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EFFECTS OF 12 WEEKS OF ESSENTIAL AMINO ACIDS (EAA)-BASED MULTI-INGREDIENT NUTRITIONAL SUPPLEMENTATION ON MUSCLE MASS, MUSCLE STRENGTH, MUSCLE POWER AND FATIGUE IN HEALTHY ELDERLY SUBJECTS: A RANDOMIZED CONTROLLED DOUBLE-BLIND STUDY.

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CYCLE XXXI
Track in Physiology

In memory of

Prof. Fulvio Marzatico

Contents

Synthesis of the thesis

While muscle aging and age-related sarcopenia are common in the older population and have huge personal and financial costs, they still have no broadly accepted clinical definition, consensus, diagnostic criteria or treatment guidelines. This has led to problems in the development of pharmacologic interventions to modify the natural history of this kind of muscle disorder. Indeed, a number of potential drug targets have been identified as a result of improved understanding of the functional and structural changes seen in sarcopenia at the molecular level, but at present no pharmacological therapies with regulatory approval are available.

More promising research in this field has extensively explored the role of physical activity interventions and nutrition strategies (frequently coupled together) with interesting growing data. However, during aging, medical conditions often prevent subjects from carrying out physical activity and the need for effective nutritional supplements *per se* becomes essential. So far, on the latter point literature is still scarce and the present thesis has been developed based on the need for an advancement of knowledge.

In the *background* section, mechanisms involved in the development of muscle aging have been summarily described to better introduce the scope of this work. Furthermore, a description of the current methods used to measure muscle mass and function in

elderly subjects has been included with the aim of explaining why the procedures we used to assess muscle strength, muscle power and fatigue can be effectively adopted to establish the effects of a nutritional supplement.

The *introduction to the thesis* describes the available data on the use of nutritional supplements for the prevention and treatment of age-related muscle impairment and defines the aim of the study, oriented to evaluate the efficacy of a multi-ingredient supplement to counterbalance progression of muscle mass and function loss.

In the *methods* paragraph we described primary and secondary outcomes measured in detail. Similarly to the few previous studies, our *results* showed that an EAA-based multi-ingredient supplement can promote muscle mass, strength and power, with positive effects on metabolic markers (vitamin D blood levels and visceral adipose tissue), and represent an effective strategy to prevent sarcopenia and its functional consequences. Conversely, muscle fatigue was not affected by the treatment. The *results* have been widely described and interpreted in the *discussions* paragraph.

In *conclusion*, based on the results presented in this work we can consider the tested supplement to be very promising in offsetting the age-related decline of muscle mass and function and suitable for future investigations on bioavailability assessment and biochemical responses of combined components in the formula.

Abstract of the thesis

Objective: to counteract muscle mass, muscle strength and power loss during aging, and to study age-related change of neuromuscular manifestation of fatigue in relation to nutritional supplementation. *Design:* randomized controlled double-blind study. *Setting:* twice-daily consumption for 12 weeks of an Essential Amino Acids (EAA)-based multi-ingredient nutritional supplement containing EAA, creatine, vitamin D and Muscle Restore Complex®. *Participants:* 38 healthy elderly subjects (8 male, 30 female; age: 68.91±4.60 years; body weight: 69.40±15.58 kg; height: 1.60±0.09 m) were randomized and allocated in supplement (SUPP) or placebo (PLA) group. *Mean Measurements:* vitamin D blood level; Appendicular Lean Mass (ALM); Visceral Adipose Tissue (VAT); Maximal Voluntary Contraction (MVC) and Peak Power (PP); myoelectric descriptors of fatigue: Fractal Dimension and Conduction Velocity initial values (FD iv, CV iv), their rates of change (FD slopes, CV slopes) and the Time to perform the Task (TtT). *Mean Results:* significant changes were found in SUPP compared to baseline: vitamin D (+8.73 ng/ml; $p<0.001$); ALM (+0.34 kg; $p<0.001$); VAT (-76.25 g; $p<0.001$); MVC (+0.52 kg; $p<0.001$); PP (+4.82 W; $p<0.001$). Between group analysis (SUPP Vs. PLA) showed improvements: vitamin D blood levels (+11,72 ng/ml; $p<0.001$); Legs FFM (+443.7 g; $p<0.05$); ALM (+0.53 kg; $p<0.05$); MVC (+1.38 kg; $p<0.05$); PP (+9.87 W; $p<0.05$). No statistical changes were found for FD iv, CV iv, FD and CV slopes and TtT, either

compared to baseline or between groups. Significant correlations between mean differences in SUPP group were also found. *Conclusion:* the study demonstrates that in healthy elderly subjects an EAA-based multi-ingredient nutritional supplementation of 12 weeks is not effective to change myoelectric manifestation of fatigue and TtT failure but can positively affect muscle mass, muscle strength, muscle power and VAT, counterbalancing more than one year of age-related loss of muscle mass and strength.

Keywords: essential amino acids, creatine, vitamin D, antioxidants, muscle aging, muscle strength, muscle power, muscle fatigue.

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1. Background of aging

1.1. The physiology of aging

Aging is a dynamic biological process characterized by continuous remodeling [1] which depends on a complex interaction between genetic, environmental and stochastic factors [2]. Theories describe aging as the result of cumulative homeostatic imbalances: cells progressively lose their morphological specificity and the ability to function correctly, leading to the deterioration of tissues, organs, systems and of the organism as a whole. This progressive and cumulative deterioration with aging is often referred to as *senescence*. Therefore, senescence is the expression of the physiological human aging in which the organ reserve capacity and resistance to stressors are generally decreased, with differences that vary significantly between individuals. Although senescence is distinct from disease, it can increase the risk of developing a disease, reduce the ability to recover from a disease, and in general it can reduce the quality of life [3]. Advanced senescence can eventually turn to *frailty*, a more severe state of physiological vulnerability. Frailty is a common clinical syndrome in much older adults that carries an increased risk for poor health outcomes including falls, accidents, disabilities, infections, hospitalization, and mortality [4]. In contrast to the process of senescence, frailty is characterized by specific objective criteria such as generalized fatigue, weakness, and weight loss [3].

In relation to the level and severity of cellular dysfunction, senescence may be divided into *primary* or *secondary* aging. Primary aging involves an unavoidable decline of cellular structure and function independent of disease and/or environment. In this context, aging is inevitable. In contrast, secondary aging involves cellular deterioration due to preventable lifestyle and environmental exposures. Thus, secondary aging offers promising opportunities for interventions, especially to prevent or delay conditions of frailty, based on a reduction of inactivity and sedentary behavior, increase of regular exercise, correct nutritional intake and the use of specific dietary supplements [3].

Although senescence can affect all tissues, organs, and physiological systems in the body (Figure 1), the deterioration of some specialized cell types has more profound effects on the physical ability to perform tasks of daily life and maintain independence. Among the body's systems, combined aging of the musculoskeletal and cardiorespiratory systems appears to produce the most significant functional limitations. Indeed, as skeletal muscle function declines and cardiorespiratory capacity is impaired, body composition shifts toward fat accumulation, and older adults sense more fatigue and weakness in daily activities [3].

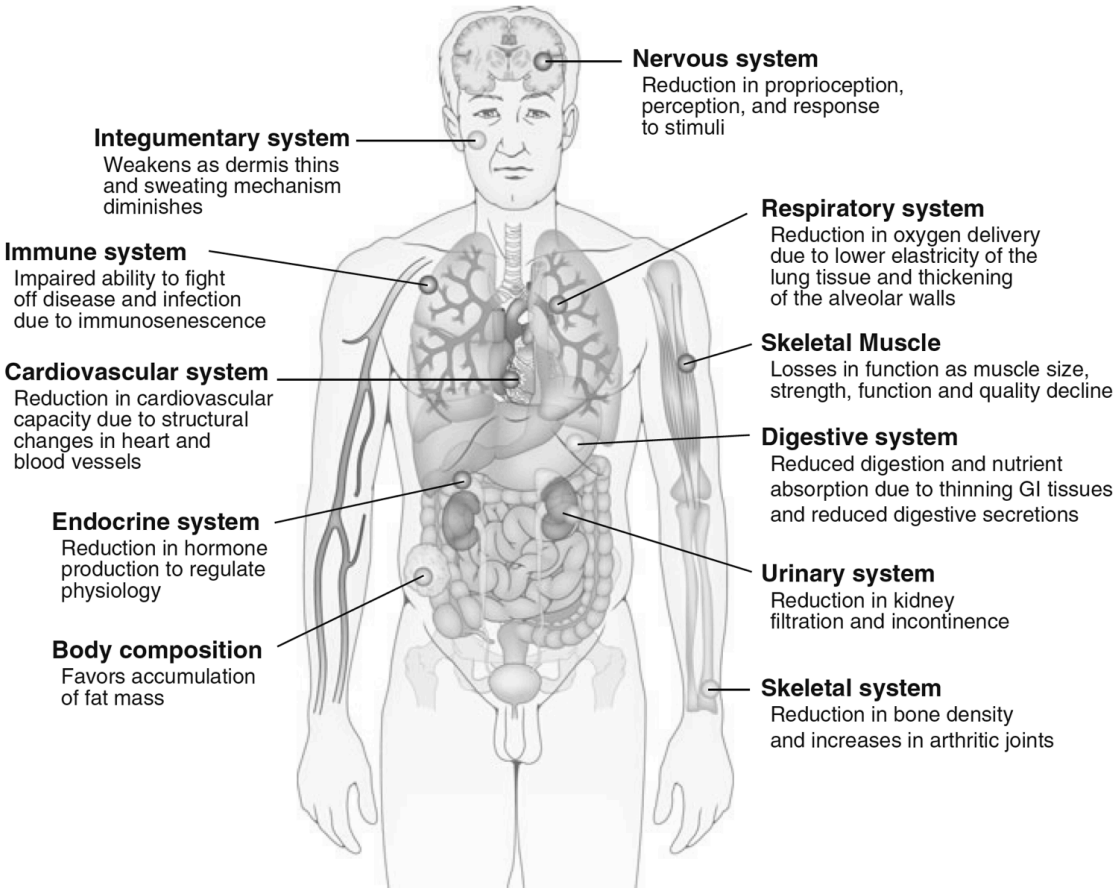

Figure 1 – Common age-related changes to body's physiological systems. Modified from Fragala, 2015 [1].

In order to best perform anti-aging interventions, the causes of cellular senescence must be carefully studied with a particular focus on the reduction in cumulative cellular damages and relative homeostatic imbalances. From this point of view, theories based on structural damage attribute aging to molecular dysfunctions that accumulate in cells over time and result in their breakdown and malfunctioning. The most widely held

structural damage theory is the *free radical theory*, which is based on the oxidative cell hypothesis. According to this theory, the cumulative exposure to free radicals over a lifetime damages cells so that their functioning becomes impaired. Free radical exposure can result in cellular damage in the form of wear and tear, faulty reconstruction, mitochondrial damage and consequent impairment of sub cellular molecular trafficking [3]. Besides the free radical theory, one of the most recent theories on aging focuses on immune response, and takes into consideration the activation of subclinical, chronic low-grade inflammation which occurs with aging, named *inflammaging* [1]. Long-lived people, especially centenarians, seem to counter chronic subclinical inflammation through an anti-inflammatory response, thus called anti-inflammaging [1]. Even though the rate of progression of inflammaging is currently recognized as the main force driving aging and one of the main risk factors for clinical morbidity and mortality in the elderly, current knowledge on the causal agents is still incomplete and the clinical evaluation of inflammaging has not yet been standardized [2].

1.2. The muscle aging

The origins of muscle decline during aging are multifactorial and include muscle disuse, neuropathic processes, endocrine dysfunction, chronic disease, inflammation and nutritional deficiencies [5] (Figure 2).

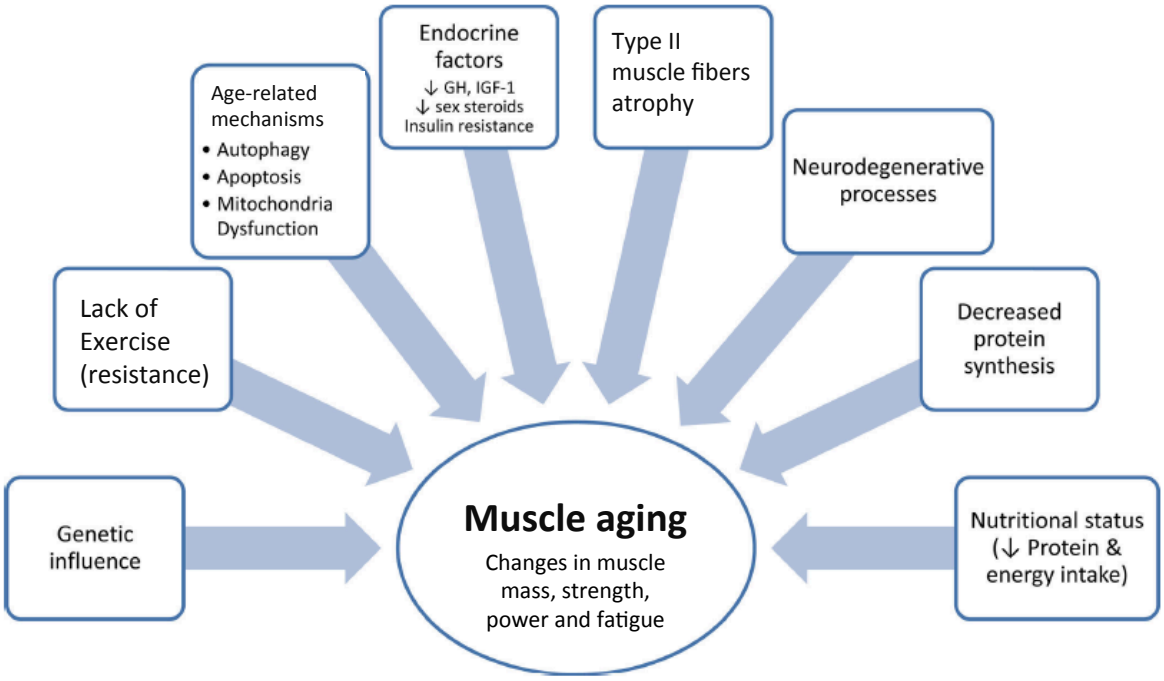

Figure 2 – Schematic representation of the factors involved in muscle aging. Modified from Ali et al., 2014 [6].

All of these possible causes change the regulation of skeletal muscle protein metabolism leading to a condition of negative protein balance and resulting in a gradual net loss of skeletal muscle protein [7]. Muscle mass and functions are regulated by many factors that are susceptible to change during the aging process, including hormone status (i.e. insulin, growth hormone, testosterone, and IGF-1), mechanical forces (i.e. physical activity and exercise), and nutrition (e.g. amino acids intake) [7]. Muscle loss is

also frequently associated with insufficient vitamin D serum level [8] and reduction in resting metabolic rate (RMR) [9].

1.2.1. Muscle mass and strength

Progressive and generalized loss of skeletal muscle mass and strength during aging [5] occurs from about the fourth decade of life [10] and contributes to various negative health outcomes, such as metabolic disorders (i.e. insulin resistance with subcutaneous and visceral body fat deposition) and progression to frailty [11] with a high risk of physical disability, poor quality of life and death [5]. After about age 50, muscle mass decreases at an annual rate of about 1% [12], the cross-sectional area of skeletal muscle is reduced by 25–30% at age 70 (Figure 3), and muscle strength declines by about 1.5% per year between ages 50 and 60 and by 3% per year thereafter [12, 13].

The decrease in muscle mass and muscle strength in elderly subjects, when compared with young people, is linked to the reduction in muscle fibers size, which is fiber-type specific. Evidence suggests that type II fibers are more vulnerable to the aging process (decreasing by up to 40%) than type I fibers [14, 15]. Furthermore, considering the subtypes of type II fibers, greater atrophy of type IIX fibers than of type IIA fibers has been found in older men (22%, versus 13%) and women (30% and 24%). Also satellite cell numbers decrease during aging, reducing muscle regeneration capacity [14,

15]. The loss of muscle strength, in particular in elderly men, is positively correlated with the loss of muscle mass, as well as the decrease of type II muscle fibers cross-sectional area, myonuclear and satellite cell content [16].

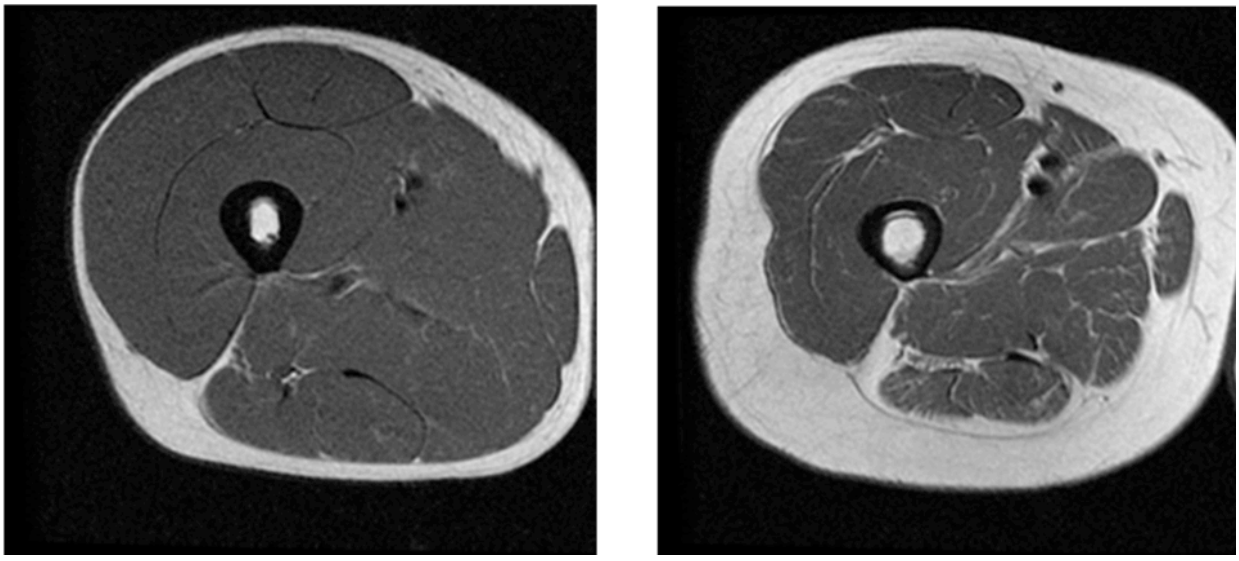

Figure 3 – Changing of the cross-sectional area (magnetic resonance imaging) of a muscle (thigh) during aging: 25 years old on the left; 65 years old on the right.

Although early changes in muscle mass and strength may be subtle and symptom-free, their age-related progression can lead to the development of a severe muscle decline condition defined as *sarcopenia* [5]. Sarcopenia (from Greek *sarx* or flesh + *penia* or loss) can be profoundly debilitating, impairing a person's ability to perform

routine activities of daily life (e.g. getting out of a chair, walking up stairs, lifting loads) [5]. On average, in the absence of a disease or injury, it is estimated that 5–13% of elderly people aged 60–70 years are affected by sarcopenia, and the numbers increase by up to 50% for those aged 80 or above. Sarcopenia can be considered *primary* (or age-related) when no other cause is evident but ageing itself, while sarcopenia is named *secondary* when one or more other causes (e.g. diseases, cachexia) are evident. In many older people, the aetiology of sarcopenia is multi-factorial so that it may not be possible to characterize each individual as having a primary or secondary condition. This situation is consistent with recognizing sarcopenia as a multi-faceted geriatric syndrome [5].

1.2.1.1. Measure of muscle mass and strength

A wide range of methods can be used to evaluate muscle mass [17]. They differ greatly in accuracy and precision and this determines whether the tools and assessment are better suited to clinical practice or research (Table 1).

Anthropometric methods (e.g. body mass index, arm and calf circumference and skinfold thickness) are simple but little precise and are prone to overestimation [18]. Bioelectrical impedance is a popular alternative and easy to use in both research and clinical settings [19], despite a lack of a standardized methodology [20, 21]. Air-displacement plethysmography is a highly reproducible method of measuring body

composition, but it relies on the assumption that the density of fat mass and fat-free mass are the same in all patients [22].

Table 1 - Techniques utilized for the assessment of muscle and fat mass. Modified from Cooper et al., 2013 [18].

	Muscle	
	Accuracy	Precision
Anthropometry	+/−	+/−
Bioelectrical impedance	+/−	+
Air-displacement plethysmography	NA	NA
DXA	++	++
CT / MRI	++	++(+)

Notes: DXA, Dual Energy X-ray Absorptiometry; CT, Computed Tomography; MRI, Magnetic Resonance Imaging.

Three imaging techniques have been used for estimating muscle mass or lean body mass: Computed Tomography (CT scan), Magnetic Resonance Imaging (MRI) and Dual Energy X-ray Absorptiometry (DXA). CT and MRI are considered to be very precise imaging systems that can separate fat from other soft tissues of the body, making these methods gold standard for estimating muscle mass in research. However, high costs, limited access to equipment at some sites and concerns about radiation exposure limit

the use of these whole-body imaging methods for routine clinical practice [5]. DXA is a validated alternative method both for research and for clinical use to distinguish fat, bone mineral and lean tissues. This whole-body scan exposes the patient to minimal radiation. Muscle mass is a well-characterized end point that can easily be measured using DXA. Through a DXA scan it is possible to obtain the Skeletal Muscle Mass Index (SMI): defined as the sum of the muscle mass of the four limbs (Appendicular Lean Muscle Mass, ALM) divided by height squared (ALM/H^2). An SMI of two standard deviations below the mean SMI of young male (7.26 Kg/m^2) and female (5.5 kg/m^2) reference groups was defined as the gender-specific cut point for sarcopenia [5].

Strength can be measured isokinetically or isometrically. Isokinetic dynamometry is the recognized gold standard for measuring muscle strength, but its use is limited by the cost and availability of special equipment [18]. Conversely, isometric strength testing of Maximal Voluntary Contractions (MVCs) can be measured with relatively more simple custom-made equipment. Isometric handgrip strength is a well-validated technique test, which is strongly related to lower extremity muscle strength and structure (knee extension torque and calf cross-sectional muscle area) [5]. The handgrip is widely used both in clinical practice and research setting, while other isometric tests (one leg/arm) are better designed for investigation purposes and their use in clinical practice is limited by the need for special equipment and training. In our lab we adopt a validated research

method [23, 24] based on the use of an isometric-ergometer developed for the measurement of upper limb MVC (Figure 4).

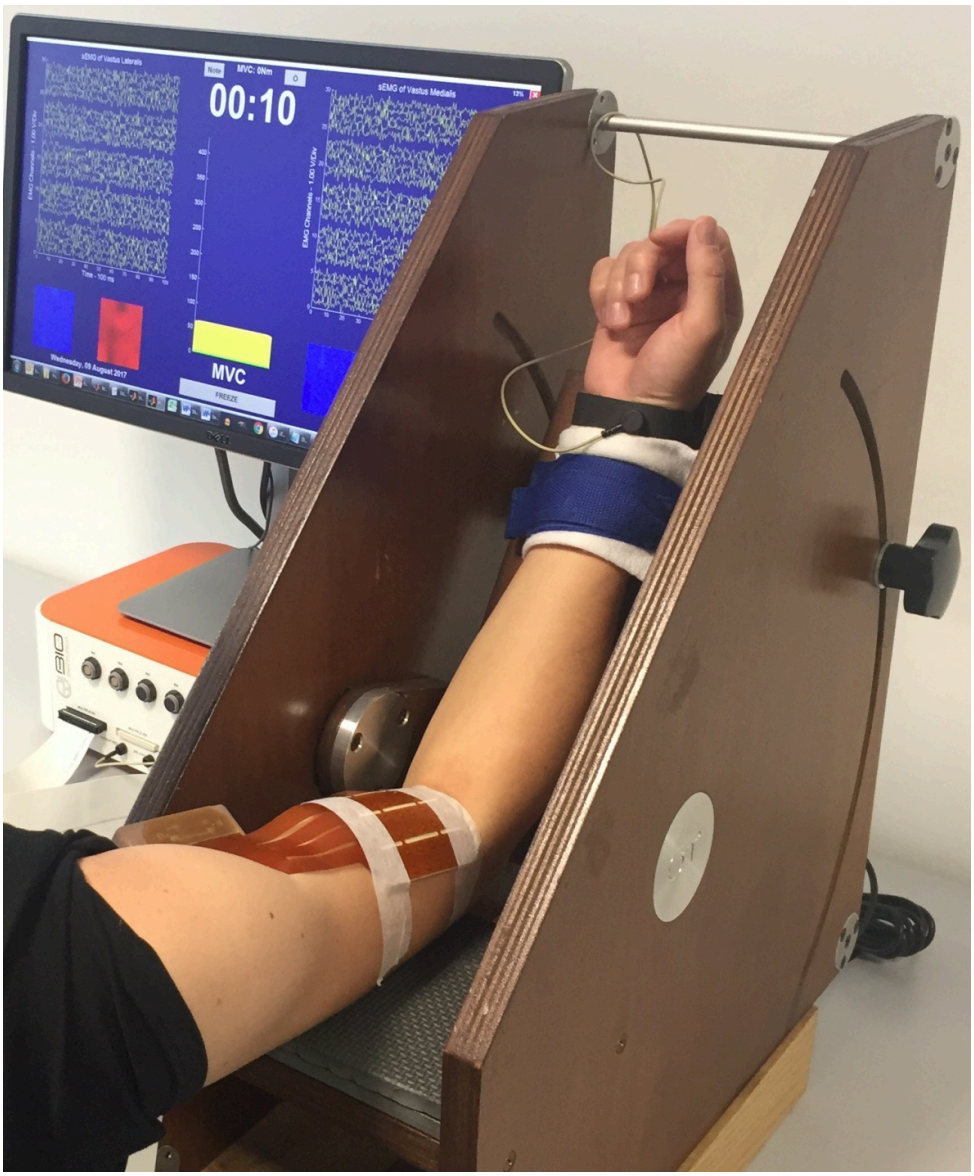

Figure 4 – Isometric ergometer for strength test on upper limb equipped with surface electromyography array. Picture modified from Negro et al., 2018 [24].

1.2.2. Muscle power

Alongside the loss of muscle mass and strength, it has been demonstrated that the decrease of functional performance in elderly people is also related to the decline of neuromuscular power. Muscle power (the maximum rate of work undertaken by a muscle per unit of time) appears to be better still at predicting functional status as it includes a neuromuscular component that provides information from pathways that are not captured by measures of muscle mass and strength [25]. Muscle power is a strong predictor of functional mobility and risk of falling among older adults [26]. Many fundamental motor tasks measured in elderly subjects reported the decline of muscle power, such as concentric contractions, explosive isometric contractions, and jumps and this has been linked to age-related impairments in neuromuscular activation [27], tendon stiffness [28], muscle contractile speed [29], and changes in muscle architecture [28]. In particular, changes in neuromuscular activation are due to a decrease in maximal discharge frequency of Motor Units (MUs) and in the percentage of MUs that exhibit doublets (i.e. a double discharge at intervals <5 ms) [30]. The reduction of MU firing rates seems to be related to a "fast-to-slow" remodeling of MUs, which is observable during aging [31]. In fact, age-related remodeling of MUs appears to involve denervation of type II (fast) skeletal muscle fibers with reinnervation of some, but not all, denervated fibers through collateral sprouting of nearby surviving motor axons

(usually slow twitch type). Although this mechanism allows the slow MUs to gain control of the population of denervated type II muscle fibers, preventing their atrophy, it creates very large remodeled MUs, which are able to produce fewer maximal firing rates, force and velocity production, contributing to the loss in muscle power [31, 32]. The decrease in muscle power with age is higher than the decrease in maximal strength, and declines are more pronounced from 70 to 90 years [33] (Figure 5).

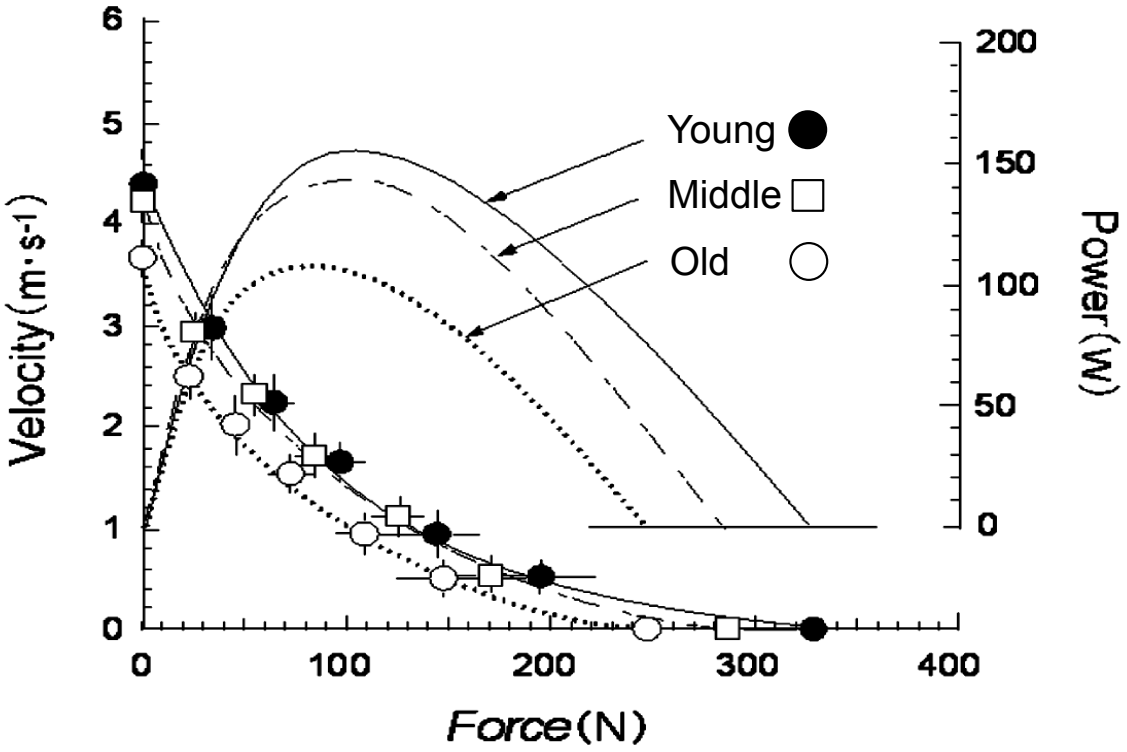

Figure 5 - Force-velocity and force-power relationships (elbow flexors) in men: elderly (70 years old), middle-aged (50 years old), and young subjects (20 years old). Modified from Toji et al., 2007 [34].

1.2.2.1. Measure of muscle power

Peak skeletal muscle Power (PP) achieved during different exercise tests (e.g. leg press, knee extension or elbow flexion) has been validated as a reliable and functionally relevant outcome in older populations [25, 34]. However, as an outcome measure of muscle decline for use in clinical practice, muscle power is potentially limited by the need for expensive equipment and, furthermore, measures of PP are inappropriate for use in people with arthritis or other mobility problems. To overcome this, the European Working Group on Sarcopenia in Older People (EWGSOP) recommended a wide range of tests of physical performance which are useful in detecting muscle power in a clinical setting, including the gait speed test, 6-min walk test and the stair climb power test [5]. Although these tests are easy to put into practice, they only evaluate the velocity of the motion and do not provide information about the force produced; from this point of view studies that investigate the force-velocity relationship with regard to aging are lacking [34]. In our lab we use a submaximal multiple trials-based muscle power test suitable for creating a force-velocity curve and calculating the PP value in elderly subjects too (Figure 6).

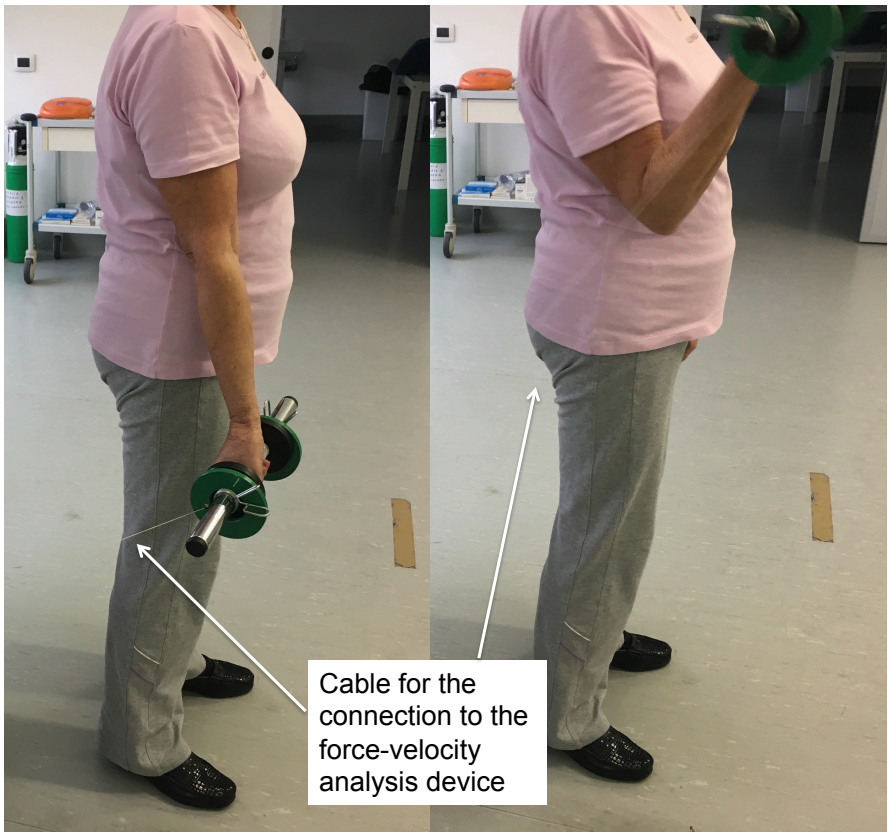

Figure 6 - Muscle power assessment performed on dominant upper limb with Musclelab™ 4000 equipment (Ergotest Innovation A.S., Norway).

1.2.3. Muscle fatigue

Neuromuscular fatigue is also affected by aging. Muscle fatigue is defined as the "muscle's inability to maintain an expected force" [35]. Based on this definition, many mechanical protocols have been proposed to investigate muscle fatigue. The Time to Task (TtT) failure in a prolonged submaximal isometric contraction could offer an index of fatigability: old adults exhibit a longer TtT failure than young adults when

performing a submaximal isometric contraction [36]. This unexpected phenomenon is known as the "fatigue paradox": elderly subjects seem to be more fatigue resistant than young adults. Several factors can be attributed to this process in aged muscles: selective atrophy of type II fibers, slowing in the contractile properties, lower MU firing rates, and greater reliance on oxidative metabolism [36].

Muscle fatigue can be divided into central and peripheral components. Central fatigue originates in structures above the neuromuscular junctions from the central nervous system to the peripheral nerves leading to changes in MU recruitment behavior including decline in the MU recruitment threshold and progressive recruitment of new MU without change to the recruitment order [37]. Peripheral aspects of fatigue include local changes at the skeletal muscle level, namely metabolic acidosis and protons accumulation [38] that may impair sarcolemmal excitability, electromechanical coupling, acto-myosin myofibrillar interaction and metabolic fuelling of the myofibers.

Fatigue can be measured by a variety of methods (Table 2). However, detection of central and peripheral components of fatigue [39-41] and their relative contribution during exercise are not particularly suitable for quantification or measurement [42]. In our lab we use an accurate and continuous recording of local muscle fatigue during tasks by evaluating myoelectric activity of a selected muscle by surface electromyography (sEMG).

Table 2 - Methods for assessment of muscle fatigue. Modified from Cooper et al., 2013 [18].

Method	Assessment	Protocol
MVC and SVC	Measures MVC and SVC until exhaustion	Sustainment of MVC or SVC at 20–60 % of MVC until failure (↓50 %)
Isokinetic measurements	Measures isometric torque, isokinetic torque, and total work performed	Five contractions at an angular velocity of 60–90°/s; 15–30 contractions at a velocity of ∼300°/s
Surface electromyography	Analyzes the myoelectrical manifestation of fatigue during muscle contractions	Used during MVC and SVC
Twitch interpolation	Differentiates fatigue of central origin from that of peripheral origin	MVC associated with nerve stimulation; failure if the difference between MVC and twitch is >15 %
Critical power	Assesses the ability to sustain exercise under anaerobic conditions	Series of short-duration, high-intensity exercises determines critical power (fatigue threshold)
Borg scale	Assesses the perception of fatigue using scales	Borg scale
^{31}P-MRS	Directly and noninvasively measures intramuscular metabolism	Repetitive localized exercise of MMII, in the MRS system assesses high-energy compounds
Biopsy	Identifies the microstructural and bioenergy characteristics of the muscles	Collection of vastus lateralis muscle samples
Determination of lactate and ammonia levels	Assesses the inability to convert oxygen into energy in acid solutions	Collection of venous, arterial, or arterialized blood samples at rest, during exercise, and during recovery

Notes: MVC, Maximal Voluntary Contraction; SVC, Submaximal Voluntary Contraction; MMII, Muscle Mass of lower limb; MRS, Magnetic Resonance Spectroscopy.

1.2.3.1. Measure of myoelectric manifestations of fatigue

Biochemical and physiological changes in muscles during fatiguing contractions are also reflected in properties of myoelectric signals recorded on the surface of the skin above the muscle(s) concerned, leading to what is commonly defined as myoelectric manifestations of fatigue [43].

sEMG can analyze the myoelectric manifestations of fatigue, mainly linked to two physiological exercise-related phenomena: 1) the slowing of Motor Unit Action Potentials (MUAPs) as they travel along muscle fibers, that is the reduction of their Conduction Velocity (CV) [44-46], and 2) the synchronization of MU by the central nervous system, which is described as a higher occurrence of simultaneous discharge of action potentials from various MUs to increase the force production when the whole MU pool is recruited [47], as observed in trained subjects [48] and in presence of central lesions [49]. Therefore, to assess peripheral components of fatigue, CV rate of change (i.e. slope) might be measured during isometric muscular tasks [50-57], whereas to evaluate central components of fatigue, sEMG descriptors of MU synchronization may be used. A sEMG descriptor of the MU synchronization level is Fractal Dimension (FD), which shows high reliance on MU synchronization [58, 59].

Changes in sEMG myoelectric manifestations of fatigue anticipate mechanical muscle fatigue from the beginning of the contraction [60]. Therefore, modifications of

sEMG highlight neuromuscular fatigue before mechanical failure: this is particularly suitable in the elderly population since the sEMG estimation of indices of fatigue avoids discomfort and danger possibly related with exhaustive efforts.

To obtain high-quality sEMG signals, in our lab we select the biceps brachii as a target muscle due to the isolation of the muscle contraction, fluency of movement, and fibers orientation [23, 24]. A setup of the sEMG recording procedure we use is shown in Figure 4.

2. Background of countermeasures to prevent muscle aging

To attenuate muscle aging and prevent the development of sarcopenia's adverse consequences [61], current scientific and clinical approaches are based on the combination of regular exercise programs and proper nutrition strategies combined with the use of supplements. Studies in this field have extensively explored several types of interventions with interesting and promising data [62, 63], while alternative treatments based on administration of hormone preparations such as testosterone, GH, and estrogens are still not universally accepted and require further investigation [63].

2.1. Physical activity

Physical activity is an important strategy to counter many of the observed age-related physiological declines. Overall, physical exercise participation slows physiological changes of aging that impair functional capacity. Studies show that exercise can alleviate age-related changes in body composition, promote psychological and cognitive well-being, reduce risks of falls, frailty and disability, and increase general longevity [3]. Furthermore, physical exercise is able to regulate and improve several aspects of aged muscle: intramuscular adipose infiltration, expression of strength and power, muscle fiber area and muscle quality, glucose tolerance and insulin sensitivity [3].

2.1.1. Aerobic exercise

Notably, Aerobic Exercise (AE) is particularly effective in improving the circulatory function, which typically declines with age, acting through positive effects on cardiovascular diastolic capacity, blood vessel elasticity and endothelial function. In skeletal muscle, programs of AE can increase important cellular function such as mitochondrial content and biogenesis, mitochondrial protein gene transcripts, muscle oxidative capacity and enzyme activities, muscle protein synthesis rates in type I myofibers [3]. Limiting mitochondrial changes in skeletal muscle during aging is an important recognized process in maintaining general metabolic health in elderly subjects [64]. Thus AE can positively regulate the expression of peroxisome proliferator activated receptor gamma 1 (PGC-1), particularly the alpha isoform (PGC-1α), a key player implicated in mitochondrial biogenesis [64].

2.1.2. Resistance exercise

Resistance Exercise (RE) is considered the most effective strategy to offset sarcopenia, promoting gains in strength, power and muscle mass [65]. Regular practice of RE results in an increased type II muscle fiber area [65], and this is particularly important because, as described above, type II muscle fiber atrophy and loss predominates during muscle aging and development of sarcopenia. The muscle

anabolic effect of RE is obtained through a fiber-type specific stimulation of Muscle Protein Synthesis (MPS) regulated in particular by the mammalian Target of the Rapamycin Complex 1 and the ribosomal protein of 70-kDa S6 kinase 1 (mTORC1-p70S6K1) pathway [65]. Over time, persistent stimulation of this pathway via loaded contractions and combined with adequate protein ingestion leads to lean mass accretion. For example, in a 16-wk training trial involving older adults between the ages of 65 and 75, RE increased muscle mass by 1.5 kg and overall strength by 60% [66]. Studies on RE in the elderly, using traditional slower movement speeds, reported greater increases in maximum strength compared with power [67-69]; however, by incorporating higher-velocity training protocols, other studies suggest that the gains in power may be either comparable [70-72] or greater [73] to gains in maximum strength/force production.

2.1.3. Exercise recommendations and limitations

In 2009 the American College of Sports Medicine (ACSM) published the physical activity recommendations for older adults [74]. These recommendations include various types of physical exercise: AE (walking or swimming), RE (lifting weights or using weight machines), flexibility (stretching), and neuromotor (balance exercises such as yoga or tai chi). However, despite the exercise benefits described, studies show that

physical activity participation decreases with aging both in the amount of physically active time and in the intensity of activities performed, especially for RE. A review from Franco et al. (2015) [75] shows that around 45% of people aged over 60 years do not meet the ACSM's recommended level of physical activity and the proportion of those who do not meet the recommended guidelines increases to 75% for those aged 75 and over. A rapidly increasing problem to be taken into account, considering that the number of people aged over 65 in the world is expected to triple in the next 30 years [75].

2.2. Nutrition guidelines

As a modifiable risk factor, nutrition is a potential target to improve or prevent the loss of physical function in older adults. In particular, consumption of dietary proteins (e.g. meat, fish, eggs) can act synergistically with RE to enhance the MPS response [65]. Proteins can also promote the rates of MPS independently of exercise, but this ability is blunted in older adults [63, 65]. Based on this statement, to maintain muscle mass and functions, elderly subjects need to have a greater protein intake compared with younger subjects; older people should have an average intake of protein of 1.2/g/kg/day of bodyweight/day [63, 65]. Furthermore, the threshold for anabolic meal intake of protein/amino acids must be greater in elderly subjects (i.e., 25 to 30 g of protein per

meal, containing approximately 2.5–2.8 g of leucine), compared with young adults [63, 65].

2.3. Dietary supplements

Specific nutrients are of particular interest because of their demonstrated role in the muscular system, and have been the object of several studies, either as single supplements or in combination with other supplements. In particular, these include proteins or amino acids, especially those rich in leucine (which is the most potent branched-chain amino acid able to stimulate the MPS), vitamin D and creatine. The role of these compounds in muscle mass and function will be briefly described below, with the aim of better informing the reader of the thesis's methods, results and discussion.

2.3.1. Protein and amino acids

It is known that proteins and amino acids, in particular Essential Amino Acids (EAA), are necessary for the maintenance of muscle health in the elderly [63, 65]. Compared with younger subjects, the elderly require a larger amount of protein to obtain the same stimulation of MPS. In fact, in older adults the ingestion of 40 g of protein at rest [76] and after resistance exercises [77], compared with 20 g in young individuals [78], was needed to maximally promote MPS (Figure 7). Similarly, data

show that a low dose (5 g) EAA [79] was less effective than a higher dose (15 g) of EAA [80] in stimulating MPS in the elderly and, more importantly, that the rates of MPS achieved in older adults, when ingesting 15 g of EAA, were no different than those seen in the young individuals.

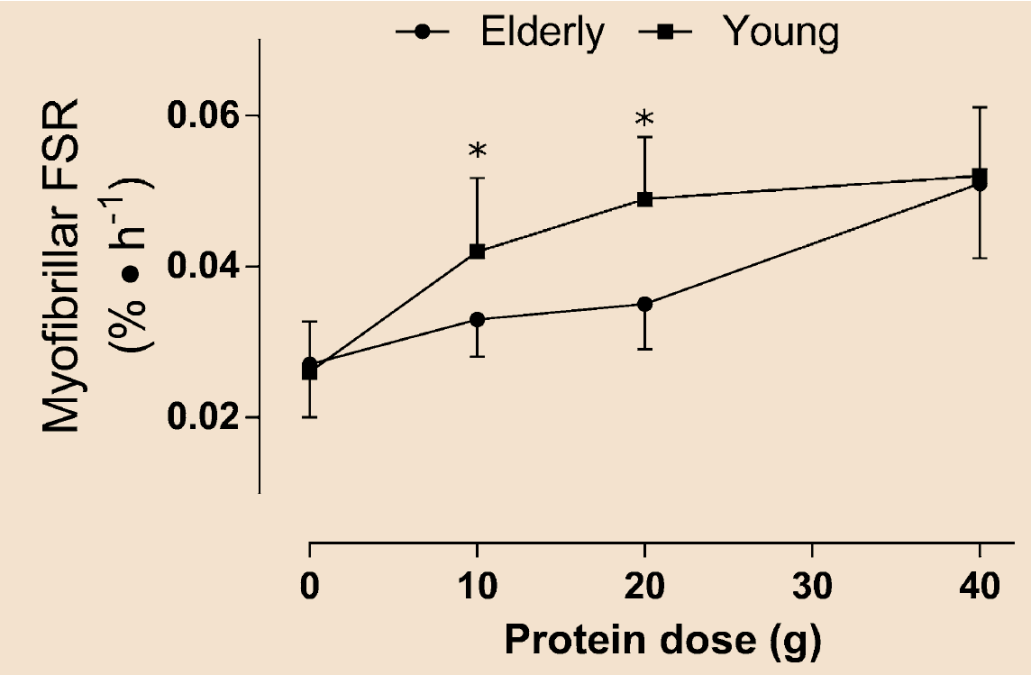

Figure 7 - Absolute protein dose-responses of skeletal muscle myofibrillar protein synthesis in older (71±1 y; n = 43) and younger (22±4 y; n = 65) men. *Significantly different between groups, P<0.05; FSR, Fractional Synthetic Rate [81].

Leucine is the most potent amino acid for stimulating MPS from activation of the nutrient and growth factor-sensing mTORC1 and in turn, its downstream targets

p70S6K1, translation initiation factor 4EBP-1, and elongation factor 2 [82, 83]. There is general agreement that older individuals have a higher leucine threshold and that they would benefit from larger amounts of leucine to stimulate MPS and muscle mass, either within a meal or as a protein/EAAs supplement [63, 65, 84, 85]. However, despite this, in the studies available, the population heterogeneity, study design, and the type of supplements studied preclude firm conclusions. Leucine was either given as pure crystalline powder, or as part of essential amino acid drinks, complete medical formula, or whey protein, at doses ranging from 2 g/day to 17.6 g/day. In many studies positive effects on MPS were obtained with a leucine dosage of about 3 g/day [63, 65, 86].

Despite several positive findings in relation to the effect of protein and/or amino acids supplementation on MPS, randomized clinical trials have reported opposing results on muscle mass or strength [87]. Heterogeneity was evident for trials that differed in the studied population (healthy, frail, diabetic, or sarcopenic individuals), duration, and supplement forms and doses. Importantly, usual dietary protein intake was not measured in all of the studies, thus limiting data interpretation as to additional protein effect. Most of the studies available assessed the impact of a combined protein supplement and exercise intervention on the muscle adaptation [88] of elderly people, whereas only few trials analyzed the effect of EAA.

2.3.2. Vitamin D

There has been growing interest in the implications of vitamin D status in the physical function of older adults given the high prevalence of vitamin D deficiency in this population [89]. The ubiquity of vitamin D receptors (VDRs) in various tissues, including muscles, is well recognized [90]. From its binding to VDRs, vitamin D mediates genomic and non-genomic effects in muscle cells; it namely promotes muscle contractility through calcium influx, myoblast differentiation, and the insulin sensitivity of muscles [91]. Large cross-sectional studies corroborate a relationship between insufficient level of serum 25(OH)D (<50 nmol/L) and low physical performance [91-96], mobility [92, 94-96], muscle strength [92, 93, 95, 96, 98, 99], and greater disability [92, 99] in free-living older adults. Vitamin D insufficiency has also been longitudinally associated with greater risks of disability [95, 100, 101], decline in physical performance [97, 102], and handgrip strength [103] in healthy older adults. The importance of considering baseline serum 25(OH)D concentrations has been emphasized, since individuals with vitamin D deficiency appear to be more responsive to supplementation [104]. Most of studies available showed that benefits are observed with vitamin D supplementation doses within the range of 800–2000 IU/day [87].

2.3.3. Creatine

It has been speculated that creatine supplementation could elicit gains in muscle mass through the activation of a number of anabolic signaling molecules. Initial studies investigating the role of Cr supplementation suggested that additional water retention in the muscle primarily contributes to the gain in mass. Differently subsequent research suggested that Cr supplementation increases body mass through greater muscle protein synthesis and therefore of the muscle fibers hypertrophy. From this point of view the pionieristic contribution by Volek et al. [105] is very interesting, demonstrating that a Cr ingestion in resistance trained males increased significantly the muscle fiber cross-sectional area in each of muscle fiber types observed: type I (35 % vs. 11 %), type IIA (36 % vs. 15 %), and type IIX (35 % vs. 6 %). Based on these findings, Willoughby and Rosene further examined the effects of Cr supplementation on gene and myosin heavy-chain protein expression of contractile filament [106, 107]. The conclusion by the authors indicated that increases in lean body mass are not solely attributable to greater water retention in the muscle, but rather to regulation of protein synthesis through a different gene expression of myogenic regulatory factors induced by Cr. These myogenic regulatory factors (e.g. MRF-4, Myf-5, Myo-D, and myogenin) act to control gene expression by binding to DNA and subsequently promoting muscle-specific gene transcription of fundamental muscular proteins such as myosin heavy chain, myosin

light chain, α-actinin, troponin I, and Cr kinase [108]. Furthermore, the influence of Cr supplementation on Satellite Cell (SC) function, based on the myonuclear domain theory, was explored by Olsen et al. [109] and Safdar et al. [110].

The existing findings generally demonstrate that post-supplementation muscle creatine may reach similar values in younger and older individuals alike, suggesting an efficient response in the elderly [111]. In a meta-analysis published in 2003, 43 out of 67 studies showed that creatine supplementation led to increased lean and/or body mass in young and middle-aged adults [112]. A more recent meta-analysis comprising 357 older adults demonstrated that creatine supplementation during resistance training can enhance muscle mass gain, strength, and functional performance over resistance training alone [113].

A recent meta-analysis revealed that resistance-trained young individuals experienced greater gains in lean body mass and muscle strength when whey proteins were consumed within a multi-ingredient supplement containing creatine, when compared to the ingestion of an iso-energetic equivalent carbohydrate or non-whey protein supplement [114]. Studies involving older individuals co-supplementing with creatine and proteins are scarce and contradictory, possessing small samples, with short-term follow-ups, and with heterogeneous outcomes and experimental designs [111]. The potential role of co-supplementation with creatine, amino acids and/ or

proteins in sparing muscle mass and improving functionality in older individual merits further investigation. Several findings suggested a dose of about 5 g/day for creatine ingestion to attenuate muscle mass and function decline [65].

3. Introduction to the thesis

As we discussed above, studies have evaluated the combined effects of resistance exercise training and dietary supplementation, suggesting a potential additional effect to counter muscle mass and strength loss. However, during aging, medical conditions often prevent subjects from carrying out physical activity and the need for effective supplements *per se* becomes essential to slow down the progression of muscle mass and function loss. In this direction, some authors, using a single nutritional supplement [115-117], have shown a beneficial effect on neuromuscular performance and muscle protein synthesis in older adults, independently of exercise, but others could not observe positive results [118, 119]. To overcome this discrepancy, which is likely to be related to the heterogeneity of the response to supplementation in older subjects [120], several trials were conducted by employing a multi-ingredient approach [120-133], based on the rationale that a combination of ingredients could be more effective in regulating multiple aging-related relevant mechanisms than the use of single compounds [120]. However, almost all the multi-ingredient studies included physical activity programs [120-130], while only few data [120, 131-133] come from protocols that investigated the effect of targeted nutritional supplements independently of combined physical intervention.

Therefore, in an effort to produce further advancement of knowledge regarding sarcopenia prevention by means of a multi-ingredient supplementation without physical exercise, we hypothesized that a twice-daily consumption of a mix containing EAA, creatine, vitamin D and Muscle Restore Complex® (MRC®: Alpha Lipoic Acid (ALA), Coenzyme Q_{10} (CoQ_{10}), resveratrol) for 12 weeks would result in the improvement of primary outcomes including Fat Free Mass (FFM), Appendicular Lean Mass (ALM), ALM index (ALM/H^2), muscle strength (Maximal Voluntary Contraction, MVC) and muscle power (Peak Power, PP), in non-sarcopenic well-nourished elderly subjects. Furthermore, since data on the possible effects of multi-ingredient supplementation on myoelectric descriptors of fatigue (peripheral and/or central) are completely lacking in aging literature, we evaluated whether the treatment can affect the sEMG-derived TtT failure, as a measure of endurance, and CV, FD (initial values and slopes), as a measure of peripheral and central myoelectric manifestations of fatigue, respectively, during submaximal isometric contractions (60% MVC) to exhaustion. Secondary outcomes we considered including vitamin D serum levels, Resting Metabolic Rate (RMR), Respiratory quotients of different substrates (R) and their fasting utilization rates (CHO%; FAT%), Fat Mass (FM) and Visceral Adipose Tissue (VAT).

Some of the ingredients we used have been shown to independently affect aspects of sarcopenia in elderly and thus have a rational basis for inclusion in a mixture: as we

described in the previous paragraph, protein/amino acids enhance muscle anabolic response and MPS [134-136]; creatine improves muscle strength and power [137, 138]; vitamin D stimulates muscle function and reduce the risk of falls [139, 140]. For ALA, CoQ_{10} and resveratrol, although there is a bulk of references of their use to counteract oxidative stress and inflammation in skeletal muscle *in vitro* models and animal studies [141-145], their therapeutic potential on muscle mass, muscle functions and metabolic outcomes during aging in humans is not well documented and needs to be further clarified.

Based on the above, the aim of this study was to evaluate the efficacy of an EAA-based multi-ingredient supplement on primary and secondary outcomes in the elderly, independently of exercise, comparing results to the few available studies and to increase the overall knowledge of how nutrients can affect muscle aging and sEMG-derived fatigue expression.

4. Materials and Methods

4.1. Study design and trial organization

A total of 50 healthy elderly individuals (aged 65-80 years) were initially identified, following which 38 eligible subjects were recruited in a randomized controlled design study and received either a multi-ingredient nutritional supplement (SUPP; 3 men and 16 women) or a placebo (PLA; 5 men and 14 women). Recruitment phase took place between November 2016 and January 2017. Potential participants were contacted first by enrollment meetings and then involved in a one-to-one interview at our medical facility. All potential participants completed a medical screening in February 2017, to determine the inclusion/exclusion criteria of enrollment. Habits regarding diet and physical activity were assessed through food and physical activity diaries.

All participants were informed of the nature and possible risks of the experimental procedures before their written informed consent was obtained. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of the Department of Internal Medicine and Medical Therapy at the University of Pavia with the code 001A0012.

The enrolled subjects ingested the supplement twice daily (two sachets) before lunch and dinner for 12 weeks: from the beginning of April 2017 to the end of June 2017. In all subjects experimental procedures and measurements were performed at the

baseline (March 2017) and at the end of the study (July 2017). An accurate simulation of the experimental procedures to measure muscle strength, muscle power and fatigue was performed before the baseline. The simulation was conducted to allow the volunteers to familiarize themselves with all the procedures and to avoid an impairment of results caused by a "learning effect." Full details concerning the flow of participants through this study can be found in Figure 8.

In the previous 6 months, potential participants had not participated in any structured high-level resistance or aerobic training; they were instructed not to begin any exercise program and not to change their physical activity habits (based on daily life) for the duration of the study. Subjects were also instructed to avoid any other supplements or remedies to counteract sarcopenia during the entire duration of the study and until the end of the measurements (July 2017).

For each subject (SUPP and PLA) an appropriate diet plan was prepared to guarantee an average intake of protein of 1.2 g/kg of bodyweight/day, in accordance with the recommended amount of protein intake for healthy elderly people [146]. The equivalent amount of protein introduced by the supplement (~20 g/day) in SUPP was excluded from the average intake of protein recommended per day.

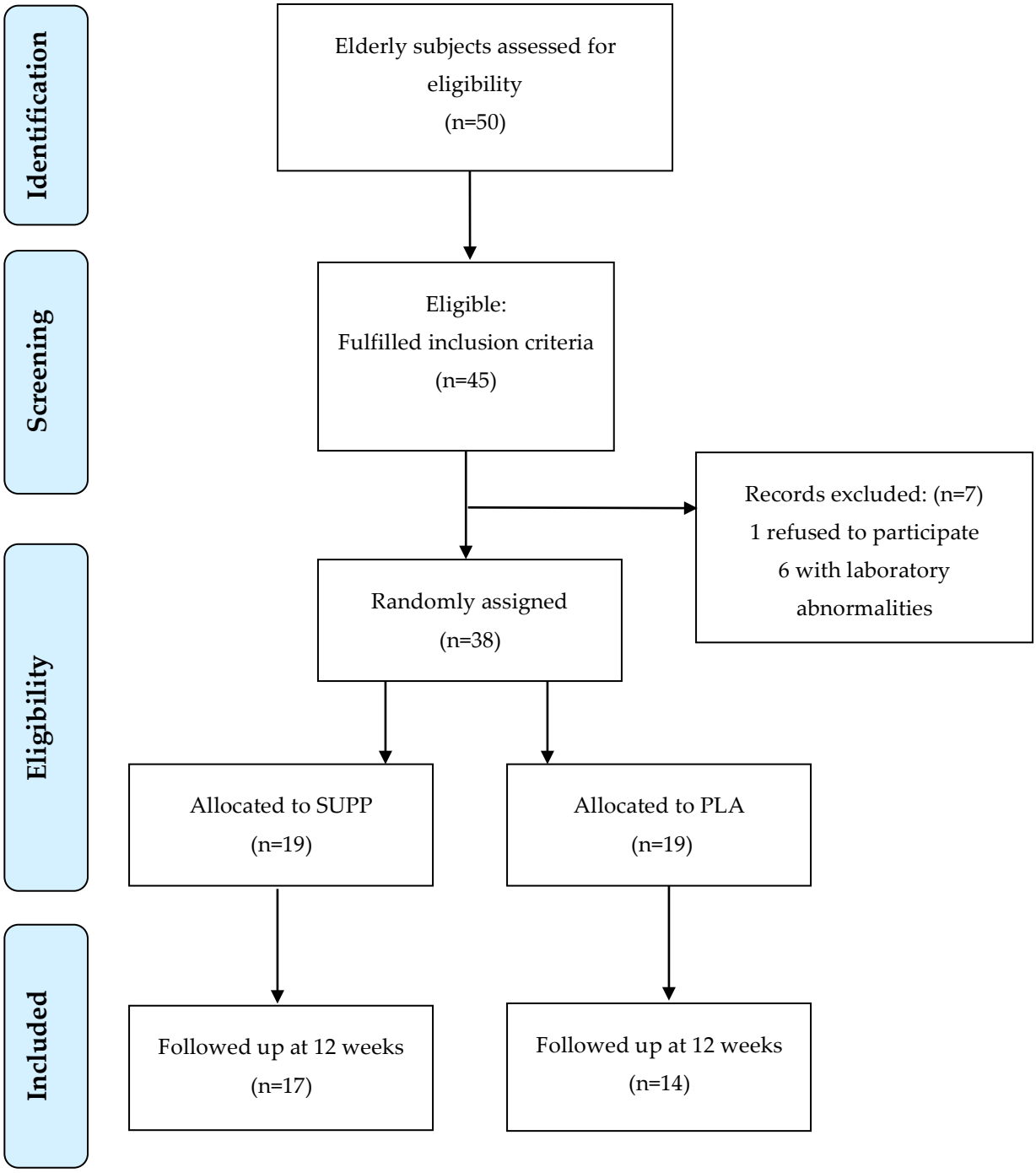

Figure 8. Flow diagram of the study: multi-ingredient supplementation (SUPP) compared to placebo (PLA) in elderly subjects. The diagram indicates the total number of subjects assessed from the identification phase to the group allocation and the number of subjects included in the final statistical analysis after 12 weeks of treatment.

During treatment, weekly meetings based on self-report and questionnaires were held with the aim to ascertain compliance to treatment and to suggested nutrition and physical activity instructions. The study was conducted at CRIAMS-Sport Medicine Centre laboratory of the University of Pavia, located in Voghera.

4.2. Selection of population

Eligible subjects were aged 65 years or older. Subjects included in the study were not affected by acute illness, severe liver disease, heart disease, respiratory or kidney dysfunction, or severe dementia, and had a body weight that had been stable for 6 months. Moreover, subjects with uncontrolled diabetes, disthyroidism and other endocrinopathies, neoplasia, neuromuscular conditions, as well as patients treated with steroids, statins or other anti-sarcopenic supplements in the 6 months before the trial were excluded.

4.3. Sample size and random assignment

We based our sample size calculation on the findings of Bell et al. [120] and considered an expected mean of 0.4 kg increase of ALM in the SUPP group, and 0 kg in the PLA group, with a power of 80% and a level (2-tailed) of 5%, as well as 10% attrition.

This gave a sample size of 40 patients (20/group). A random-blocks 1:1 random assignment list was prepared by a statistician.

4.4. Nutritional supplement

The supplement (a product of Laborest Italia S.r.l., Italy and manufactured by S.I.I.T. S.r.l., Italy) was composed of EAA, creatine, vitamin D and MRC® (ALA, CoQ_{10}, resveratrol). All compounds in powder form were packaged in individual sachets and administrated to SUPP group. SUPP daily ingested two sachets prepared at home by mixing the contents of each sachet with 200 mL water and consuming as follows: first beverage during the morning/before lunch, and second beverage late in the afternoon/before dinner. The before-meal rationale for the supplement-feeding pattern was based on preliminary tests, targeted to avoid dislike of taste and gastrointestinal discomfort, which revealed that assumption of the mix during meals was generally not appreciated by the subjects.

The placebo administrated to PLA was composed of maltodextrine. Compared to the active forms, the placebo powder was isocaloric and undistinguishable for flavor, color and odor. All the sachets were labeled in a blinded manner and double blindness was accurately maintained during each step of the experimental design. The composition of the supplement is described in Table 3.

Table 3. EAA-based multi-ingredient supplement composition.

Compounds	Mean quantity for single-dose/sachet	
EAA	5000	mg
L-Leucine	1400	mg
L-Phenylalanine	600	mg
L-Lysine	700	mg
L-Isoleucine	670	mg
L-Valine	700	mg
L-Threonine	450	mg
L-Methionine	290	mg
L-Tryptophan	190	mg
Creatine (from creatine citrate)	1500	mg
Vitamin D	1000	UI
MRC®		
ALA	300	mg
CoQ_{10}	50	mg
Resveratrol	50	mg

Notes: EAA, Essential Amino Acids; MRC®, Muscle Restore Complex®; ALA, Alpha Lipoic Acid; CoQ_{10}, Coenzyme Q_{10}.

4.5. Anthropometric and body composition assessment

Body weight and height were measured using a periodically calibrated scale equipped with a statimeter (SECA 700, SECA GmbH & co, Germany). Subjects were measured in light clothes (underwear) and without shoes, feet joined and parallel to each other with the head horizontally aligned to the Frankfurt plane. Body Mass Index

(BMI) was calculated by the ratio between body weight and the square of height in meters.

Fat Free Mass (FFM), Fat Mass (FM), gynoid and android fat distribution (%) were measured with the use of Dual Energy X-Ray Absorptiometry (DXA) equipped with Lunar Prodigy DXA technology (GE Healthcare Medical Systems, USA). The *in vivo* coefficients of variations were 0.89% and 0.48% for FM and FFM, respectively [147].

4.5.1. Diagnosis of Sarcopenia

Appendicular Lean Mass (ALM) was taken as the sum of the fat-free soft tissue mass of arms plus legs and Appendicular Lean Mass index (ALM/H^2) was obtained by dividing ALM by height squared. ALM/H^2 cutoffs for men and women were then used to assess the condition of sarcopenia [5].

4.5.2. Diagnosis of Visceral Adipose Tissue (VAT) with Core Scan

Diagnosis of VAT was estimated within the android region. FM data from DXA core Scan was transformed into X-ray computed tomography (CT) adipose tissue volume using a constant correction factor (0.94 g/cm^3). FM, android fat and visceral fat data were derived from DXA using the DXA Prodigy enCORE software (version 17; GE Heathcare, USA). The software automatically places a quadrilateral box, that represents

android region, outlined by the iliac crest and with a superior height equivalent to 20% of the distance from the top of the iliac crest to the base of the skull [148].

4.6. Blood sample measurement (Vitamin D)

For the assessment of 25-hydroxyvitamin D, fasting venous blood samples were drawn between 8 am and 10 am. Subjects were placed in a sitting position and the median cubital vein was used as a selected venipuncture site. Blood handling and collection were carried out under strictly standardized conditions. For the quantitative determination of vitamin D the chemiluminescent immunoassay technology was used.

4.7. Metabolic evaluation

Resting Metabolic Rate (RMR), Respiratory quotients of different substrates (R) and their fasting utilization rates (CHO%; FAT%) were measured by using a respiratory gas analyzer (Quark PFT, Cosmed, Italy). Ambient conditions were standardized (25 °C) and the analyzer was gas- and volume-calibrated each morning prior to the measurements, according to the recommendations stated in the manufacturer's user manual. Gas exchange and metabolic variables were measured continuously using the breath-by-breath method. After an overnight fast, participants were instructed to lie down quietly for 10 min, wearing a two-way breathing mask covering their nose and

mouth (V2 Mask™, Hans Rudolph Inc, USA). Thereafter, the measurement period started by connecting the mask to the gas analyzer and data collection continued for a total of 20 min.

4.8. Isometric muscle strength and fatigue assessment with sEMG technique

sEMG recording procedure was carried out as follows: subjects' dominant upper limb was fastened in a isometric-ergometer (MUC1, OT Bioelettronica, Turin, Italy) fitted with a load cell (CCT Transducer, linear, full scale 100 kg), in order to isolate the action of the biceps brachii. Participants were sitting, with the elbow at 120 degrees (Figure 4).

A 64-channel bidimensional array (10 mm IED, 8 lines, 8 columns) was positioned between the distal tendon and the innervation zone of the biceps brachii, with electrode columns parallel to the orientation of the muscle fibers in order to have a pure propagation of MUAPs. Biceps brachii was selected primarily to obtain high-quality sEMG signals due to the isolation of the muscle contraction, fluency of movement, and fiber orientation. The adhesive array was applied following muscle fiber leanings in correspondence to the muscle belly previously localized by ultrasound scan (Phillips CX-30). The sEMG signals were amplified (EMG-USB2+, OT Bioelettronica, Turin, Italy) and sampled at 2048 Hz.

Following 5 min rest, two isometric Maximal Voluntary Contractions (MVCs) were completed, separated by 2 min rest. Two contractions were performed in order to consider the highest MVC value. Participants were instructed to increase the force as maximum as they can, and to hold it as steady as possible, for 2–3 s. Participants were given verbal stimulation.

Following 2 min rest, a low intensity sustained contraction (20% MVC) was performed for 90 s.

Following 4 min rest, subjects were asked to execute a high level sustained contraction (60% MVC) until exhaustion, during which they were verbally stimulated to keep the force level as long as possible, until the force value decreased to below 5% of the target [149]. At 60% of MVC, CV iv and FD iv (initial values), their slopes and the Time to perform the Task (TtT) were registered.

4.8.1 Signal processing

Data were divided into 0.5 s periods and each variable was computed for each period. Exhaustion time was defined as the moment when force was below 5% of the target [23]. The regression line was computed for all the values from the beginning of contraction to exhaustion time. For each acquisition, the channels for the analysis were selected through visual inspection. The column showing the largest portion of

propagating channels with the biggest amplitude was selected, and the channels between innervation zones and tendons were selected for CV computation. FD was computed for each selected channel and then averaged. FD initial value was estimated using the box counting method. Briefly, as expressed in Gitter and Czerniecki [150], a grid of square boxes was used to cover the signal, and the number of boxes that the sEMG waveform passed through was counted. When decreasing the side of the boxes in a dichotomic process, the number of boxes required to cover the signal increased exponentially. However, by plotting the logarithm of the number of boxes counted (log N) vs. the logarithm of the inverse of the box size (log 1/S), the exponential relationship became linear. The slope of the interpolation line (estimated using the least mean squared procedure) is the FD. CV initial value (m/s) was estimated using a multichannel algorithm on double differential signals, based on the matching between signals filtered in the temporal and in the spatial domains [151]. CV values outside the physiological range (3–6 m/s) [152], were excluded from the analysis. CV and FD slopes were measured as rate of change (%) of CV iv and FD iv.

4.9. Muscle power assessment

Muscle power was measured by a force-velocity device analysis (Musclelab™ 4000, Ergotest Innovation A.S., Norway). Participants performed 3 tests of biceps curling (3

sets each, to choose the best execution) with a dumbbell (Technogym S.p.A., Italy) loaded at 30%, 40% and 50% of the 1 Repetition Maximum (1 RM), respectively, connected to the device by a cable (Figure 6). A rest of 90 s between sets and 180 s between tests was held. Indirect 1RM tests to establish the dumbbell load (with the use of Brzycki's equation) were performed one week before the muscle power assessment. A week was considered appropriate to exclude any individual variability in relation to the time required for a complete muscle recovery and to resolve the Delayed Onset Muscle Soreness (DOMS) that could compromise the maximum speed of muscle contraction. Participants were instructed to execute each contraction as fast as they could and were given verbal stimulation. Muscle power values registered from each test were computed to create a force-velocity curve. Based on this curve the peak power (PP) value was obtained. Muscle power assessment was performed on dominant upper limb.

4.10. Statistical analyses

All analyses were performed using statistical package SPSS, version 21.0 (SPSS Inc., USA). Descriptive statistics representing raw data for each of the three categories and the full sample were provided, including means, standard deviations (sd), and frequencies, where appropriate. After the verification of the normal distribution of the continuous variables, data were analyzed as descriptive statistics. We carried out a

paired t-tests and 95% Confidence Intervals (CI) to evaluate statistical significance on model parameters at baseline (supplement versus placebo). P-values <0.05 were considered significant.

Linear Mixed Model (LMM) for repeated measures [153] was applied to assess all differences for the variables considered (Table 5) among individuals at pre- and post-treatment (post-pre). These data were presented as mean differences with 95% CI.

Non-normally distributed data were checked by Shapiro-Wilk test and log transformed for parametric statistics.

For each outcome we fitted a LMM where age, sex, BMI and time (pre=0, post=1) were the explanatory variables. A random effect was used to adjust the models for intra-subject variability produced by two different measurements carried out on same patients. The time LMM parameters were interpreted as adjusted mean changes from baseline.

To compare changes between groups, a general linear regression model was fitted with FFM as the dependent variable, and treatment, time, and the interaction of treatment with time were used as independent variables.

A Pearson's correlation analysis was used to assess the relationships between mean differences in all markers investigated.

5. Results

5.1. Participants

38 elderly subjects were randomized: 31 completed the study and 7 dropped out (n=2 in SUPP: 1 for dislike of taste of the supplement drink, 1 for gastrointestinal discomfort probably related to supplement intake; n=5 in PLA: 3 for medical conditions which occurred over the course of the study, 1 who moved, 1 for hospitalization). The main details on participants' baseline characteristics are shown in Table 4.

Baseline outcomes observed showed an inadequate level of vitamin D (<30 ng/ml is considered insufficient) and a slightly high BMI (>24.9 kg/m^2). SUPP and PLA were similar on all counts and this means that randomization was correctly carried out. Based on the score obtained from the compliance questionnaires, for the subjects that completed the study we reached a compliance percentage which was close to 100%.

5.2. Treatment effect compared to baseline

The complete results for all variables considered are presented in Table 5. The main variations observed are described as follows.

Primary outcomes (FFM; ALM, ALM/H^2; MVC; PP and myoelectric manifestation of fatigue): 1) no statistical difference was found in total FFM in either group; 2) a statistically significant increase in all index of sarcopenia (ALM: +0.34 kg and ALM/H^2:

+0.12 kg/m^2; p<0.001) were found in SUPP, with no changes in PLA; 3) increases of MVC (+0.52 kg; p<0.001) and PP (+4.82 W; p<0.001) were significantly observed in the SUPP, whereas the same variables showed a negative change in PLA, with a significant decrease registered for MVC: -0.86 kg; p<0.001; 4) no statistical changes were found for all sEMG descriptors of fatigue (FD iv, CV iv, FD and CV slopes) and TtT.

Secondary outcomes (vitamin D blood levels; RMR, R, CHO%, FAT%; FM and VAT): 1) we measured a significant increase of vitamin D levels in SUPP (+8.73 ng/ml; p<0.001) with a negative change in PLA (-2.98 ng/ml; p<0.001) (Figure 9); 2) a statistically significant increase was observed for fasting FAT oxidation rate (+12%; p<0.001), whereas no other changes of fasting metabolic markers (RMR, R, CHO%) were measured; 3) no changes were found for FM absolute values or FM gynoid or android distribution (FM%) in either group, but we highlighted a statistical decrease of VAT (-76.25 g; p<0.001) in SUPP.

5.3. Treatment effect between groups

The results of intergroup analysis (PLA vs. SUPP) are shown in Table 5. The variables that showed significant changes between the two groups over the course of the study are indicated as follows. Primary outcomes: 1) a positive legs FFM response, with a mean difference of 443.70 g (p<0.05); 2) an increase of ALM (+0.53 kg; p<0.05) and

ALM/H^2 (+0.19 kg/m^2; $p<0.05$); 3) an increase of MVC (+1.38 kg; $p<0.05$) and PP (+9.87 W; $p<0.05$). Secondary outcomes: we registered an increase of vitamin D level of 11.72 ng/ml ($p<0.001$).

5.4. Pearson's correlations between mean differences in treatment group

A significant correlation was found between vitamin D and ALM/H^2 ($r=0.706$; $p<0.001$) (Figure 10A), between VAT/FM and MVC ($r=-0.572$; $p<0.001$) (Figure 10B) and between Legs FFM and ALM/H^2 ($r=0.857$; $p<0.001$) (Figure 10C). R is computed as the partial correlation, adjusted for age, sex and BMI.

Table 4. Baseline characteristics and descriptive statistics of the 38 participants (mean ± sd).

Variables	PLA	SUPP	Total	P-value
General data				
Gender (men, women)*	19 (5, 14)	19 (3, 16)	38 (8, 30)	0.26
Age (years)	70.09±4.22	68.34±4.75	68.91±4.60	0.29
Blood analysis				
Vitamin D (ng/ml)	23.92±9.28	25.59±8.73	25.05±8.80	0.62
Anthropometric measures				
Height (m)	1.60±0.11	1.60±0.09	1.60±0.09	0.94
BMI (kg/m^2)	28.79±3.72	25.72±4.80	26.70±4.64	0.09
DXA measures				
DXA weight (kg)	74.42±13.73	67.04±16.21	69.40±15.58	0.25
FFM (g)	44046.00±10589.38	41915.78±9538.50	42561.30±9750.74	0.32
Arms FFM (g)	4795.90±1642.47	4533.56±1511.04	4618.44±1534.66	0.66
Legs FFM (g)	16453.09±3727.66	15736.00±3443.33	15968.00±3497.30	0.59
ALM (kg)	20.87±5.44	20.26±4.80	20.45±4.92	0.76
ALM/H^2 (kg/m^2)	8.06±1.20	7.82±1.09	7.89±1.11	0.60
FM (g)	25672.60±8320.66	22614.13±9756.25	23540.94±9324.62	0.37
Gynoid FM %	36.61±9.58	36.93±9.60	36.83±9.44	0.93
Android FM %	43.50±8.15	38.99±13.53	40.36±12.20	0.25
VAT (g)	1522.57±824.75	960.76±915.61	1124.62±910.32	0.16
VAT/FM	0.05±0.02	0.04±0.02	0.04±0.02	0.11
Indirect calorimetry				
RMR (kcal)	1292.73±264.19	1235.41±244.60	1254.51±248.65	0.55
R	0.84±0.07	0.85±0.05	0.85±0.06	0.79
FAT (%)	51.42±25.11	48.84±18.91	49.70±20.81	0.77
CHO (%)	48.97±25.12	51.57±18.88	50.70±20.80	0.76
Strength and Power assessment				
MVC (kg)	9.97±4.11	9.06±3.46	9.51±3.78	0.53
PP (W)	41.04±26.40	29.72±17.45	35.38±21.92	0.21
sEMG fatigue assessment				
FD iv	1.60±0.50	1.62±0.40	1.62±0.05	0.32
FD slopes (%/s)	-0.05±0.02	-0.04±0.03	-0.05±0.03	0.78
CV iv (m/s)	4.27±0.93	4.07±0.61	4.15±0.77	0.44
CV slopes (%/s)	-0.38±0.32	-0.18±0.19	-0.24±0.25	0.15
TtT (s)	66.50±17.81	61.00±17.38	62.67±17.42	0.42

Notes: BMI, Body Mass Index; DXA, Dual Energy X-Ray Absorptiometry; FFM, Fat Free Mass; ALM, Appendicular Lean Mass; FM, Fat Mass; VAT, Visceral Adipose Tissue; RMR, Resting Metabolic Rate; R,

Respiratory quotient; MVC, Maximal Voluntary Contraction; PP, Peak Power; sEMG, surface electromyography; FD iv, Fractal Dimension initial value; CV iv, Conduction Velocity initial value; TtT, Time to perform the Task. *X^2 : 1.27

Table 5. Treatment effect from baseline and between groups refers to subjects that completed the study.

Variables	Mean changes from baseline	P-value	95% CI	Mean changes from baseline	P-value	95% CI	Mean difference between groups and (CI 95%)	P-value
	PLA			SUPP				
Blood analysis								
Vitamin D (ng/ml)	-2.98	**<0.001**	-5.39;-0.58	8.73	**<0.001**	7.12; 10.35	11.72 (8.74; 14.70)	**<0.001**
Anthropometric measures								
BMI (kg/m^2)	-0.12	ns	-0.77; 0.53	-0.01	ns	-0.46; 0.42	0.12 (-0.69; 0.90)	ns
DXA measures								
DXA weight (kg)	0.25	ns	-0.79; 1.30	0.18	ns	-0.48; 0.84	-0.07 (-1.34; 1.19)	ns
FFM (g)	58.30	ns	-809.67; 926.27	232.76	ns	-313.72; 779.24	174.45 (-870.23; 1219.15)	ns
Arms FFM (g)	37.63	ns	-203.45; 278.71	148.23	ns	-13.33; 309.80	110.60 (-187.61; 408.82)	ns
Legs FFM (g)	-235.37	ns	-604.28; 133.54	208.33	ns	-23.94; 440.60	443.70 (0.326; 887.72)	***<0.05***
ALM (kg)	-0.18	ns	-0.58; 0.21	0.34	**<0.001**	0.09; 0.59	0.53 (0.05; 1.01)	***<0.05***
ALM/H^2 (kg/m^2)	-0.06	ns	-0.22; 0.09	0.12	**<0.001**	0.02; 0.22	0.19 (0.00; 0.37)	***<0.05***
FM (g)	240.70	ns	-665.29; 1146.69	-77.05	ns	-647.47; 493.36	-317.75 (-1408.20; 772.69)	ns
Gynoid FM (%)	1.35	ns	0.03; 2.66	0.17	ns	-0.65; 1.00	-1.17 (-2.76; 0.41)	ns
Android FM (%)	0.35	ns	-1.92; 2.62	-0.65	ns	-2.09; 0.77	-1.01 (-3.74; 1.73)	ns
VAT (g)	-39.83	ns	-130.23; 50.56	-76.25	**<0.001**	-136.84; -15.67	-34.11 (-139.49; 71.27)	ns
VAT/FM	0.00	ns	-0.00; 0.00	-0.00	ns	-0.00; 0.00	-0.001 (-0.003; +0.002)	ns
Indirect calorimetry								
RMR (kcal)	-97.17	ns	-227.90; 33.56	12.89	ns	-69.41; 95.20	110.07 (-47.28; 267.42)	ns
R	0.00	ns	-0.04; 0.05	-0.03	ns	-0.06; 0.00	-0.04 (0.95; 0.23)	ns
FAT (%)	0.06	ns	-16.60; 16.74	12.00	**<0.001**	1.51; 22.50	11.94 (-8.13; 32.01)	ns
CHO (%)	-3.05	ns	-20.46; 14.36	-6.27	ns	-17.49; 4.94	-3.22 (-25.30; 18.85)	ns

Strength and Power assessment								
MVC (kg)	-0.86	**<0.001**	-1.51; -0.20	0.52	**<0.001**	0.08; 0.96	1.38 (0.57; 2.19)	***<0.05***
PP (W)	-5.04	ns	-10.13; 0.04	4.82	**<0.001**	1.41; 8.23	9.87 (3.58; 16.15)	***<0.05***
sEMG fatigue assessment								
FD iv	0.04	ns	-0.02; 0.10	-0.03	ns	-0.06; 0.00	-0.07 (-0.14; 0.00)	ns
FD slopes (%/s)	-0.03	ns	-0.08; 0.02	-0.01	ns	-0.04; 0.02	0.02 (-0.05; 0.08)	ns
CV iv (m/s)	-0.98	ns	-2.01; 0.05	0.04	ns	-0.48; 0.56	1.02 (-0.06; 2.21)	ns
CV slopes (%/s)	0.04	ns	-0.23; 0.31	0.01	ns	-0.13; 0.16	-0.26 (-0.34; 0.28)	ns
TtT (s)	-12.53	ns	-30.58; 5.51	0.58	ns	-8.90; 10.07	13.11 (-7.48; 33.71)	ns

Notes: BMI, Body Mass Index; DXA, Dual Energy X-Ray Absorptiometry; FFM, Fat Free Mass; ALM, Appendicular Lean Mass; FM, Fat Mass; VAT, Visceral Adipose Tissue; RMR, Resting Metabolic Rate; R, Respiratory quotient; MVC, Maximal Voluntary Contraction; PP, Peak Power; sEMG, surface electromyography; FD iv, Fractal Dimension initial value; CV iv, Conduction Velocity initial value; TtT, Time to perform the Task. ns, not significant. In bold the statistically significant evidences.

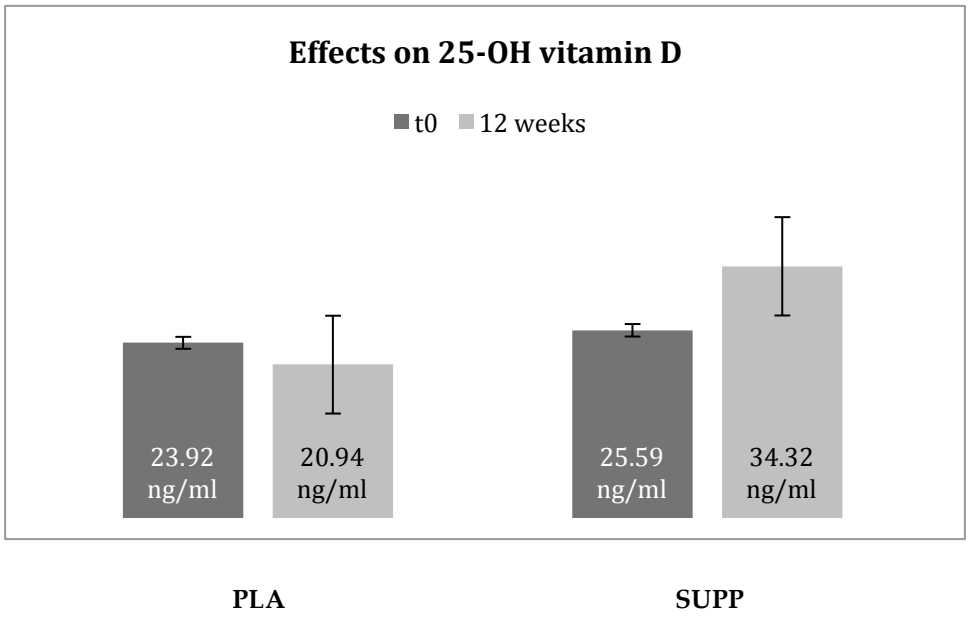

Figure 9. Mean variation of vitamin D blood levels in PLA (-2.98 ng/ml) and SUPP (+ 8.73 ng/ml) after 12 weeks of treatment. Error bars indicate standard error of the mean.

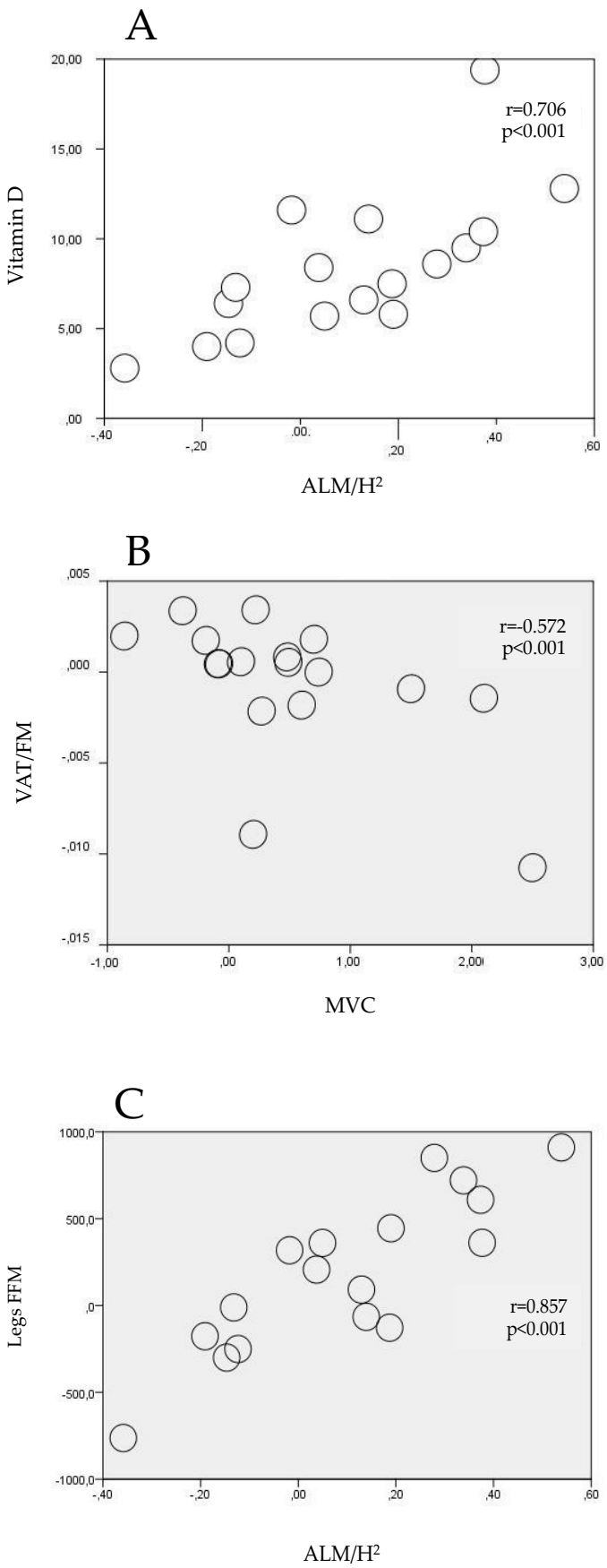

Figure 10. Pearson's correlations between mean differences in SUPP group (n=17): Vitamin D Vs. ALM/H² (A); VAT/FM Vs. MVC (B); Legs FFM Vs. ALM/H² (C). Notes: ALM/H², Appendicular Lean Mass index; VAT/FM, Visceral Adipose Tissue to Fat Mass ratio; FFM, Fat Free Mass; MVC, Maximal Voluntary Contraction.

6. Discussion

6.1. Effects on muscle mass, muscle strength and muscle power

The experimental results agree with comparable previously published works in which the use of a similar mixture of compounds has shown enhancements of muscle mass (ALM) with no exercise intervention [120, 131]. In particular, Bauer et al. [131] after 13 weeks of supplementation using a formula containing leucine-enriched whey protein (40 g) with 6 g of leucine, vitamin D (1600 IU) and carbohydrates, showed an absolute increase of 0.25 kg and a relative increase of 0.17 kg compared to control. More recently, Bell et al. [120] after 6 weeks of supplementation using a multi-ingredient mix including whey protein (60 g), vitamin D (1000 IU), creatine (5 g) and n-3 PUFA, observed an improvement of ALM of 0.40 kg in treated group, whereas the difference with matched controls was not described. In our experimental conditions (ALM: + 0.34 kg from baseline; + 0.53 kg between groups), compared to the study by Bauer et al. [131] which shows lower ALM improvement after treatment and compared to placebo, the higher ALM gain may be attributed to the coexistence of EAA and creatine in the mixture, which is probably more effective than a higher dose of amino acids and leucine alone. In fact, although the leucine-enriched whey protein blend seems to be an appropriate approach to preserving muscle mass and function in older sarcopenic adults [154], a recent systematic review [155] found this effect only in 3 out of 12 Randomized Control Studies (RCTs) whereas an additional

anabolic action of creatine was found in 4 out of the 5 RCTs considered. In the study by Bell et al. [120] comparable results were obtained in half the time (6 weeks). However, the authors used a very high dose of protein/amino acids and creatine compared to our formula and the subjects involved were male. Although it is not known, at present, whether elderly males and females respond differently to a multi-ingredient supplementation, we should consider a "gender effect" of based on recent findings [156] underlying that aged females' muscle displays higher heterogeneity in myofibers size and phenotype composition compared to males' (about 5-fold).

Although it is not possible to isolate which compounds in the supplement were responsible for the outcomes assessed, we believe that the observed reversing of vitamin D inadequacies (Figure 9) may have contributed to the overall favorable effect on muscle strength (MVC). This hypothesis is based on previous meta-analysis revealing a small but significant positive effect of vitamin D supplementation on global muscle force expression [157]. Furthermore, published data indicated that serum 25-hydroxyvitamin D concentrations between 60 and 75 nmol/L (24.04 and 30.05 ng/ml, respectively) correlate with lower-extremity strength [158] and, possibly, with the amelioration of Legs FFM. This correlation could explain the increase of Legs FFM we observed after supplementation (Table 5) and the positive correlation between Vitamin D and ALM/H^2 (Figure 10A), Legs FFM and ALM/H^2 (Figure 10C) we observed by the treatment effect.

In the present study a gain in muscle power (PP) was found as an important functional outcome of nutrient supplementation. Considering that an additional effect of EAA and vitamin D on muscle power tests is improbable, as outlined by recent meta-analysis [157] and systematic review [155], we suppose that creatine in the mixture could be highly effective in increasing power-based functional tests [159] and may have contributed to the observed effect.

6.2. Potential muscle and metabolic role of Muscle Restore Complex® (ALA, CoQ10 and resveratrol)

With the aim of obtaining greater insight for the design of "the most effective formula" - capable of maximally preventing muscle wasting due to ageing - and considering a likely role of free radical production and inflammation in its development and progression, compounds with antioxidants and anti-inflammatory properties (ALA, CoQ10 and resveratrol) [141-145, 160-164] were added to the mix. This is the first time that a similar blend has been added to an EAA-based formula for the prevention of aged-related loss of muscle mass and function. However, considering that the bioavailability of each single component was not measured, and sub-group analysis is also missing, at this stage we can only speculate that their presence in the formula may have played a potential synergic role leading to the obtained results. In particular, compared to the study of Bauer et al. [131], the greater

ALM improvement compared to placebo may be due at least in part to antioxidant and/or anti-inflammatory mechanisms.

The anti-inflammatory properties of ALA, although rarely investigated in humans, have shown a 15% significant decrease in serum interleukin-6 levels following 4 weeks of supplementation [165]. CoQ10 blood levels were recently correlated with muscle strength in two independent humans cohorts studies [162], and modulating effects of CoQ10 supplementation on inflammatory [163] and chronic oxidative stress response [164] were found after 4 weeks of treatment in the elderly. Other interesting data suggest that cellular energy delivery may be positively conditioned by a combination of creatine, ALA and CoQ10 use in subjects carrying mitochondrial dysfunctions [166]. Resveratrol, a plant-derived polyphenol, is probably the most promising of such compounds as it proved: 1) to protect skeletal muscle from aging-induced oxidative stress [167]; 2) to enhance, at least in rodents, skeletal muscle fiber size (type IIA and IIB fibers) and myonuclear number thus leading to hypertrophy [144]; 3) to reverse the atrophy, on isolated myotubes, caused by TNF-α, through the regulation of the Akt-mTORC1-FoxO1 signaling pathways and inhibition of the atrophy-related ubiquitin ligase [168]; 4) to improve mitochondrial capacity by activating the AMPK-SIRT1-PGC1α pathway [169-171]. On this latter point, Most et al. [172] showed that a 12-week supplementation with 80 mg/day of resveratrol (a dosage similar to our experimental condition) can improve skeletal muscle oxidative capacity leading to amelioration of fasting substrate

oxidation. Just as we found, the authors [172] underlined that although body weight, total FM and resting R were not affected by treatment, a significant decrease of VAT (~11%) was found. Considering that VAT is detrimental to metabolic health [173], the reduction we observed may be of clinical importance in the long term. Furthermore, we found that the negative association between VAT and MVC (Figure 10B) is in agreement with previously published data [174].

6.3. Effects on Time to perform the Task (TtT) and myoelectric manifestations of fatigue

We measured the effect of supplementation on TtT failure, as a measure of endurance, and on myoelectric descriptors as reliable indicators of peripheral (CV initial values and slopes) and central fatigue (FD initial values and slopes), during submaximal isometric contractions at 60% MVC to exhaustion. No statistical changes were found for all the measured outcomes, either compared to baseline or between groups. These data demonstrate, for the first time, that 12 weeks of an EAA-based multi-ingredient nutritional supplementation failed to positively affect muscle endurance capacity (TtT) and myoelectric manifestations of central and peripheral fatigue in older adults. We consider this finding to be indirect confirmation of a preferential effect of the mixture on the structure and function of type II fibers. In fact, changes in muscle mass, strength and power are mainly attributable to relative expression of fast fibers within the muscle without amelioration in endurance and relative proportion of slow type I fibers.

This is the first study to have assessed the effects of a multi-ingredient supplementation on myoelectric manifestation of fatigue in the elderly and this precludes further considerations on the subject. So far, only two middle-term studies have investigated the effects of amino acids-based supplementations on fatigue in healthy elderly people but different procedures to induce and detect fatigue and the combination with a specific training program was used. In particular, Reule et al. [175] documented a reduction of fatigue after 12 weeks of leucine-rich (3.2 g/day) amino acid supplementation by measuring the capacity of this treatment to counteract the loss of strength measured as MVC, and not during submaximal contractions, in the acute phase recovery (0-3 hours) after an eccentric stress test (downhill walking). Gryson et al. [176] described the effect of 16 weeks of a leucine-fortified milk protein supplementation on TtT failure during a sustained isometric contraction (dominant leg until exhaustion at 75% MVC) performed after a fatiguing protocol (3 isometric MVCs). Authors referred that the TtT failure improved in the trained participants receiving a 10 g/day of the protein compared to controls. However, it is important to highlight that in this study TtT was measured at % MVC higher than those generally described in literature to assess the arising of peripheral fatigue [177] and the findings from Gryson et al. probably describe the overall contribution of type II fibers to peripheral fatigue rather than giving information on the role of type I fibers within the muscle. In fact, since fiber type composition has been proposed as a major determinant of CV rate of change during

submaximal isometric contractions [178], it is well known that 70% of MVC, with a higher decrease of CV, may indicate a major recruitment of type II fibers [179] compared to 60% MVC, more suitable to detecting the contribution of type I fibers to TtT [177].

6.4. Limitations

This study has limitations. As other authors have outlined [120], it is very difficult to create an experimental design finalized to statistically discriminate the effects of each single compound included in a multi-ingredient supplement. To obtain reliable results, this would require a very large sample size and several subgroups to be analyzed. Therefore, we consider the results obtained to be suitable for further investigations towards the effectiveness of each compound and their bioavailability.

Furthermore, compared to the two studies we mainly analyzed in the discussion [120, 131], we used different procedures for evaluating upper limb muscle strength and power. While on the one hand this represent an unconventional method to measure the muscle function, increasing the possibilities of studying muscular strength and power compared to classical methods used on the elderly (handgrip test, gait speed test, sit-to-stand chair test or other lower extremity function tests), on the other hand it limits the comparison to results obtained with more validated tests available in literature.

7. Conclusions, potential applications and future directions

The study demonstrate that a mixture with EAA, creatine, vitamin D and MRC® (ALA, CoQ10, resveratrol) may improve muscle aging-related outcomes, such as muscle mass, muscle strength and muscle power in a medium-short period and without physical activity programs. In particular, given that from the fifth decade of life muscle mass and strength decline at rates of ~0.5-1% and ~1-3% annually, respectively [180], the changes in ALM (+ 1.68%) and MVC (+5.22%) observed in SUPP after 12 weeks of treatment are to be considered clinically relevant. In fact, in absolute values the increase of these variables is equivalent to an offsetting of more than one year of age-related decline, suggesting that this formula, similarly to previous studies [120, 131], can effectively counterbalance progression of muscle mass and strength loss.

The importance of these results should be take into account not only for healthy aging (physiologic), but also for pathologic aging, considering that altered muscle mass has a recognized key role in the genesis of many common medical conditions and chronic diseases observable over the course of life [181]. From this last point of view, the maintenance of muscle mass and function during aging may be effective in the prevention of several metabolic disorders [181]: a) *sarcopenia* and related frailty, with a loss of quality of life and ultimately institutionalization, or other chronic diseases, such as *cardiac* or *cancer cachexia*, in which the loss of muscle mass and strength is an important determinant of survival; b) *obesity*, by the preservation of

muscle protein turnover and consequent energy requirements to sustain it; c) *insulin resistance* and *diabetes*, because the metabolic function of muscles is central to their development and progression; d) *osteoporosis*, because the conservation of adequate bone strength and density with aging is highly dependent on the maintenance of adequate muscle mass, strength and power. Furthermore, an adequate muscle mass is absolutely necessary to supply a greater demand for amino acids, from muscle protein breakdown, to support cellular metabolism during *critical illness* (sepsis, advanced cancer, traumatic injury).

As a preliminary study, in this frame we first aimed to compare our original formula with others available in literature and we now hope for future studies that imply much more effort in terms of bioavailability assessment (measuring blood profile changes in levels of multi-ingredient components after ingestion), biochemical responses (with analysis of outcomes related to free radical production and inflammation, on blood samples, and outcomes related to cellular anabolic regulation, based on evaluation of muscle samples) and number of enrolled subjects (to create a sub-groups design trial able to discriminate the effects of each singular compound of the formula).

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References

1. Minciullo, P.L.; Catalano, A.; Mandraffino, G.; Casciaro, M.; Crucitti, A.; Maltese, G.; Morabito, N.; Lasco, A.; Gangemi, S.; Basile, G. Inflammaging and Anti-Inflammaging: The Role of Cytokines in Extreme Longevity. *Arch Immunol Ther Exp (Warsz).* **2016**, 64, 111-26. DOI: 10.1007/s00005-015-0377-3.
2. Fulop T, Witkowski JM, Olivieri F, Larbi A. The integration of inflammaging in age-related diseases. *Semin Immunol* **2018**, 40, 17-35. DOI: 10.1016/j.smim.2018.09.003.
3. Fragala, M.S. The Physiology of Aging and Exercise. In "G.M. Sullivan, A.K. Pomidor (eds.): Exercise for Aging Adults", pp 1-11; DOI 10.1007/978-3-319-16095-5_1.
4. Xue, Q.L. The Frailty Syndrome: Definition and Natural History. *Clin Geriatr Med* **2011**, 27, 1–15. DOI: 10.1016/j.cger.2010.08.009.
5. Cruz-Jentoft, A.J.; Baeyens, J.P.; Bauer, J.M.; Boirie, Y.; Cederholm, T., Landi, F.; Martin, F.C.; Michel, J.P.; Rolland, Y.; Schneider, S.M.; et al. Sarcopenia: European consensus on definition and diagnosis Report of the European Working Group on Sarcopenia in Older People. *Age and Aging* **2010**, *39*, 412–423. DOI: 10.1093/ageing/afq034.
6. Ali, S.; Garcia, J.M. Sarcopenia, cachexia and aging: diagnosis, mechanisms and therapeutic options - a mini-review. *Gerontology* **2014**, 60, 294-305. DOI: 10.1159/000356760.
7. Dillon, E.L. Nutritionally essential amino acids and metabolic signaling in aging. *Amino Acids* **2013**, 45, 431-41.
8. Bhattoa, H.P.; Konstantynowicz, J.; Laszcz, N.; Wojcik, M.; Pludowski, P. Vitamin D: Musculoskeletal health. *Rev Endocr Metab Disord* **2017**, *18*, 363-371. DOI: 10.1007/s11154-016-9404-x.
9. Karakelides, H.; Nair, K.S. Sarcopenia of aging and its metabolic impact. *Curr Top Dev Biol* **2005**, *68*, 123-148. DOI: 10.1016/S0070-2153(05)68005-2.
10. Grimby, G.; Saltin, B. The aging muscle. *Clin. Physiol.* **1983**, *3*, 209.
11. Curtis, E.; Litwic, A.; Cooper, C.; Dennison, E. Determinants of Muscle and Bone Aging. *J Cell Physiol* **2015**, *230*, 2618-2625, DOI: 10.1002/jcp.25001.
12. Von Haehling, S.; Morley, J.E.; Anker, S.D. An overview of sarcopenia: facts and numbers on prevalence and clinical impact. *J Cachexia Sarcopenia Muscle*. **2010**, 1, 129-33.
13. Lenk, K.; Schuler, G.; Adams, V. Skeletal muscle wasting in cachexia and sarcopenia: molecular pathophysiology and impact of exercise training. *J Cachexia Sarcopenia Muscle* **2010**, 1, 9–21.
14. Narici, M.V.; Maffulli, N. Sarcopenia: characteristics, mechanisms and functional significance. *Br Med Bull* **2010**, 95, 139-59.

15. Snijders, T.; Verdijk, L.B.; van Loon, L.J. The impact of sarcopenia and exercise training on skeletal muscle satellite cells. *Ageing Res Rev* **2009**, 8, 328–338.
16. Verdijk, L.B.; Snijders, T.; Beelen, M.; Savelberg, H.H.; Meijer, K.; Kuipers, H.; Van Loon, L.J. Characteristics of muscle fiber type are predictive of skeletal muscle mass and strength in elderly men. *J Am Geriatr Soc* **2010**, 58, 2069–2075.
17. Lukasi, H.; Heymsfield, M.; et al, ed. Assessing muscle mass. Human body composition. Champaign, IL, USA: *Human Kinetics*, **2005**.
18. Cooper, C.; Fielding, R.; Visser, M.; van Loon, L.J.; Rolland, Y.; Orwoll, E.; Reid, K.; Boonen, S.; Dere, W.; Epstein, S.; et al. Tools in the Assessment of Sarcopenia. *Calcif Tissue Int* **2013**, 93, 201-10. doi: 10.1007/s00223-013-9757-z.
19. Kyle, U.G.; Bosaeus, I.; De Lorenzo, A.D.; Deurenberg, P.; Elia, M.; Go´mez, J.M.; Heitmann, B.L.; Kent-Smith, L.; Melchior, J.C.; Pirlich, M.; et al. Bioelectrical impedance analysis—part I: review of principles and methods. *Clin Nutr* **2004**, 23, 1226–1243.
20. Janssen, I.; Heymsfield, S.B.; Baumgartner, R.N.; Ross, R. Estimation of skeletal muscle mass by bioelectrical impedance analysis. *J Appl Physiol* **2000**, 89, 465–471.
21. Kyle, U.G.; Genton, L.; Hans, D.; Pichard, C. Validation of a bioelectrical impedance analysis equation to predict appendicular skeletal muscle mass (ASMM). *Clin Nutr* **2003**, 22, 537–543.
22. Fields, D.A.; Goran, M.I.; McCrory, M.A. Body-composition assessment via air-displacement plethysmography in adults and children: a review. *Am J Clin Nutr* **2002**, 75, 453–467.
23. Meduri, F.; Beretta-Piccoli, M.; Calanni, L.; Segreto, V.; Giovanetti, G.; Barbero, M.; Cescon, C.; D'Antona, G. Inter-Gender sEMG Evaluation of Central and Peripheral Fatigue in Biceps Brachii of Young Healthy Subjects. *PLoS One* **2016**, 11, e0168443. DOI: 10.1371/journal.pone.0168443.
24. Negro, M.; Segreto, V.; Barbero, M.; Cescon, C.; Castelli, L.; Calanni, L.; D'Antona, G. Essential Amino Acids (EAA) Mixture Supplementation: Effects of an Acute Administration Protocol on Myoelectric Manifestations of Fatigue in the Biceps Brachii After Resistance Exercise. *Front Physiol* **2018**, 9, 1140. DOI: 10.3389/fphys.2018.01140.
25. Reid, K.F.; Fielding, R.A. Skeletal muscle power: a critical determinant of physical functioning in older adults. *Exercise Sport Sci Rev* **2012**, 40, 4–12.
26. Reid, K.F.; Callahan, D.M.; Carabello, R.J.; Phillips, E.M.; Frontera, W.R.; Fielding, R.A. Lower extremity power training in elderly subjects with mobility limitations: a randomized controlled trial. *Aging Clin Exp Res* **2008**, 20, 337–343.
27. Clark, D.J.; Patten, C.; Reid, K.F.; Carabello, R.J.; Phillips, E.M.; Fielding, R.A. Muscle performance and physical function are associated with voluntary rate of neuromuscular activation in older adults. *J Gerontol A Biol Sci Med Sci* **2011**, 66, 115–121.

28. Reeves, N.D.; Narici, M.V.; Maganaris, C.N. Myotendinous plasticity to ageing and resistance exercise in humans. *Exp Physiol* **2006**, 91, 483–498.
29. Roos, M.R.; Rice, C.L.; Connelly, D.M.; Vandervoort, A.A. Quadriceps muscle strength, contractile properties, and motor unit firing rates in young and old men. *Muscle Nerve* **1999**, 22, 1094–1103.
30. Klass, M.; Baudry, S.; Duchateau, J. Age-related decline in rate of torque development is accompanied by lower maximal motor unit discharge frequency during fast contractions. *J Appl Physiol (1985)* **2008**, 104, 739–746.
31. Deschenes, M.R. Effects of aging on muscle fibre type and size. *Sports Med* **2004**, 34:809-24.
32. Kwon, Y.N.; Yoon, S.S. Sarcopenia: Neurological Point of View. *J Bone Metab.* **2017**, 24, 83-89. DOI: 10.11005/jbm.2017.24.2.83.
33. McNeil, C.J.; Vandervoort, A.A.; Rice, C.L. Peripheral impairments cause a progressive age related loss of strength and velocity-dependent power in the dorsiflexors. *J Appl Physiol (1985)* **2007**, 102, 1962–1968.
34. Toji, H.; Kaneko, M.J. Effects of aging on force, velocity, and power in the elbow flexors of males. *Physiol Anthropol* **2007**, 26, 587-92.
35. Barry, B.K.; Enoka, R.M. The neurobiology of muscle fatigue: 15 years later. *Integr Comp Biol* **2007**, 47, 465–473.
36. Griffith, E.E.; Yoon, T.; Hunter, S.K. Age and load compliance alter time to task failure for a submaximal fatiguing contraction with the lower leg. *J Appl Physiol* **2010**, 108, 1510–1519.
37. De Luca, C.J. Myoelectrical manifestations of localized muscular fatigue in humans. *Crit Rev Biomed Eng* **1984**,11, 251–279.
38. Enoka, R.M.; Duchateau, J. Muscle fatigue: what, why and how it influences muscle function. *J Physiol* **2008**, 586, 11–23. doi: 10.1113/jphysiol.2007.139477.
39. Bigland-Ritchie, B.; Jones, D. A.; Hosking, G. P.; Edwards, R. H. Central and peripheral fatigue in sustained maximum voluntary contractions of human quadriceps muscle. *Clin Sci Mol Med* **1978**, 54, 609–614. DOI: 10.1042/cs0 540609.
40. Kent-Braun, J. A. Central and peripheral contributions to muscle fatigue in humans during sustained maximal effort. *Eur J Appl Physiol Occup Physiol* **1999**, 80, 57–63. DOI: 10.1007/s004210050558.
41. Schillings, M. L.; Hoefsloot, W.; Stegeman, D. F.; Zwarts, M. J. Relative contributions of central and peripheral factors to fatigue during a maximal sustained effort. *Eur J Appl Physiol* **2003**, 90, 562–568. DOI: 10.1007/s00421-003-0913-4.
42. Merletti, R.; Bottin, A.; Cescon, C.; Farina, D.; Gazzoni, M.; Martina, S.; Mesin, L.; Pozzo, M.; Rainoldi, A.; Enck, P. Multichannel surface EMG for the non-invasive assessment of the anal sphincter muscle. *Digestion* **2004**, 69, 112–122. DOI: 10.1159/000077877.

43. Merletti, R.; Holobar, A.; Farina, D. Analysis of motor units with high-density surface electromyography. *J Electromyogr Kinesiol* **2008**, 18, 879–890.
44. Komi, P. V.; Tesch, P. EMG frequency spectrum, muscle structure, and fatigue during dynamic contractions in man. *Eur J Appl Physiol Occup Physiol* **1979**, 42, 41–50. DOI: 10.1007/BF00421103.
45. Bouissou, P.; Estrade, P. Y.; Goubel, F.; Guezennec, C. Y.; Serrurier, B. Surface EMG power spectrum and intramuscular pH in human vastus lateralis muscle during dynamic exercise. *J Appl Physiol* **1989**, 67, 1245–1249. DOI: 10.1152/jappl.1989.67.3.1245.
46. Brody, L. R.; Pollock, M. T.; Roy, S. H.; De Luca, C. J.; Celli, B. pH-induced effects on median frequency and conduction velocity of the myoelectric signal. *J Appl Physiol* **1991**, 71, 1878–1885. DOI: 10.1152/jappl.1991.71.5.1878.
47. Holtermann, A.; Gronlund, C.; Karlsson, J. S.; Roeleveld, K. Motor unit synchronization during fatigue: described with a novel sEMG method based on large motor unit samples. *J Electromyogr Kinesiol* **2009**, 19, 232–241. DOI: 10.1016/j.jelekin.2007.08.008.
48. Semmler, J. G. Motor unit synchronization and neuromuscular performance. *Exerc Sport Sci Rev* **2002**, 30, 8–14. DOI: 10.1097/00003677-200201000- 00003.
49. Datta, A. K.; Farmer, S. F.; Stephens, J. A. Central nervous pathways underlying synchronization of human motor unit firing studied during voluntary contractions. *J Physiol* **1991**, 432, 401–425. DOI: 10.1113/jphysiol.1991.sp018391.
50. Linssen, W. H.; Stegeman, D. F.; Joosten, E. M.; van't Hof, M. A.; Binkhorst, R. A.; Notermans, S. L. Variability and interrelationships of surface EMG parameters during local muscle fatigue. *Muscle Nerve* **1993**, 16, 849–856. DOI: 10.1002/mus.880160808.
51. Bilodeau, M.; Arsenault, A. B.; Gravel, D.; Bourbonnais, D. EMG power spectrum of elbow extensors: a reliability study. *Electromyogr Clin Neurophysiol* **1994**, 34, 149–158.
52. Ng, J. K.; Richardson, C. A. Reliability of electromyographic power spectral analysis of back muscle endurance in healthy subjects. *Arch Phys Med Rehabil* **1996**, 77, 259–264. DOI: 10.1016/S0003-9993(96)90108-2.
53. Merletti, R.; Fiorito, A.; Lo Conte, L. R.; Cisari, C. Repeatability of electrically evoked EMG signals in the human vastus medialis muscle. *Muscle Nerve* **1998**, 21, 184–193.
54. Kollmitzer, J.; Ebenbichler, G. R.; Kopf, A. Reliability of surface electromyographic measurements. *Clin Neurophysiol* **1999**, 110, 725–734. DOI: 10.1016/S1388-2457(98)00050-9.
55. Dedering, A.; Roos af Hjelmsater, M.; Elfving, B.; Harms-Ringdahl, K.; Nemeth, G. Between-days reliability of subjective and objective assessments of back extensor muscle fatigue in subjects without lower-back pain. *J Electromyogr Kinesiol* **2000**, 10, 151–158. DOI: 10.1016/S1050-6411(00) 00009-2.

56. Rainoldi, A.; Bullock-Saxton, J. E.; Cavarretta, F.; Hogan, N. Repeatability of maximal voluntary force and of surface EMG variables during voluntary isometric contraction of quadriceps muscles in healthy subjects. *J Electromyogr Kinesiol* **2001**, 11, 425–438. DOI: 10.1016/S1050-6411(01)00022-0.
57. Gonzalez-Izal, M.; Malanda, A.; Gorostiaga, E.; Izquierdo, M. Electromyographic models to assess muscle fatigue. *J Electromyogr Kinesiol* **2012**, 22, 501–512. DOI: 10.1016/j.jelekin.2012.02.019.
58. Troiano, A.; Naddeo, F.; Sosso, E.; Camarota, G.; Merletti, R.; Mesin, L. Assessment of force and fatigue in isometric contractions of the upper trapezius muscle by surface EMG signal and perceived exertion scale. *Gait Posture* **2008**, 28, 179–186. DOI: 10.1016/j.gaitpost.2008.04.002.
59. Mesin, L.; Cescon, C.; Gazzoni, M.; Merletti, R.; Rainoldi, A. A bidimensional index for the selective assessment of myoelectric manifestations of peripheral and central muscle fatigue. *J Electromyogr Kinesiol* **2009**, 19, 851–863. DOI 10.1016/j.jelekin.2008.08.003.
60. Merletti, R.; Knaflitz, M.; De Luca, C.J. Myoelectric manifestations of fatigue in voluntary and electrically elicited contractions. *J Appl Physiol* **1990**; 69, 1810–1820.
61. Sirven, N.; Rapp, T.; Coretti, S.; Ruggeri, M.; Cicchetti, A. Preventing mobility disability in Europe: a health economics perspective from the SPRINTT study. *Aging Clin Exp Res* **2017**, *29*, 75-79. DOI: 10.1007/s40520-016-0713-4.
62. Vlietstra, L.; Hendrickx, W.; Waters, D.L. Exercise interventions in healthy older adults with sarcopenia: A systematic review and meta-analysis. *Austral J Ageing* **2018**, 37, 169-183. DOI: 10.1111/ajag.12521.
63. Rondanelli, M.; Faliva, M.; Monteferrario, F.; Peroni, G.; Repaci, E.; Allieri, F.; Perna, S. Novel insights on nutrient management of sarcopenia in elderly. *Biomed. Res. Int.* **2015**, DOI: 10.1155/2015/524948.
64. Cartee, G.D.; Hepple, R.T.; Bamman, M.M.; Zierath, J.R. Exercise Promotes Healthy Aging of Skeletal Muscle. *Cell Metab* **2016**, 23, 1034-1047. DOI: 10.1016/j.cmet.2016.05.007.
65. Phillips, S.M. Nutritional supplements in support of resistance exercise to counter age-related sarcopenia. *Adv Nutr* **2015**, 6, 452-60. DOI: 10.3945/an.115.008367.
66. Yaraskeski, K.E.; Zachwieja, J.J.; Campbell, J.A.; Bier, D.M. Effect of growth hormone and resistance exercise on muscle growth and strength in older men. *Am J Physiol* **1995**, 268, E268–76.
67. Fiatarone, M.A.; O'Neill, E.F.; Ryan, N.D.; et al. Exercise training and nutritional supplementation for physical frailty in very elderly people. *N Engl J Med* **1994**, 330, 1769–75.
68. Jozsi, A.C.; Campbell, W.W.; Joseph, L.; Davey, S.L.; Evans, W.J. Changes in power with resistance training in older and younger men and women. *J Gerontol A Biol Sci Med Sci* **1999**, 54, M591–6.

69. Skelton, D.A.; Young, A.; Greig, C.A.; Malbut, K.E. Effects of resistance training on strength, power, and selected functional abilities of women aged 75 and older. *J Am Geriatr Soc* **1995**, 43, 1081–7.
70. Earles, D.R.; Judge, J.O.; Gunnarsson, O.T. Velocity training induces power-specific adaptations in highly functioning older adults. *Arch Phys Med Rehabil* **2001**, 82, 872–8.
71. Izquierdo, M.; Hakkinen, K.; Ibanez, J.; et al. Effects of strength training on muscle power and serum hormones in middle-aged and older men. *J Appl Physiol* **2001**, 90, 1497–507.
72. Newton, R.U.; Hakkinen, K.; Hakkinen, A.; McCormick, M.; Volek, J.; Kraemer W.J. Mixed-methods resistance training increases power and strength of young and older men. *Med Sci Sports Exerc* **2002**, 34, 1367–75.
73. Fielding, R.A.; LeBrasseur, N.K.; Cuoco, A.; Bean, J.; Mizer, K.; Fiatarone, M.A. High-velocity resistance training increases skeletal muscle peak power in older women. *J Am Geriatr Soc* **2002**, 50, 655–62.
74. American College of Sports Medicine, Chodzko-Zajko, W.J.; Proctor, D.N.; Fiatarone, M.A.; Minson, C.T.; Nigg, C.R.; Salem, G.J.; Skinner, J.S. American College of Sports Medicine position stand. Exercise and physical activity for older adults. *Med Sci Sports Exerc* **2009**, 4, 1510-30. DOI: 10.1249/MSS.0b013e3181a0c95c.
75. Franco, M.R.; Tong, A.; Howard, K.; et al. Older people's perspectives on participation in physical activity: a systematic review and thematic synthesis of qualitative literature. *Br J Sports Med* **2015**, 0, 1–9.
76. Pennings, B.; Groen, B.; de Lange, A.; Gijsen, A.; Zorenc, A.; Senden, J.; van Loon, L. Amino acid absorption and subsequent muscle protein accretion following graded intakes of whey protein in elderly men. *Am J Physiol Endocrinol Metab* **2012**, 302, E992–9.
77. Yang, Y.; Breen, L.; Burd, N.; Hector, A.; Churchward-Venne, T.; Josse, A.; Tarnopolsky, M.; Phillips S. Resistance exercise enhances myofibrillar protein synthesis with graded intakes of whey protein in older men. *Br J Nutr* **2012**, 108, 1780–8.
78. Witard, O.C.; Jackman, S.R.; Breen, L.M.; Smith, K.; Selby, A.; Tipton, K.D. Myofibrillar muscle protein synthesis rates subsequent to a meal in response to increasing doses of whey protein at rest and after resistance exercise. *Am J Clin Nutr* **2014**, 99, 86–95.
79. Katsanos, C.S.; Kobayashi, H.; Sheffield-Moore, M.; Aarsland, A.; Wolfe, R.R. A high proportion of leucine is required for optimal stimulation of the rate of muscle protein synthesis by essential amino acids in the elderly. *Am J Physiol Endocrinol Metab* **2006**, 291, E381–7.
80. Paddon-Jones, D.; Sheffield-Moore, M.; Zhang, X.J.; Volpi, E., Wolf, S.E.; Aarsland, A., Ferrando, A.A., Wolfe, R.R. Amino acid ingestion improves muscle

protein synthesis in the young and elderly. *Am J Physiol Endocrinol Metab* **2004**, 286, E321–8.
81. Moore, D.R.; Churchward-Venne, T.A.; Witard, O.; Breen, L., Burd, N.A.; Tipton, K.D., Phillips, S.M. Protein ingestion to stimulate myofibrillar protein synthesis requires greater relative protein intakes in healthy older versus younger men. *J Gerontol A Biol Sci Med Sci* **2015**, 70, 57–62.
82. Anthony, J.C.; Yoshizawa, F.; Anthony, T.G.; Vary, T.C.; Jefferson, L.S.; Kimball, S.R. Leucine stimulates translation initiation in skeletal muscle of post-absorptive rats via a rapamycin-sensitive pathway. *J Nutr* **2000**, 130, 2413–2419.
83. Crozier, S.J.; Kimball, S.R.; Emmert, S.W.; Anthony, J.C.; Jefferson, L.S. Oral leucine administration stimulates protein synthesis in rat skeletal muscle. *J Nutr* **2005**, 135, 376–382.
84. Ispoglou, T.; White, H.; Preston, T., McElhone, S.; McKenna, J.; Hind, K. Double blind, placebo-controlled pilot trial of L-leucine-enriched amino-acid mixtures on body composition and physical performance in men and women aged 65-75 years. *Eur J Clin Nutr* **2016**, 70, 182–8.
85. Komar, B.; Schwingshackl, L.; Hoffmann, G. Effects of leucine-rich protein supplements on anthropometric parameter and muscle strength in the elderly: a systematic review and meta-analysis. *J Nutr Health Aging* **2015**, 19, 437–46.
86. Devries, M.C.; McGlory, C.; Bolster, D.R.; Kamil, A.; Rahn, M.; Harkness, L.; Baker, S.K.; Phillips, S.M. Leucine, not total protein, content of a supplement is the primary determinant of muscle protein anabolic responses in healthy older women. *J Nutr* **2018**, 148, 1088–1095.
87. Tessier, A.J.; Chevalier, S. An Update on Protein, Leucine, Omega-3 Fatty Acids, and Vitamin D in the Prevention and Treatment of Sarcopenia and Functional Decline. *Nutrients* **2018**, 10, pii: E1099. DOI: 10.3390/nu10081099.
88. Cermak, N.M.; Res, P.T.; de Groot, L.C.; Saris, W.H.; van Loon, L.J. Protein supplementation augments the adaptive response of skeletal muscle to resistance-type exercise training: a meta-analysis. *Am J Clin Nutr* **2012**, *96*, 1454-1464. DOI: 10.3945/ajcn.112.037556.
89. van Schoor, N.M.; Lips, P. Worldwide vitamin D status. *Best Pract Res Clin Endocrinol Metab* **2011**, 25, 671–680.
90. Simpson, R.U.; Thomas, G.A.; Arnold, A.J. Identification of 1,25-dihydroxyvitamin D3 receptors and activities in muscle. *J Biol Chem* **1985**, 260, 8882–8891.
91. Dirks-Naylor, A.J.; Lennon-Edwards, S. The effects of vitamin D on skeletal muscle function and cellular signaling. *J Steroid Biochem Mol Biol* **2011**, 125, 159–168.
92. Houston, D.K.; Tooze, J.A.; Davis, C.C.; Chaves, P.H.; Hirsch, C.H.; Robbins, J.A.; Arnold, A.M.; Newman, A.B.; Kritchevsky, S.B. Serum 25-hydroxyvitamin D and

physical function in older adults: The cardiovascular health study all stars. *J Am Geriatr Soc* **2011**, 59, 1793–1801.
93. Houston, D.K.; Cesari, M.; Ferrucci, L.; Cherubini, A.; Maggio, D.; Bartali, B.; Johnson, M.A.; Schwartz, G.G.; Kritchevsky, S.B. Association between vitamin D status and physical performance: The inCHIANTI study. *J Gerontol A Biol Sci Med Sci* **2007**, 62, 440–446.
94. Bischoff-Ferrari, H.A.; Dietrich, T.; Orav, E.J.; Hu, F.B.; Zhang, Y.; Karlson, E.W.; Dawson-Hughes, B. Higher 25-hydroxyvitamin D concentrations are associated with better lower-extremity function in both active and inactive persons aged > or =60 y. *Am J Clin Nutr* **2004**, 80, 752–758.
95. Houston, D.K.; Tooze, J.A.; Neiberg, R.H.; Hausman, D.B.; Johnson, M.A.; Cauley, J.A.; Bauer, D.C.; Cawthon, P.M.; Shea, M.K.; Schwartz, G.G.; et al. 25-hydroxyvitamin D status and change in physical performance and strength in older adults: The health, aging, and body composition study. *Am J Epidemiol* **2012**, 176, 1025–1034.
96. Toffanello, E.D.; Perissinotto, E.; Sergi, G.; Zambon, S.; Musacchio, E.; Maggi, S.; Coin, A.; Sartori, L.; Corti, M.C.; Baggio, G.; et al. Vitamin D and physical performance in elderly subjects: The pro.V.A study. *PLoS One* **2012**, 7, e34950.
97. Dam, T.-T.L.; von Mühlen, D.; Barrett-Connor, E.L. Sex specific association of serum 25-hydroxyvitamin D levels with physical function in older adults. *Osteoporos Int* **2009**, 20, 751–760.
98. Mowe, M.; Haug, E.; Bohmer, T. Low serum calcidiol concentration in older adults with reduced muscular function. *J Am Geriatr Soc* **1999**, 47, 220–226.
99. Zamboni, M.; Zoico, E.; Tosoni, P.; Zivelonghi, A.; Bortolani, A.; Maggi, S.; Di Francesco, V.; Bosello, O. Relation between vitamin D, physical performance, and disability in elderly persons. *J Gerontol A Biol Sci Med Sci* **2002**, 57, M7-11.
100. Sohl, E.; de Jongh, R.T.; Heijboer, A.C.; Swart, K.M.; Brouwer-Brolsma, E.M.; Enneman, A.W.; de Groot, C.P.; van der Velde, N.; Dhonukshe-Rutten, R.A.; Lips, P.; et al. Vitamin D status is associated with physical performance: The results of three independent cohorts. *Osteoporos Int* **2013**, 24, 187–196.
101. Houston, D.K.; Neiberg, R.H.; Tooze, J.A.; Hausman, D.B.; Johnson, M.A.; Cauley, J.A.; Bauer, D.C.; Shea, M.K.; Schwartz, G.G.; Williamson, J.D.; et al. Low 25-hydroxyvitamin D predicts the onset of mobility limitation and disability in community-dwelling older adults: The health abc study. *J Gerontol A Biol Sci Med Sci* **2013**, 68, 181–187.
102. Wicherts, I.S.; van Schoor, N.M.; Boeke, A.J.; Visser, M.; Deeg, D.J.; Smit, J.; Knol, D.L.; Lips, P. Vitamin D status predicts physical performance and its decline in older persons. *J Clin Endocrinol Metab* **2007**, 92, 2058–2065.
103. Visser, M.; Deeg, D.J.; Lips, P.; Longitudinal Aging Study, A. Low vitamin D and high parathyroid hormone levels as determinants of loss of muscle strength and

muscle mass (sarcopenia): The longitudinal aging study amsterdam. *J Clin Endocrinol Metab* **2003**, 88, 5766–5772.
104. Annweiler, C.; Schott, A.M.; Berrut, G.; Fantino, B.; Beauchet, O. Vitamin D-related changes in physical performance: A systematic review. *J Nutr Health Aging* **2009**, 13, 893–898.
105. Volek, J.S.; Duncan, N.D.; Mazzetti, S.A.; Staron, R.S.; Putukian, M.; Gomez, A.; Pearson, D.R.; Fink, W.J.; Kraemer, W.J. Performance and muscle fiber adaptations to creatine supplementation and heavy resistance training. *Med Sci Sports Exerc* **1999**, 31, 1147–56.
106. Willoughby, D.S.; Rosene, J.M. Effects of oral creatine and resistance training on myosin heavy chain expression. *Med Sci Sports Exerc* **2001**, 33, 1674–81.
107. Willoughby, D.S.; Rosene, J.M. Effects of oral creatine and resistance training on myogenic regulatory factor expression. *Med Sci Sports Exerc* **2003**, 35, 923–9.
108. Lowe, D.A.; Lund, T.; Always, S.E. Hypertrophy stimulated myogenic regulatory factor mRNA increases are attenuated in fast muscle of aged quails. *Am J Physiol* **1998**, 275, C155–62.
109. Olsen, S.; Aagaard, P.; Kadi, F.; Tufekovic, G.; Verney, J.; Olesen, J.L.; Suetta, C.; Kjaer, M. Creatine supplementation augments the increase in satellite cell and myonuclei number in human skeletal muscle induced by strength training. *J Physiol* **2006**, 573, 525-534.
110. Safdar, A.; Yardley, N.J.; Snow, R.; Melov, S.; Tarnopolsky, M.A. Global and targeted gene expression and protein content in skeletal muscle of young men following short-term creatine monohydrate supplementation. *Physiol Genomics* **2008**, 32, 219-228.
111. Gualano, B.; Rawson, E.S.; Candow, D.G.; Chilibeck, P.D. Creatine supplementation in the aging population: effects on skeletal muscle, bone and brain. *Amino Acids* **2016**, *48*, 1793-1805. DOI: 10.1007/s00726-016-2239-7.
112. Branch, J.D. Effect of creatine supplementation on body composition and performance: a meta-analysis. Int. *J Sport Nutr Exerc Metab* **2003**, 13, 198–226
113. Devries, M.C.; Phillips, S.M. Creatine supplementation during resistance training in older adults: a meta-analysis. *Med Sci Sports Exerc* **2014**, *46*, 1194-1203. DOI: 10.1249/MSS.0000000000000220.
114. Naclerio, F.; Larumbe-Zabala, E. Effects of whey protein alone or as part of a multi-ingredient formulation on strength, fat free mass, or lean body mass in resistance-trained individuals: a meta-analysis. *Sports Med* **2015**, 46, 125-37. DOI:10.1007/s40279-015-0403-y
115. Dhesi, J.K.; Jackson, S.H.; Bearne, L.M.; Moniz, C.; Hurley, M.V.; Swift, C.G.; Allain, T.J. Vitamin D supplementation improves neuromuscular function in older people who fall. *Age Ageing* **2004**, *33*, 589-595. DOI: 10.1093/ageing/afh209.

116. Gotshalk, L.A.; Volek, J.S.; Staron, R.S.; Denegar, C.R.; Hagerman, F.C.; Kraemer, W.J. Creatine supplementation improves muscular performance in older men. *Med Sci Sports Exerc* **2002**, *34*, 537-543.
117. Pennings, B.; Boirie, Y.; Senden, J.M.G.; Gijsen, A.P.; Kuipers, H.; van Loon, L.J. Whey protein stimulates postprandial muscle protein accretion more effectively than do casein and casein hydrolysate in older men. *Am J Clin Nutr* **2011**, *93*, 997–1005. DOI: 10.3945/ajcn.110.008102.
118. Bermon, S.; Venembre, P.; Sachet, C.; Valour, S.; Dolisi, C. Effects of creatine monohydrate ingestion in sedentary and weight-trained older adults. *Acta Physiol Scand* **1998**, *164*, 147-155. DOI: 10.1046/j.1365-201X.1998.00427.x.
119. Zhu, K.; Kerr, D.A.; Meng, X.; Devine, A.; Solah, V.; Binns, C.W., Prince, R.L. Two-Year Whey Protein Supplementation Did Not Enhance Muscle Mass and Physical Function in Well-Nourished Healthy Older Postmenopausal Women. *J Nutr* **2015**, *145*, 2520-2526. DOI: 10.3945/jn.115.218297.
120. Bell, K.E., Snijders, T.; Zulyniak, M.; Kumbhare, D.; Parise, G.; Chabowski, A.; Phillips, S.M. A whey protein-based multi-ingredient nutritional supplement stimulates gains in lean body mass and strength in healthy older men: A randomized controlled trial. *PLoS One* **2017**, *12*, e0181387. DOI: 10.1371/journal.pone.0181387.
121. Bonnefoy, M.; Cornu, C.; Normand, S.; Boutitie, F.; Bugnard, F.; Rahmani, A.; Lacour, J.R.; Laville, M. The effects of exercise and protein-energy supplements on body composition and muscle function in frail elderly individuals: a long-term controlled randomised study. *Br J Nutr* **2003**, *89*, 731–739. DOI: 10.1079/BJN2003836.
122. Fiatarone, M.A.; O'Neill, E.F.; Ryan, N.D.; Clements, K.M.; Solares, G.R.; Nelson, M.E.; Roberts, S.B.; Kehayias, J.J.; Lipsitz, L.A.; Evans, W.J. Exercise training and nutritional supplementation for physical frailty in very elderly people. *N Engl J Med* **1994**, *330*, 1769–1775. DOI: 10.1056/NEJM199406233302501.
123. Fiatarone, M.A.; O'Neill, E.F.; Doyle, N.; Clements, K.M.; Roberts, S.B.; Kehayias, J.J.; Lipsitz, L.A.; Evans, W.J. The Boston FICSIT study: the effects of resistance training and nutritional supplementation on physical frailty in the oldest old. *J Am Geriatr Soc* **1993**, *41*, 333–337.
124. Miller, M.D.; Crotty, M.; Whitehead, C.; Bannerman, E.; Daniels, L.A. Nutritional supplementation and resistance training in nutritionally at risk older adults following lower limb fracture: a randomized controlled trial. *Clin Rehabil* **2006**, *20*, 311–323. DOI: 10.1191/0269215506cr942oa.
125. Sugawara, K.; Takahashi, H.; Kashiwagura, T.; Yamada, K.; Yanagida, S.; Homma, M.; Dairiki, K.; Sasaki, H.; Kawagoshi, A.; Satake, M.; et al. Effect of anti-inflammatory supplementation with whey peptide and exercise therapy in patients with COPD. *Respir Med* **2012**, *106*, 1526–1534. DOI: 10.1016/j.rmed.2012.07.001.

126. Bunout, D.; Barrera, G.; de la Maza, P.; Avendaño, M.; Gattas, V.; Petermann, M.; Hirsch, S. The impact of nutritional supplementation and resistance training on the health functioning of free-living Chilean elders: results of 18 months of follow-up. *J Nutr* **2001**, *131*, 2441S–2446S. DOI: 10.1093/jn/131.9.2441S.
127. Bunout, D.; Barrera, G.; de la Maza, P.; Avendaño, M.; Gattas, V.; Petermann, M.; Hirsch, S. Effects of nutritional supplementation and resistance training on muscle strength in free living elders. Results of one year follow. *J Nutr Health Aging* **2004**, *8*, 68–75.
128. de Jong, N.; Chin, A.; Paw, M.J.; de Groot, L.C.; Hiddink, G.J.; van Staveren, W.A. Dietary supplements and physical exercise affecting bone and body composition in frail elderly persons. *Am J Public Health* **2000**, *90*, 947–954.
129. Rondanelli, M.; Klersy, C.; Terracol, G.; Talluri, J.; Maugeri, R.; Guido, D.; Faliva, M.A.; Solerte, B.S.; Fioravanti, M.; Lukaski, H.; et al. Whey protein, amino acids, and vitamin D supplementation with physical activity increases fat-free mass and strength, functionality, and quality of life and decreases inflammation in sarcopenic elderly. *Am J Clin Nutr* **2016**, *103*, 830-840. DOI: 10.3945/ajcn.115.113357.
130. Verreijen, A.M.; Verlaan, S.; Engberink, M.F.; Swinkels, S.; de Vogel-van den Bosch, J.; Weijs, P.J. A high whey protein-, leucine-, and vitamin D-enriched supplement preserves muscle mass during intentional weight loss in obese older adults: a double-blind randomized controlled trial. *Am J Clin Nutr* **2015**, *101*, 279-286. DOI: 10.3945/ajcn.114.090290.
131. Bauer, J.M.; Verlaan, S.; Bautmans, I.; Brandt, K.; Donini, L.M.; Maggio, M.; McMurdo, M.E.; Mets, T.; Seal, C.; Wijers, S.L.; Ceda, G.P.; De Vito, G.; Donders, G.; et al. Effects of a vitamin D and leucine-enriched whey protein nutritional supplement on measures of sarcopenia in older adults, the PROVIDE study: a randomized, double-blind, placebo-controlled trial. *J Am Med Dir Assoc* **2015**, *16*, 740-747. DOI: 10.1016/j.jamda.2015.05.021.
132. Bo, Y.; Liu, C.; Ji, Z.; Yang, R.; An, Q.; Zhang, X.; You, J.; Duan, D.; Sun, Y.; Zhu, Y.; et al. A high whey protein, vitamin D and e supplement preserves muscle mass, strength, and quality of life in sarcopenic older adults: A double-blind randomized controlled trial. *Clin Nutr* **2018**, pii: S0261-5614(18)30007-4. DOI: 10.1016/j.clnu.2017.12.020.
133. Chanet, A.; Verlaan, S.; Salles, J.; Giraudet, C.; Patrac, V.; Pidou, V.; Pouyet, C.; Hafnaoui, N.; Blot, A.; Cano, N.; et al. Supplementing breakfast with a vitamin D and leucine-enriched whey protein medical nutrition drink enhances postprandial muscle protein synthesis and muscle mass in healthy older men. *J Nutr* **2017**, 147, 2262–2271.
134. D'Antona, G., Nisoli, E. mTOR signaling as a target of amino acid treatment of the age-related sarcopenia. *Interdiscip Top Gerontol* **2010**, *37*, 115-141. DOI: 10.1159/000319998.

135. Dillon, E.L. Nutritionally essential amino acids and metabolic signaling in aging. *Amino Acids* **2013**, *45*, 431-441. DOI: 10.1007/s00726-012-1438-0.
136. Xu, Z.R.; Tan, Z.J.; Zhang, Q.; Gui, Q.F.; Yang, Y.M. Clinical effectiveness of protein and amino acid supplementation on building muscle mass in elderly people: a meta-analysis. *PLoS One* **2014**, *9*, e109141. DOI: 10.1371/journal.pone.0109141.
137. Moon, A.; Heywood, L.; Rutherford, S.; Cobbold, C. Creatine supplementation: can it improve quality of life in the elderly without associated resistance training? *Curr Aging Sci* **2013**, *6*, 251-7.
138. Gotshalk, L.A.; Kraemer, W.J.; Mendonca, M.A.; Vingren, J.L.; Kenny, A.M.; Spiering, B.A.; Hatfield, D.L.; Fragala, M.S.; Volek, J.S. Creatine supplementation improves muscular performance in older women. *Eur J Appl Physiol* **2008**, 102, 223-231. DOI: 10.1007/s00421-007-0580-y.
139. Dawson-Hughes, B. Serum 25-hydroxyvitamin D and muscle atrophy in the elderly. *Proc Nutr Soc* **2012**, *71*, 46-49. DOI: 10.1017/S0029665111003260.
140. Weaver, C.M.; Alexander, D.D.; Boushey, C.J.; Dawson-Hughes, B.; Lappe, J.M.; LeBoff, M.S.; Liu, S.; Looker, A.C.; Wallace, T.C.; Wang, D.D. Calcium plus vitamin D supplementation and risk of fractures: an updated meta-analysis from the National Osteoporosis Foundation. *Osteoporos Int* **2016**, *27*, 367-76. DOI: 10.1007/s00198-015-3386-5.
141. Shay, K.P., Moreau, R.F.; Smith, E.J.; Smith, A.R.; Hagen, T.M. Alpha-lipoic acid as a dietary supplement: Molecular mechanisms and therapeutic potential. *Biochim Biophys Acta* **2009**, *1790*, 1149–1160. DOI: 10.1016/j.bbagen.2009.07.026.
142. Goraca, A.; Huk-Kolega, H.; Piechota, A.; Kleniewska, P.; Ciejka, E.; Skibska B. Lipoic acid – biological activity and therapeutic potential. *Pharm Reports* **2011**, 63, 849-858.
143. Fischer, A.; Onur, S.; Niklowitz, P.; Menke, T.; Laudes, M.; Rimbach, G.; Döring, F. Coenzyme Q10 Status as a Determinant of Muscular Strength in Two Independent Cohorts. *PLoS One* **2016**, *11*, e0167124. DOI: 10.1371/journal.pone.0167124.
144. Bennett, B.T.; Mohamed, J.S.; Alway, S.E. Effects of Resveratrol on the Recovery of Muscle Mass Following Disuse in the Plantaris Muscle of Aged Rats. *PLoS One* **2013**, *8*, e83518. DOI: 10.1371/journal.pone.0083518.
145. Jackson, J.R.; Ryan, M.J.; Alway, S.E. Long-term supplementation with resveratrol alleviates oxidative stress but does not attenuate sarcopenia in aged mice. *J Gerontol A Biol Sci Med Sci* **2011**, *66*, 751-764. DOI: 10.1093/gerona/glr047.
146. Bauer, J.; Biolo, G.; Cederholm, T.; Cesari, M.; Cruz-Jentoft, A.J.; Morley, J.E.; Phillips, S.; Sieber, C.; Stehle, P.; Teta, D.; et al. Evidence-based recommendations for optimal dietary protein intake in older people: a position paper from the PROT-AGE Study Group. *J Am Med Direct Ass* **2013**, *14*, 542–559. DOI: 10.1016/j.jamda.2013.05.021.

147. Janssen, I.; Baumgartner, R.N.; Ross, R.; Rosenberg, I.H.; Roubenoff, R. Skeletal muscle cutpoints associated with elevated physical disability risk in older men and women. *Am J Epidemiol* **2004**, *159*, 413–421.
148. Reinhardt, M.; Piaggi, P.; DeMers, B.; Trinidad, C.; Krakoff, J. Cross calibration of two dual-energy X-ray densitometers and comparison of visceral adipose tissue measurements by iDXA and MRI. *Obesity* **2017**, *25*, 332-337. DOI: 10.1002/oby.21722.
149. Merletti, R.; Roy, S. Myoelectric and mechanical manifestations of muscle fatigue in voluntary contractions. *J Orthop Sports Phys Ther* **1996**, 24, 342–353. DOI: 10.2519/jospt.1996.24.6.342.
150. Gitter, J. A.; Czerniecki, M. J. Fractal analysis of the electromyographic interference pattern. *J Neurosci Methods* **1995**, 58, 103–108. DOI: 10.1016/0165-0270(94)00164-C.
151. Farina, D.; Merletti, R. A novel approach for estimating muscle fiber conduction velocity by spatial and temporal filtering of surface EMG signals. *IEEE Trans Biomed Eng* **2003**, 50, 1340–1351. DOI: 10.1109/TBME.2003.819847.
152. Andreassen, S. Methods for computer-aided measurement of motor unit parameters. *Electroencephalogr Clin Neurophysiol Suppl* **1987**, 39, 13–20.
153. Fitzmaurice, G.M.; Laird, N.M.; Ware, J.H. *Applied longitudinal analysis*. Wiley-Interscience, Hoboken, NJ, **2004.**
154. Adamo, M.L.; Farrar, R.P. Resistance training, and IGF involvement in the maintenance of muscle mass during the aging process. *Ageing Res Rev* **2006**, *5*, 310-331. DOI: 10.1016/j.arr.2006.05.001.
155. Beaudart, C.; Dawson, A.; Shaw, S.C.; Harvey, N.C.; Kanis, J.A.; Binkley, N.; Reginster, J.Y.; Chapurlat, R.; Chan, D.C.; Bruyère, O.; et al. Nutrition and physical activity in the prevention and treatment of sarcopenia: systematic review. *Osteoporos Int* **2017**, *28*, 1817–1833. DOI 10.1007/s00198-017-3980-9.
156. Roberts, B.M.; Lavin, K.M.; Many, G.M.; Thalacker-Mercer, A.; Merritt, E.K.; Bickel, C.S.; Mayhew, D.L.; Tuggle, S.C.; Cross, J.M.; Kosek DJ; et al. Human neuromuscular aging: Sex differences revealed at the myocellular level. *Exp Gerontol* **2018**, *106*, 116-124. DOI: 10.1016/j.exger.2018.02.023.
157. Beaudart, C.; Buckinx, F.; Rabenda, V.; Gillain, S.; Cavalier, E.; Slomian, J.; Petermans, J.; Reginster, J.Y.; Bruye`re, O. The effects of vitamin D on skeletal muscle strength, muscle mass, and muscle power: a systematic review and meta-analysis of randomized controlled trials. *J Clin Endocrinol Metab* **2014**, *99*, 4336–4345. DOI: 10.1210/jc.2014-1742.
158. Bischoff-Ferrari, H.A. Optimal serum 25-hydroxyvitamin D levels for multiple health outcomes. *Adv Exp Med Biol* **2014**, *810*, 500-525.
159. Gualano, B.; Rawson, E.S.; Candow, D.G.; Chilibeck, P.D. Creatine supplementation in the aging population: effects on skeletal muscle, bone and brain. *Amino Acids* **2016**, *48*, 1793-1805. DOI: 10.1007/s00726-016-2239-7.

160. Arivazhagan, P.; Ramanathan, K.; Panneerselvam, C. Effect of DL-Alpha-lipoic acid on mitochondrial enzymes in aged rats. *Chem Biol Interact* **2001**, 138, 189–198.
161. Arivazhagan, P.; Ramanathan, K.; Panneerselvam, C. Effect of DL-Alpha-lipoic acid on the status of lipid peroxidation and lipids in aged rats. *J Gerontol A Biol Sci Med Sci* **2003**, *58*, B788–B791.
162. Fischer, A.; Onur, S.; Niklowitz, P., Menke, T.; Laudes, M.; Rimbach, G.; Döring, F. Coenzyme Q10 Status as a Determinant of Muscular Strength in Two Independent Cohorts. *PLoS One* **2016**, *1*, e0167124. DOI:10.1371/journal.pone.0167124.
163. González-Guardia, L.; Yubero-Serrano, E.M.; Delgado-Lista, J.; Perez-Martinez, P.; Garcia-Rios, A.; Marin, C.; Camargo, A.; Delgado-Casado, N.; Roche, H.M.; Perez-Jimenez, F.; et al. Effects of the Mediterranean Diet Supplemented With Coenzyme Q10 on Metabolomic Profiles in Elderly Men and Women. *J Gerontol A Biol Sci Med Sci* **2015**, *70*, 78–84. DOI:10.1093/gerona/glu098.
164. Yubero-Serrano, E.M.; Gonzalez-Guardia, L.; Rangel-Zuñiga, O.; Delgado-Lista, J.; Gutierrez-Mariscal, F.M.; Perez-Martinez, P.; Delgado-Casado, N., Cruz-Teno, C., Tinahones, F.J.; Villalba, J.M.; et al. Mediterranean Diet Supplemented With Coenzyme Q10 Modifies the Expression of Proinflammatory and Endoplasmic Reticulum Stress–Related Genes in Elderly Men and Women. *J Gerontol A Biol Sci. Med Sci* **2012**, *67*, 3–10. DOI:10.1093/gerona/glr167.
165. Sola, S.; Mir, M.Q.; Cheema, F.A.; Khan-Merchant, N.; Menon, R.G.; Parthasarathy, S.; Khan, B.V. Irbesartan and lipoic acid improve endothelial function and reduce markers of inflammation in the metabolic syndrome: results of the Irbesartan and Lipoic Acid in Endothelial Dysfunction (ISLAND) study. *Circulation* **2005**, *111*, 343–348. DOI: 10.1161/01.CIR.0000153272.48711.B9.
166. Rodriguez, M.C.; MacDonald, J.R.; Mahoney, D.J.; Parise, G.; Beal, M.F.; Tarnopolsky, M.A. Beneficial effects of creatine, CoQ10, and lipoic acid in mitochondrial disorders. *Muscle Nerve* **2007**, *35*, 235-242. DOI: 10.1002/mus.20688.
167. Jackson, J.R.; Ryan, M.J.; Alway, S.E. Long-term supplementation with resveratrol alleviates oxidative stress but does not attenuate sarcopenia in aged mice. *J Gerontol A Biol Sci Med Sci* **2011**, *66*, 751–764. DOI: 10.1093/gerona/glr047.
168. Wang, D.T.; Yin, Y.; Yang, Y.J.; Lv, P.J.; Shi, Y.; Lu, L.; Wei, L.B. Resveratrol prevents TNF-α-induced muscle atrophy via regulation of Akt/mTOR/FoxO1 signaling in C2C12 myotubes. *Int Immunopharmacol* **2014**, *19*, 206–213. DOI: 10.1016/j.intimp.2014.02.002.
169. Baur, J.A.; Pearson, K.J.; Price, N.L.; Jamieson, H.A.; Lerin, C.; Kalra, A.; Prabhu, V.V.; Allard, J.S.; Lopez-Lluch, G.; Lewis, K.; et al. Resveratrol improves health and survival of mice on a high-calorie diet. *Nature* **2006**, *444*, 337–342. DOI: 10.1038/nature05354.
170. Lagouge, M.; Argmann, C.; Gerhart-Hines, Z.; Meziane, H.; Lerin, C.; Daussin, F.; Messadeq, N.; Milne, J.; Lambert, P.; Elliott, P.; et al. Resveratrol improves

mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha. *Cell* **2006**, *127*, 1109–1122. DOI: 10.1016/j.cell.2006.11.013.
171. Timmers, S.; Konings, E.; Bilet, L.; Houtkooper, R.H.; van de Weijer, T.; Goossens, G.H.; Hoeks, J.; van der Krieken, S.; Ryu, D.; Kersten, S.; et al. Calorie restriction-like effects of 30 days of resveratrol supplementation on energy metabolism and metabolic profile in obese humans. *Cell Metab* **2011**, *14*, 612–622. DOI: 10.1016/j.cmet.2011.10.002.
172. Most, J.; Timmers, S.; Warnke, I.; Jocken, J.W.; van Boekschoten, M.; de Groot, P.; Bendik, I.; Schrauwen, P.; Goossens, G.H.; Blaak, E.E. Combined epigallocatechin-3-gallate and resveratrol supplementation for 12 wk increases mitochondrial capacity and fat oxidation, but not insulin sensitivity, in obese humans: a randomized controlled trial. *Am J Clin Nutr* **2016**, *104*, 215–227. DOI: 10.3945/ajcn.115.122937.
173. Tchernof, A.; Despres, J.P. Pathophysiology of human visceral obesity: an update. *Physiol Rev* **2013**, *93*, 359–404. DOI: 10.1152/physrev.00033.2011.
174. Schweitzer L, Geisler C, Johannsen M, Glüer CC, Müller MJ. Associations between body composition, physical capabilities and pulmonary function in healthy older adults. *Eur J Clin Nutr* **2017**, *71*, 389-394. DOI: 10.1038/ejcn.2016.146.
175. Reule, C.A.; Scholz, C.; Schoen, C.; Brown, N.; Siepelmeyer, A.; Alt, W.W. Reduced muscular fatigue after a 12-week leucine-rich amino acid supplementation combined with moderate training in elderly: a randomised, placebo-controlled, double-blind trial. *BMJ Open Sport Exerc Med.* **2017**, 2, e000156. DOI: 10.1136/bmjsem-2016-000156.
176. Gryson, C.; Ratel, S.; Rance, M.; Penando, S.; Bonhomme, C.; Le Ruyet, P.; Duclos, M.; Boirie, Y.; Walrand S. Four-month course of soluble milk proteins interacts with exercise to improve muscle strength and delay fatigue in elderly participants. *J Am Med Dir Assoc* **2014**, 15, 958.e1-9. DOI: 10.1016/j.jamda.2014.09.011.
177. Merletti, R.; Farina, D.; Gazzoni, M.; Schieroni, M.P. Effect of age on muscle functions investigated with surface electromyography. *Muscle Nerve* **2002**; 25, 65–76.
178. Kupa, E.J.; Roy, S.H.; Kandarian, S.C.; De Luca, C.J. Effects of muscle fiber type and size on EMG median frequency and conduction velocity. *J Appl Physiol* **1995**; 79, 23–32.
179. Boccia, G.; Dardanello, D.; Rosso, V.; Pizzigalli, L.; Rainoldi A. The Application of sEMG in Aging: A Mini Review. *Gerontology* **2015**, 61,477-84. DOI: 10.1159/000368655.
180. Goodpaster, B.H.; Park, S.W.; Harris, T.B.; Kritchevsky, S.B.; Nevitt, M.; Schwartz, A.V.; Simonsick, E.M.; Tylavsky, F.A.; Visser, M.; Newman, A.B. The loss of skeletal muscle strength, mass, and quality in older adults: The Health,

Aging and Body Composition Study. *J Gerontol A Biol Sci Med Sci* **2006**; *61*, 1059-1064.
181. Wolfe, R.R. The underappreciated role of muscle in health and disease. *Am J Clin Nutr* **2006**, 84, 475–82.