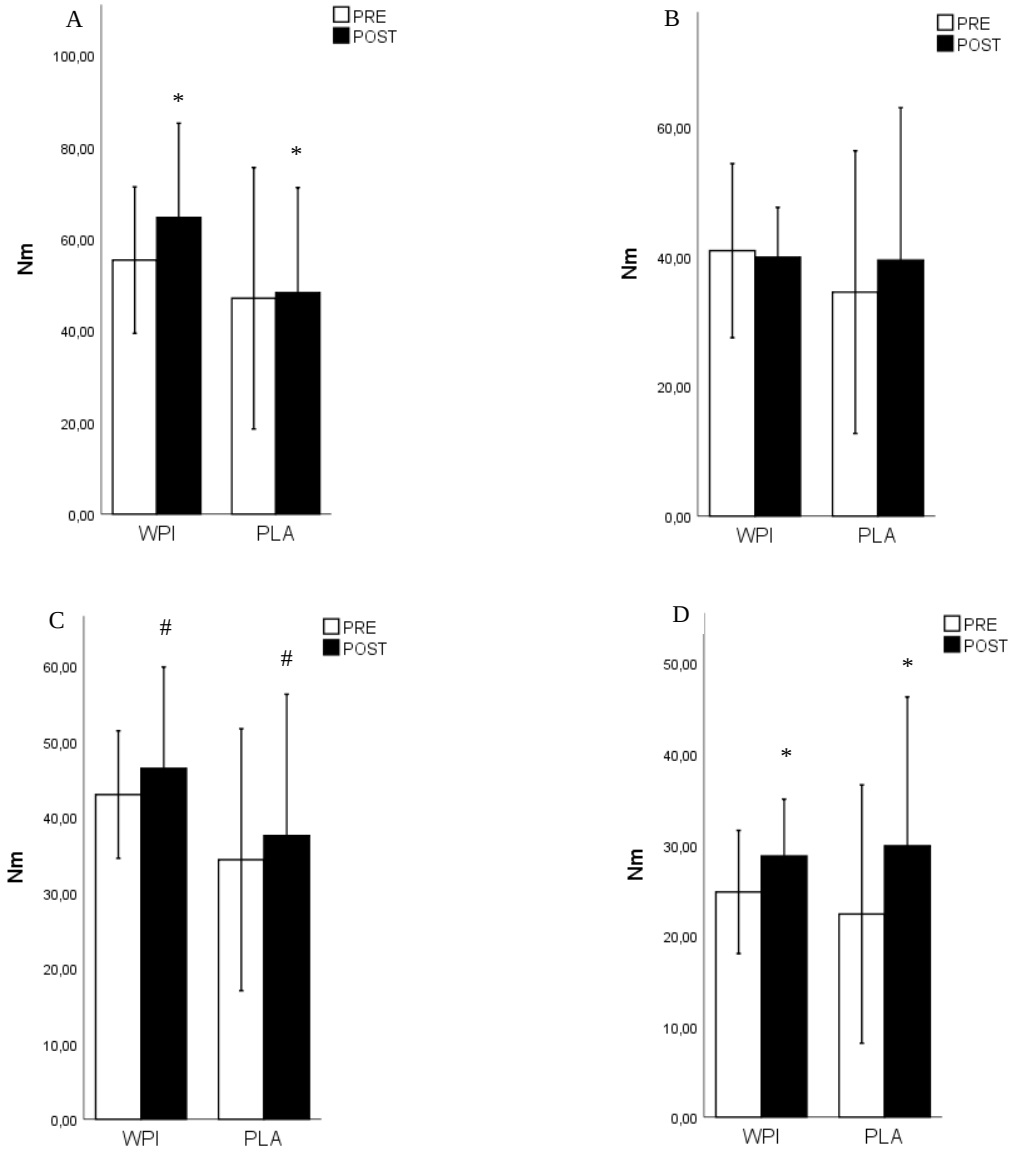

Figure 4 – Elbow extensors/flexors; Values are means ± SD. WPI, whey protein isolate; PLA, placebo. A, peak torque at 60º extension (log transformed for statistical analysis and data presented non-transformed); B, peak torque at 60º flexion; C, peak torque at 180º extension; D, peak torque at 180º flexion. * Training effect within participants (p<0,05); # Trend towards significance (p < 0,09).

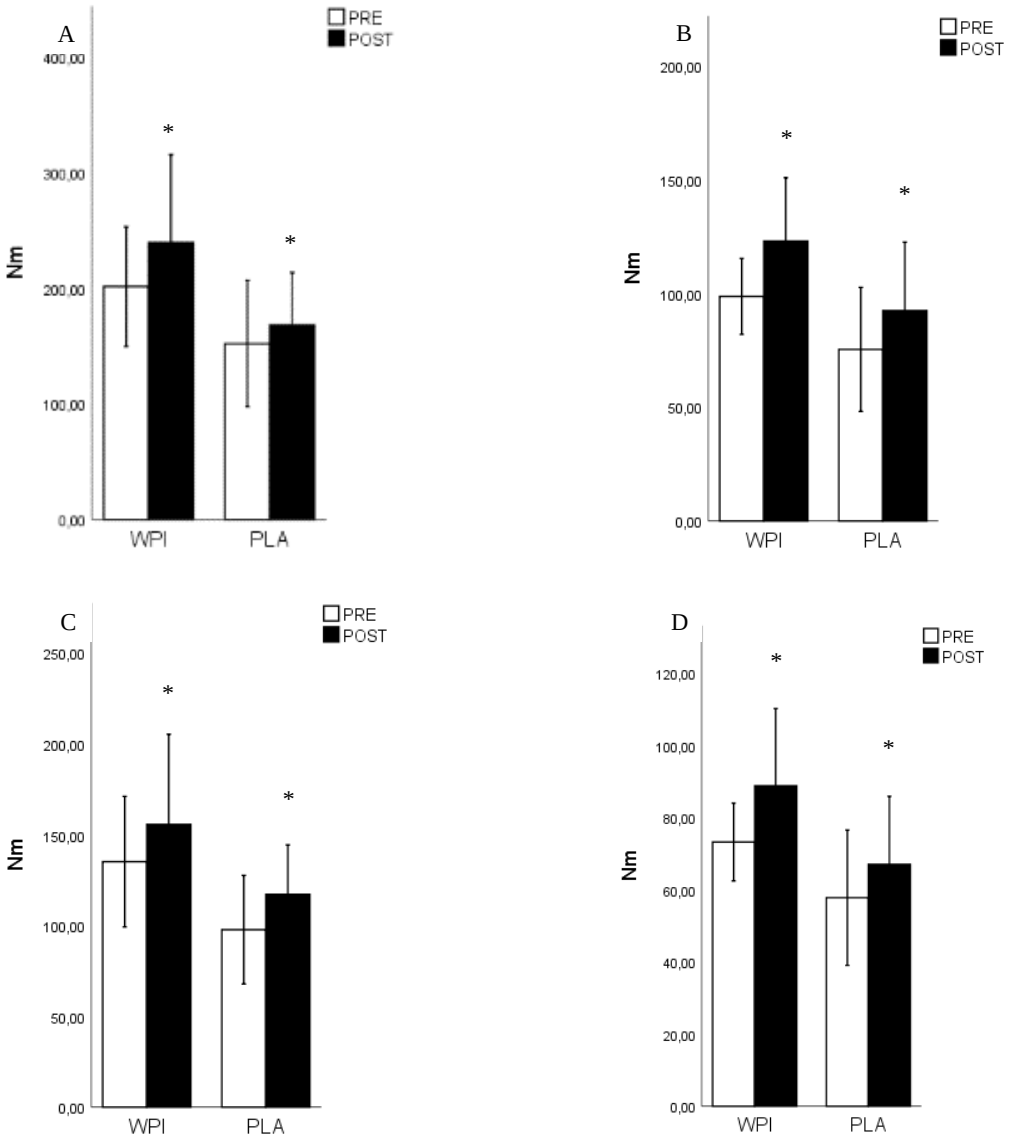

Figure 5 - **Knee extensors/flexors;** Values are means ± SD. WPI, whey protein isolate; PLA, placebo. A, peak torque at 60º extension; B, peak torque at 60º flexion; C, peak torque at 180º extension; D, peak torque at 180º flexion. * Training effect within participants (p<0,05).

Energy intake, macronutrients and body composition

Regarding energy intake, there were no differences within subjects or between WPI and PLA ($p > 0,05$). Nutrient/ body weight ratio is maintained during the trial. Noteworthy is the relatively elevated protein ratio (1,3 – 1,4 g/Kg/day) in both groups. For dependent variables of body composition, a significant main effect occurred due to training in LBM (F (1,6) = 11,480, $p = 0,015$, Power = 0,805) with training accounting for more than 65% of LBM increase ($\eta^2_p = 0,657$). When comparing %BF means, no statistical significant result was detected for training or supplementation ($p > 0,05$) along the 12-week trial. No interactions were detected ($p > 0,05$) for both variables. Values are shown in table 5.

Table 5 - Caloric intake, macronutrients and body composition

	WPI (n=4)	**PLA (n=4)**
Energy intake (Kcal)		
Week 1	1646 ± 217,6	1566 ± 236,4
Week 12	1617 ± 364,6	1747 ± 332,2
Protein (g/kg/d)		
Week 1	1,33 ± 0,19	1,43 ± 0,25
Week 12	1,31 ± 0,15	1,42 ± 0,19
Carbohydrates (g/kg/d)		
Week 1	2,27 ± 0,45	2,14 ± 0,47
Week 12	2,12 ± 0,21	2,51 ± 0,88
Lipids (g/kg/d)		
Week 1	1,01 ± 0,33	0,94 ± 0,15
Week 12	0,92 ± 0,29	1,0 ± 0,19
LBM (Kg)		
Pre	54,1 ± 13,3	54,0 ± 4,4
Post	55,3 ± 13,7*	55,5 ± 4,2*
BF (%)		
Pre	19,2 ± 10,0	17,8 ± 7,6
Post	20,3 ± 9,0	17,6 ± 6,1

Values are means ± SD. WPI, whey protein isolate; PLA, placebo; LBM, lean body mass; BF, Body fat.
*Training effect within participants (p<0,05).

Discussion

Results from our study show a significant increase in muscle mass and strength after the 12-week RT protocol, primarily due to training. These effects are well documented in literature (Gondin, Guette, Ballay, & Martin, 2005; Phillips, Tipton, Ferrando, & Wolfe, 1999) resulting in the anabolic changes seen on participants.

Muscle thickness: It is noteworthy the group effect on thickness of V.I. muscle at 50% which demonstrated that whey had a positive effect on muscle mass, although very modest. A trend was also detected in thickness of V.I. muscle at 30% ($p = 0,075$). This effect might not have been detected on other muscles and positions since the sample in this study was small. Another reason for this is the fact that participants despite being trained in RT for at least three months may not have been following a program designed to increase muscle size. This aspect lead to an overall greater response to the RT program has evidenced by the results in almost every variable of muscle thickness and strength , which probably overlapped any additional benefits provided by whey supplementation (Ahtiainen, Pakarinen, Alen, Kraemer, & Häkkinen, 2003; Phillips et al., 1999). Compliance for supplementation (75%) and RT attendance (70%) were arbitrarily defined and might have been too low, affecting the response to both co-factors. BCAA's are viewed has ingredients necessary for muscle hypertrophy, namely leucine that plays an important role in MPS (Churchward-Venne et al., 2012). In reviewed literature, the leucine threshold is established between 2-3 g to maximize MPS (Devries & Phillips, 2015). In this study leucine content is adequate for maximal MPS (2,91g) which may explain the whey superiority in V.I muscle at 50% and the trend detected for V.I. at 30% even with such a small sample.

Muscle strength: Regarding strength, no group differences were detected suggesting whey didn't had any effect. Furthermore, elbow flexors at 60º angular velocity did not show any training effect and only a trend was detected for extensors at 180º angular velocity. Training effects on quadriceps muscles however were clear on extensors and flexors at all angular velocities. Muscles with more muscle fibers and consequently more nervous receptors, develop greater responses, thus strength differences are easier to detect reflecting the achieved results (Baechle, Earle, & Wathen, 2000). Overall, strength increased on all participants independently of supplementation. In a paper by Buckley et al. 28 males engaged in 100 maximal eccentric contractions of the knee extensors and ingestion of whey supplements vs placebo revealing an increased capacity to recover from muscle damage, which in turn reveals strength increase with whey protein (Buckley et al., 2010). In contrast, Boone et al. show that a short-term RT program in untrained individuals augments muscle strength and size with no additive effect of whey (Boone, Stout, Beyer, Fukuda, & Hoffman, 2015). Furthermore muscle strength gained after a short RT period in trained men is maintained even when participants are subjected to a detraining period of two weeks (Hwang et al., 2017). Even in long

term RT periods, no effect was detected with whey (Brooks Mobley et al., 2017). These works reinforce the notion that the number of muscle fiber recruitment is the most important factor influencing strength gains with whey playing a minor role unlike in muscle hypertrophy. This is more evident in untrained individuals were neuronal adaptations are more intense, whereas in trained individuals, the whey effect on strength is more easily detectable due to less confounding effects of neuronal adaptations (Pasiakos, McLellan, & Lieberman, 2014).

Body composition: Measurements of LBM and %BF revealed a main effect of training in LBM during the 12-week RT program ($p = 0{,}015$). Other studies reported the same findings (Folland & Williams, 2007; Reidy et al., 2016), which was expected since RT exercises are major stimulus for MPS, via IGF-1-AKt-mTOR pathway (Schiaffino, Dyar, Ciciliot, Blaauw, & Sandri, 2013). Activation of this pathway via IGF-1 is directly linked to insulin release, necessary to increase glycoses availability in muscle cells during strenuous exercise. Importantly %BF did not increase during the trial ($p > 0{,}05$), albeit both groups increased their caloric intake and whey group increased even more their caloric intake. This result is consistent with other works (Brooks Mobley et al., 2017; Cribb, Williams, Carey, & Hayes, 2006) showing that RT in combination with protein supplementation may be an effective strategy to improve body composition, since participants tend to increase LBM and maintain or even decrease fat mass. We hypothesized that %BF would decrease but this did not occur, which might be explained by the training program, designed to enhance muscle size and strength instead of fat oxidation. Another aspect that could limit detection of decreases in this variable is the sensitivity of our body composition instrument (combined with the small sample size) which uses segmental multi-frequency bioelectrical impedance method instead of other more sensitive methods (Ling et al., 2011).

Conclusion

This study evaluated the effects of high doses of whey protein intake combined with a 12-week RT protocol on muscle mass, muscle strength and body composition (LBM and %BF). It was proposed that muscle mass and strength would increase significantly on participants taking whey. Body composition was also expected to improve more on whey group.

The results show an effect of whey on muscle mass but not on strength. The results on muscle mass are supported by an increase in V.I. muscle at 50% and a trend in the same muscle at 30%. These results are in line with other works and meta-analysis suggesting an ergogenic effect of whey. Despite the positive results on muscle mass, whey did not have any effect on muscle strength. Body composition was not affected by whey either. Whey group decreased their caloric intake when compared to placebo group which increased it. The protein/body weight ratio was elevated. It surpassed the 1,1 – 1,3 g/kg/day goal in PLA reinforcing the notion that whey supplementation is probably responsible for muscle mass gains, since whey supplementation might have increased this ratio marginally in whey group.

Our investigation had some limitations, namely the small sample size, undermining our ability to detect small but meaningful differences between groups. Compliance values (75% - supplementation; 70% - RT attendance) were possibly too low and may have also affected our ability to detect differences. Despite these limitations, we were still able to detect effects and trends between groups which testifies the robustness of our study design. Body composition variables were assessed with method rigors enough for clinical practice but considering the small sample size other methods may provide more expressive results.

In future investigations a bigger sample and higher compliance pre-defined values are necessary as well as another method of determining body composition such has dual x-ray absorptiometry. Another point in time measurements is also advisable (i.e. at 6 week of trial). This may help understand at which extent neuronal adaptations affect strength gains, since they occur early in RT training, allowing for the detection of a possible effect of whey on strength. It may also provide insight on the magnitude of the effect of whey on muscle mass since it is expected a less noticeable effect of whey later in the trial.

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Conflicts of interest

The authors wish to declare there are no conflict of interests.

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Annexes

Annex I

Trial Protocol

Trial Protocol

SPIHFMA

Porto School of Health Sciences
Polytechnic Institute of Porto

Intervention: Protein supplement containing Whey isolate

Title Protocol: Randomized blinded clinical trial whose intervention constitutes supplementation with whey protein isolate in amateur/recreational athlete's vs placebo.

Protocol version: SPIHFMA-3/17

Date of the Protocol: October 2017

Promoting institution - School of Health Sciences - P.Porto

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SUMMARY OF THE PROTOCOL

Intervention: Protein supplement containing Whey isolate

Title Protocol: Randomized blinded clinical trial whose intervention constitutes supplementation with whey protein isolate in amateur/recreational athlete's vs placebo.

Dates: November 1, 2017 - Elements recruitment

30 of November 2017 - End of recruitment

1 January to 14 January - Run-in period

15 January - January 21 - Variable measurement (establishment of baseline)

22 January - Start of trial

26 February 3 March - Follow-up interview and refill

143April - End of the trial

15 April – 21 April - Variable measurement (outcomes)

Main objectives: Evaluate the influence of intake of a supplement containing whey isolate in the dependent variables, muscle strength and muscle hypertrophy.

Secondary objectives: Evaluate the influence of intake of supplements containing whey isolate in body fat mass.

Study design: This study is a randomized blinded trial, whose intervention consists in protein supplement intake containing whey isolate and whose comparator is an isocaloric placebo.

The sample elements eligible for the study are divided into two groups; one will ingest the protein supplement, the other will ingest the placebo. The elements of the sample are unaware of what they are taking, but researchers have that information.

Study population: Amateur/recreational athletes

Duration of the study: The trial will take 12 weeks during which the sample will perform resistance training in gymnasiums they attend while taking the supplement / placebo daily. A period of run-in will be used to establish a baseline. All variables of interest will be measured one week before and after the test.

Ethical and safety aspects: The products used are dietary supplements; the test does not require any authorization to be carried out and only need informed consent signed by the elements of the sample. The elements of the sample may withdraw at any time of the test being always guaranteed the confidentiality of data obtained during the test. The ESS-PPorto ethics committee as approved this study.

Content

Abbreviations

α - LA - α - lactalbumin

BCAA - Branched Chain Amino Acids

IgA - immunoglobulin A

GIP - glucose-dependent insulinotropic peptide

MIT - maximum isometric torque

PRO - Protein

PLA - Placebo

AMC - arm muscle circumference

AMA - arm muscle area

CEMAH - Movement Studies Center and Human Activity

ACSM - American College of Sports Medicine

% HRmax - percentage of maximum heart rate

1. Introduction

When the milk coagulates forms a solid component called "curd" rich in casein, a milk protein, and the liquid component. This liquid component is milk serum rich in many proteins. This set of high biological value protein is named whey. In fact the "whey protein" is not a protein, but rather a set of proteins. Interestingly whey being a byproduct of fermentation of milk resulting from cheese production was considered wasteful and used to fertilize agricultural land, feeding swine or simply discarded. Today the role of nutritional whey is widely recognized and its status evolved from waste to much valued commodity (1). Nutritionally, whey is considered a mixture of proteins highly beneficial for human health. Among the many positive effects such as the effect on calcium homeostasis, effects on the immune system, antioxidant effect and change in body composition by increasing the metabolism of adipose tissue, is the anabolic effects on the skeletal muscle (1), (2). The protein contains immunoglobulin A (IgA) which strengthens the immune system. Some glycoproteins have pre and probiotic effect improving gastrointestinal athlete functionality and appear to stimulate the release of cholecystokinin (2).The effect on striated muscle is the most relevant for this study and is associated with α-lactalbumin (α - LA) isolated in the 30's. This protein containing branched chain amino acids (BCAA), is largely responsible for muscle protein synthesis effect and consequently gain of muscle mass and strength. The BCAAs are leucine, isoleucine and valine, known for their metabolic effects and signal initiation of protein synthesis in muscle fibers (1). A study demonstrated the insulinotropic effect derived from the mixture of BCAA in a beverage compared with a beverage containing whey. From this study it was concluded that although BCAA determine a similar insulinotropic response, the whey protein increases the secretion of incretins such as glucose insulinotropic peptide-dependent (GIP) as opposed to only the beverage containing BCAAs. The superior effect of whey is not negligible since it is known that the secretion of incretins like GIP hormones in the presence of carbohydrates in the gut enhance the anabolic action of insulin allowing for greater control of blood sugar levels (3). In the aspect of muscle recovery a study published by Jonathan Buckley et al. compares the use of whey isolate with whey isolate hydrolyzed and measured various variables indicative of recovery of muscle contractile capacity after eccentric contraction, being the most important, the maximum isometric torque (MIT). The MIT was evaluated on an isokinetic dynamometer and increased

after 100 knee extensions in the group taking the whey isolate hydrolyzed in contrast to the other groups (control group and group whey isolate) which showed reduction in the value of MIT (4). Another article from Michaela C. Devries and Stuart M. Phillips agglomerates all the evidence on the beneficial muscular strength and muscular hypertrophy of whey. Whey, according to the authors, has revealed robust ability to increase muscle protein synthesis and is superior to casein and soy in this context (5).

Having said this it's justified a study in a real context, in a population of amateur/recreational athletes, given the significant growth of the dietary supplements market, in particular whey protein, in this segment of the population. Moreover, consumers increasingly require the most accurate information on the efficacy, effectiveness and safety of these products, and the pharmacy professionals need to answer these questions. On the other hand, recognizing the role of pharmacy and its professionals in this field, the brands that produce these supplements need to start preparing their products for this distribution channel, which is recognized for their reliability and trustworthiness.

2. Objectives of the trial

- To evaluate the influence of the intake of supplements containing whey isolate in the dependent variables, muscle strength and muscle hypertrophy.
- To evaluate the influence of the intake of supplements containing whey isolate in body fat mass.

3. Study Design

3.1 Type of study

The SPIHFMA trial corresponds to a prospective experimental study. It is a randomized blinded trial to participants relating to the intervention and control group. The elements of the sample are distributed to two groups; one group will ingest the protein (PRO) whey isolate and the other group a placebo (PLA).

Supplement 3.2 / Placebo

3.2.1 Characteristics of products

The supplement used is GoldNutrition ISOHIDRO WHEY®, Ltd. trademark of Econutracêuticos. A label on the product composition is available (Annex I). The placebo used is a isoenergetic maltodextrin provided by Econutracêuticos Ltd. A product label is available (Annex II). All products are delivered in 2kg of powder containers containing a measuring spoon. Both products should be stored in cool, dry place in the premises of ESS P.Porto, respecting any additional information storage that is transmitted to the researchers by the supplier. The high protein supplements should be segregated from the placebo when delivered to ESS P.Porto campus.

3.2.2 Preparation and delivery of products

In preparation for delivery to sample elements, it will be registered in a control form (Annex III), the product lot number, the code / ID corresponding to the sample element, if the product is a supplement or placebo and the weight of the container. The original label will be hidden / removed to ensure that the element of the sample receiving the product doesn’t know if it is protein or placebo.

3.2.3 Management Product

Products must be prepared and self-administered by the elements of the sample at home and in the gym and they receive detailed information on how to proceed. The administrations are performed in the form of shakes according to the following preparation steps:

- Remove the measuring spoon from the package.
- Fill the measuring spoon and scrape with a kitchen knife excess powder.
- Place the powder in the mixing vessel and add about 200ml of water.
- Stir the mixing vessel.
- Take the mixture.

The administration of the shake must be performed according to the following scheme:

1 serving (~34g) = scoop of WHEY PRO ISOLATE® = 30g Whey protein

1 serving (~55g) = scoop of Placebo (70% Amilopectin + 30% Chocolate)

- Training days - 3x week up to 1 hour after resistance training (1 serving)
- Other days of the week - After breakfast or lunch or dinner (1 serving)

3.2.4 Control adhesion of the protocol and refill

The administration protocol is controlled by weighing the containers delivered to the sample elements. All containers before being delivered are weighted and registration in a specific form allows, together with the dosing schedule defined above, determine if a dose has not been ingested. All of the sample data showing a lower adhesion than 75% are excluded.

The process of product refill takes place in the 6th week of the trial 26 February to 3 March together with the follow-up interview. The empty containers are delivered to

researchers by sample elements who receive new filled containers. The containers collected or delivered are weighted and registration is performed by the researchers.

4. Sample

4.1 Target Population

The target population of this trial is amateur/recreational athletes who perform resistance training. Since this population is very heterogeneous and in order to avoid the sample selection bias, we defined a subpopulation of amateur/recreational athletes who meet certain inclusion and exclusion criteria for the selection of the sample. The sample should contain between 20 and 40 individuals.

4.2 Recruitment Strategy and informed consent

Athletes are selected from two gyms in Porto. They must respond to a quiz which is available via online to determine their eligibility for the study. If eligible, they are then informed of the possibility to participate in the trial (6).

All sample elements selected expressing desire to participate are fully informed in writing and verbally about the study, and may ask questions about it. Each element of the sample must sign and date the informed consent provided, which will be duly filed (Annex IV).

4.3 Criteria for inclusion and exclusion

The elements of the sample can participate in the study if they meet the following inclusion criteria:

- Healthy men or women aged 18 years to 30 years.
- Perform resistance training for at least 3 months.
- Do not ingest sports supplements for at least 3 months.
- Have a body mass index between 19 and 26 inclusive.
- Have fat mass less than or equal to 30% of body weight (females) and 25% of body weight (males).
- Be available to take measurements of the variables in the premises of ESS P.Porto.

The elements of the sample are excluded from the study for the following reasons:

- Subjects with renal insufficiency of any degree.
- Individuals who do not comply with the standardization of diet and training.
- Individuals with neuro-muscular-skeletal injuries.
- Individuals with chronic joint or muscle pain.
- Pregnant or breast-feeding.

4.4 Withdrawal

The elements of the sample may withdraw at any time without indicating the justification. In the act of withdrawal of the trial, sample elements should return the product provided.

5. Control confounding

5.1 Diet

The standardization of diet aims to control the influence of intake of various nutrients in the study results. Each selected element is consulted by a nutritionist and fellow researcher on this study**.** It is prescribed for each of the elements an isocaloric diet. The sample elements should follow the prescribed diet while participating in the trial which will be periodically evaluated. Participants must log on diet diaries their food intake in specified dates (in the beginning and in the end of the trial).

5.2 Resistance training

The standardization of training is intended to control the influence of different types of training and exercises performed during the trial, on study outcomes.

The training schedule is 3 times a week for 12 weeks. The elements of the sample cannot perform any more workout during the week in order to avoid interference confounders. Training divides into:

- Warm-up (10-15 minutes).
- Resistance training with machines/weights (40-60 minutes).

The warm-up consists of light to moderate aerobic endurance exercise like bicycle or treadmill for the lower limbs and cycle ergometer or rowing for the upper limbs. The warm-up, classified as "mild" and "moderate" complies with the recommendations of the American College of Sports Medicine (ACSM). It uses the percentage of maximum heart rate (%HRmax) since it is an easy method to apply. The mild to moderate warm-up must be carried out between 57-76% HRmax (7).

Resistance training is standardized and consists of four compulsory exercises required for all training sessions and must be performed at 60-80% of one repetition maximum (1 RM). The athlete must perform 3 sets of 8-12 reps (8). The 1RM is adjusted every two weeks

by qualified professionals in the first session of the week (adjusted at the 3rd week; 5th week; 7th week, 9th week, 11th week) for compulsory exercises.

The 1RM for each exercise is determined using the Brzycki formula:

$$1\,RM = w \cdot \frac{36}{37 - r} = \frac{w}{\frac{37}{36} - \frac{1}{36}r} \approx \frac{w}{1.0278 - 0.0278r}$$

w = weight used; r = number of repetitions

The player can customize the rest of the workout, but should focus on exercises that do not involve or minimally involve muscle groups already exercised (e.g. abductor exercises, adductors, trunk).

As for compulsory exercises, they must be carried out on machines for more security for the participant in the execution of movements and to determine 1RM. The four compulsory exercises, movements, positions and velocities are described below and follow the general recommendations for healthy adults on intermediate resistance training according to the ACSM (8):

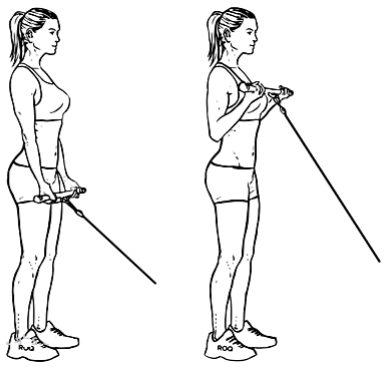
Muscular group: Biceps.

Exercise to perform: low pulley.

Starting position: shoulder in neutral position, elbow near the trunk, forearm perpendicular to the ground.

Movement: concentric elbow flexion (duration: approx. 2s).

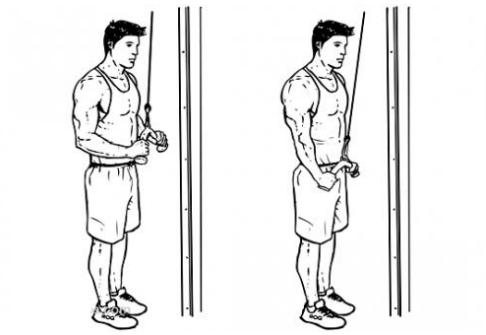
Final position: full flexion of the elbow.



Muscular group: Triceps.

Exercise to perform: High pulley.

Starting position: shoulder in neutral position, elbow near the trunk, forearm parallel to the ground.



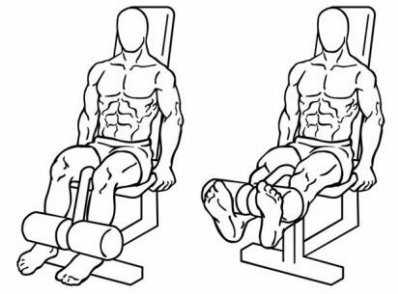
Movement: concentric elbow extension (duration: approx. 2s).

Final position: full extension of the elbow.

Muscle Group: Quads.

Exercise to perform: leg extension machine.

Starting position: sitting on the machine supported with the back and the cushion of the lever above the ankle. Thighs resting on the seat, parallel to the floor, knees off the seat with your legs perpendicular to the floor. Hands should hold the handles on each side of the machine.



Movement: concentric knee extension (approx. duration: 2s).

Final position: full knee extension.

Muscular group: Hamstrings.

Exercise to perform: legs bending machine.

Starting position: lying prone on the lever machine with the pad supported on the legs just below the calf. Legs parallel to the floor, knees in full extension. Hands should hold the handles on each side of the machine.

Movement: concentric knee flexion (approx. duration: 2s).

Final position: full knee flexion.

6. Variables measurement

The variables measurement takes place on the premises of ESS P.Porto on the dates set forth in this Protocol, and by agreed time between researchers and the sample elements but always take place in the morning between 9 am and 12 pm. The variables of interest are measured using validated protocols. The primary variables to be measured are muscle hypertrophy and muscle strength. The secondary variables are fat mass and lean body mass.

6.1 Measuring variable "muscle hypertrophy"

Muscle hypertrophy corresponds to the accretion of muscle mass. In clinical practice the evaluation of muscle mass is performed using anthropometric measurements and validated formulas. The muscle mass of the sample elements is evaluated by determining the arm muscle circumference (AMC) and arm muscle area (AMA) corrected. It will also be registered thigh circumference. This limb circumference measurements are carried out according validated protocols. If available an ultrasound equipment well be used to determine thigh and arm muscle thickness.

6.2 Measuring variable "muscle strength"

The muscular strength is measured using the isokinetic dynamometer from the Center of Studies and Movement of Human Activity (CEMAH), an investigation center of ESS P.Porto, determining the maximum torque per body mass. The measurement considers the standardization of training and it's followed by a validated protocol that mimics the compulsory exercises performed. The evaluations are conducted to the lower and upper dominant limb in dynamic movement to 60º/s motion (CON/CON). Exercises to perform on the isokinetic dynamometer are the extension and flexion of the elbow and extension and flexion of the knee.

6.3 Measuring variable "fat mass" and "lean body mass"

The fat mass and lean body mass are measured using a body composition analyser (segmental multi-frequency bioelectrical impedance method).

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Annex’s

Annex I

Declaração nutricional	Por 100 g	Por porção (34 g)	%DR**
Energia	373kcal / 1585 kJ	127 kcal / 539 kJ	6
Lípidos	1,2 g	0,4 g	<1
Dos quais saturados	1,0 g	0,3 g	2
Hidratos de carbono	1,9 g	0,6 g	<1
Dos quais açúcares	1,1 g	0,4 g	<1
Fibra	0,86 g	0,29 g	-
Proteínas	87 g	30 g	60
Sal	1 g	0,3 g	5
Do qual Sódio	334 mg	114 mg	-
Cálcio	481 mg	164 mg (21%*)	-

Annex II

Amilopectin

Declaração nutricional	Por 100 g	Por porção (50 g)	% *
Energia	400 kcal / 1674 kJ	200 kcal / 837 kJ	10
Lípidos	0 g	0 g	0
Dos quais: saturados	0 g	0 g	0
Hidratos de carbono	100 g	50 g	19
Dos quais: açúcares	0 g	0 g	
Fibra	0 g	0 g	
Proteínas	0 g	0 g	
Sal	0 g	0 g	

Chocolate

DECLARAÇÃO NUTRICIONAL	POR 100g DE PRODUTO	POR PORÇÃO (20g)	%DR*	DR*
ENERGIA	1690kJ 401kcal	338kJ 80kcal	4	8400 kJ 2000kcal
LÍPIDOS	6,5g	1,3g	2	70g
DOS QUAIS: ÁCIDOS GORDOS SATURADOS	4,5g	0,9g	5	20g
HIDRATOS DE CARBONO	74g	15g	6	260g
DOS QUAIS: AÇÚCARES	68g	14g	15	90g
FIBRA	9g	1,8g		
PROTEÍNAS	7g	1,4g	3	50g
SAL	0g	0g	0	6g

Annex III

identification code	Lot number	Group		Weight emb + prod. (gg)		Net Weight (g)		Consumption
		PRO	PLA	start	end	start	end	

Annex IV

P.PORTO

ESCOLA
SUPERIOR
DE SAÚDE
POLITÉCNICO
DO PORTO

TERMO DE CONSENTIMENTO INFORMADO

Declaração de Consentimento Informado

Conforme a lei 67/98 de 26 de outubro e a "Declaração de Helsínquia" da Associação Médica Mundial (Helsínquia 1964; Tóquio 1975; Veneza 1983; Hong Kong 1989; Somerset West 1996, Edimburgo 2000; Washington 2002, Tóquio 2004, Seul 2008, Fortaleza 2013)

Suplementação com proteínas isoladas *Whey* e sua influência na hipertrofia e força muscular em atletas amadores (SPIHFMA)

Eu, abaixo-assinado _____:

Fui informado de que o Estudo de Investigação acima mencionado se destina a avaliar os efeitos da suplementação com proteína *whey* sobre a massa muscular, força muscular e massa gorda.

Sei que neste estudo está prevista a realização de uma intervenção que consiste na ingestão de um suplemento desportivo alimentar hiperproteico ou de um suplemento alimentar controlo isocalórico tendo-me sido explicado em que consistem e quais os seus possíveis efeitos.

Também sei que alguns exames serão realizados em dois momentos diferentes nas instalações da Escola Superior de Saúde do Porto e que consistem em medições corporais da massa muscular, força muscular e massa gorda.

Foi-me garantido que todos os dados relativos à identificação dos Participantes neste estudo são confidenciais e que será mantido o anonimato.

Sei que posso recusar-me a participar ou interromper a qualquer momento a participação no estudo, sem nenhum tipo de penalização por este facto.

Compreendi a informação que me foi dada, tive oportunidade de fazer perguntas e as minhas dúvidas foram esclarecidas.

Aceito participar de livre vontade no estudo acima mencionado.

Concordo que sejam efetuados os exames que fazem parte deste estudo comprometendo-me a estar disponível para os realizar nas datas pré-determinadas.

Também autorizo a divulgação dos resultados obtidos no meio científico, garantindo o anonimato.

Investigador:
Nuno Miguel Silva Duarte
Professor Assistente Convidado da Licenciatura em Farmácia
M: RUA DR ANTÓNIO BERNARDINO DE ALMEIDA, 400. 4200-072 PORTO. PORTUGAL
T: +351 222 061 051 (Gabinete) TLMVL: 912099732
E: nsd@ess.ipp.pt

_____/_____/_____ DATA

_____ ASSINATURA

Annex II

Ethics Committee trial approval

ESCOLA SUPERIOR DE SAÚDE
POLITÉCNICO DO PORTO

ESCOLA SUPERIOR DE SAÚDE P. PORTO
DATA 10 JAN 2018
N.º 000120
ENTRADA

P.PORTO

PARECER DA COMISSÃO DE ÉTICA

3901

Número de Registo da Comissão de Ética

15.12.2017

Data receção do Documento

Sim

Existência de entradas anteriores

TÍTULO DO TRABALHO

Suplementação com proteínas isoladas Whey e sua influencia na hipertrofia e força muscular em atletas amadores

INVESTIGADOR RESPONSÁVEL

Nuno Duarte

DATA PREVISTA PARA A REALIZAÇÃO DO TRABALHO

Início 1 de janeiro de 2018; Fim Abril de 2018

RESUMO DO ESTUDO

OBJETIVOS

Presentes

AMOSTRA

Descrição presente. Forma de contacto voluntária

FORMULÁRIO DE DADOS A RECOLHER

Presente mas com dados sensíveis (nome, contactos)

MATERIAL

Presentes

MÉTODOS

Descrição presente. Com referência a garantia do anonimato e confidencialidade, através de codificação.

RISCOS

Sem referência a riscos.

CONSENTIMENTO INFORMADO

Presente

AUTORIZAÇÃO PELOS RESPONSÁVEIS LOCAIS

Autorização presente do ISEPGym

APRECIAÇÃO DA COMISSÃO DE ÉTICA

Reúne condições para parecer favorável.

PARECER FINAL DA COMISSÃO DE ÉTICA

Reúne condições para parecer favorável.

DATA: 19 dezembro de 2017

ASSINATURAS