Review

Anabolic resistance in the pathogenesis of sarcopenia in the elderly

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Posted Date: 8 August 2023

doi: 10.20944/preprints202308.0516.v1

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Review

Anabolic Resistance in the Pathogenesis of Sarcopenia in the Elderly

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Abstract: The development of sarcopenia in the elderly is associated to many potential factors and/or processes, that impair the renovation and the maintenance of skeletal muscle mass and strength as ageing progresses. Among them, a defect by skeletal muscle to respond to anabolic stimuli is to be considered. Common anabolic stimuli/signals in skeletal muscle are hormones (insulin, growth hormones, IGF-1, androgens, β-agonists such epinephrine), substrates (amino acids as protein precursors on top, but also glucose and fat, as source of energy), metabolites (such as β-agonists and HMB), some cytokines, various biochemical/ intracellular mediators), physical exercise, neurogenic and immune-modulating factors, etc. Each of them may exhibit a reduced effect upon skeletal muscle as ageing progresses. In this review article, we will concisely overview the effects of anabolic signals on muscle metabolism, as well as currently available evidence of a resistance, at skeletal muscle level, to any of the above-mentioned anabolic factors, from both in vitro and in vivo studies.

Keywords: exercise; intracellular signals; nutrition; protein foods; protein synthesis; sarcopenia; skeletal muscle

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Abbreviations

AA: amino acid; EAA: essential amino acids; HMB: β-hydroxyl, β-methylbutyrate; hGH: human Growth Hormone; MPS: muscle protein synthesis; PD: protein degradation; PS: protein synthesis; WP: whey protein.

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Introduction

The term “sarcopenia” (from the Greek words “sarx”. i.e., flesh, and “penia” i.e., loss) was firstly proposed by Rosenberg in 1989 to describe the age-related loss of muscle mass [1]. Later, this term was referred to the decrease in muscle mass and/or strength. Sarcopenia is an almost unavoidable process associated to ageing, occurring at variable rates and different magnitudes in each subject, despite the influence of a variety of factors on its progression rate as well as in the ultimate extent of the functional impairment. Although the criteria for screening and diagnosing sarcopenia are currently under debate [2], its prevalence in the 7th decade would range between 5–13%, increasing up to ≥50% after 80 yrs [3,4]. Muscular loss has been estimated to occur at a rate of 1–3% yearly [5]. Factors associated with the development of sarcopenia with ageing are a progressive decrease of physical activity, the coexistence of subtle or overt malnutrition and age-related diseases, genetic factors, pharmacological therapies, and a chronic (sub)-inflammatory condition [6,7]. Each of these factors may impair muscle mass, function (i.e., strength, speed of contraction, overall power) or both. These factors interact with each other, so that often it cannot be entirely clear which one comes first. A subjective reaction to perceived fatigue may also lead to an involuntary reduction of daily physical activity, thus amplifying muscle disuse in a negative loop. The mechanisms(s) for the

 doi:10.20944/preprints202308.0516.v1

increased fatigability occurring with ageing are yet incompletely understood [8]. Maintenance, wasting and the recovery of muscle mass depend on the relative rates of two opposite, ongoing processes, i.e., protein degradation and synthesis, in other words, of protein turnover. These two processes occur simultaneously although at variable rates, and their regulation depends on different factors operating in a complex network. Therefore, net protein anabolism (or "accretion"), i.e., the net increase of muscle mass, occurs anytime when synthesis exceeds degradation.

PART 1: SKELETAL MUSCLE METABOLISM AND GROWTH

1. PHYSIOLOGICAL REGULATION OF MUSCLE METABOLISM AND GROWTH

1a. The molecular mechanisms behind muscles growth in young subjects: exercise, nutrients and hormones

Muscle growth, or muscle hypertrophy, is a complex process regulated by several molecular pathways. The primary pathways that promote muscle growth in response to exercise, nutrients, and growth signals include IGF-1/PI3K (phosphoinositide 3-kinase)/Akt/mTOR pathway and hormones like testosterone and GH. The IGF-1/PI3K/Akt/mTOR pathway is a vital signaling cascade in muscle growth that involves various interconnected mechanisms. Its activation increases protein synthesis, reduces protein degradation, and improves cell growth. The molecular mechanisms and interactions within this pathway are still being studied in human muscle physiology. When activated, mTOR promotes muscle hypertrophy by stimulating protein synthesis and cell growth [9] The mTOR kinase is the main regulator of muscle protein synthesis and responds to the availability of nutrients, particularly amino acids and growth factors. Indeed, resistance exercise and nutrient intake, especially leucine-rich amino acids, activate the mTOR pathway essential for exercise-induced muscle protein synthesis, thus, necessary for increasing muscle protein synthesis following resistance exercise in young men [10]. The key importance of mTOR is exemplified by an experiment, where young subjects were treated with a potent mTORC1 inhibitor (rapamycin) before performing a series of high-intensity muscle contractions to demonstrate this activation. Rapamycin treatment blocked the early acute (1-2h) contraction-induced increase in human muscle protein synthesis. Other studies have also shown that a high-protein diet enhances mTOR-mediated protein synthesis in young men following endurance exercise [11]. These studies /indicate that mTOR activation is crucial in mediating the anabolic response to exercise and diet in humans (Figure 1).

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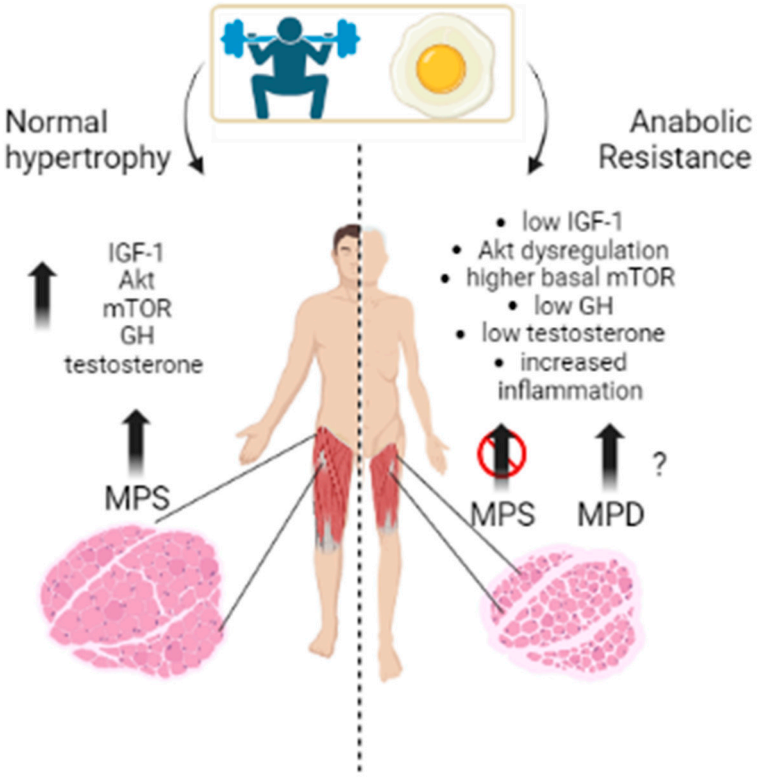

Figure 1. Here is a diagram showing the typical triggers for muscle growth in young people, which includes exercise and high-quality protein intake. These stimuli activate various molecular pathways that lead to muscle hypertrophy. However, in the right-hand side of the diagram, you can see that the same stimuli do not have the same effect on skeletal muscle in cases of anabolic resistance.

Akt activation is crucial in promoting muscle protein synthesis in response to exercise and nutrient intake in young individuals [12]. In response to resistance training, a significant hypertrophy (+10%) in the human quadriceps was demonstrated, with a parallel increase in phospho-Akt, phospho-GSK-3beta, and phospho-mTOR protein and a decrease in the nuclear protein content of Foxo1, which is the master regulator of the atrophy program [13]. Akt is also influenced by nutrition, as excess leucine intake has been shown to enhance Akt signaling in young individuals, suggesting that Akt is sensitive to dietary amino acids and can modulate protein synthesis accordingly [14]. The timing of exercise and protein intake also affect Akt activation and subsequent muscle protein synthesis. While exercise alone did not increase Akt and mTOR phosphorylation, protein ingestion afterward did so in a dose-dependent manner. Akt activation is a complex process influenced by exercise, nutrition, and specific amino acids, and further research is being conducted to fully understand its role in muscle growth and adaptation [15].

The upstream controller in this axis is Insulin-like growth factor-1 (IGF-1), which is crucial in promoting growth and anabolic processes in skeletal muscles [9]. IGF-1 is a key growth factor that regulates both anabolic and catabolic pathways in skeletal muscle. Studies indicate that IGF-1 induces hypertrophy of human myotubes in vitro, characterized by an increase in the mean number of nuclei per myotube, an increase in the fusion index, and an increase in myosin heavy chain (MyHC) content [16]. IGF-1 contributes to muscle protein turnover, protein synthesis, and the adaptation of skeletal muscles to resistance training [17]. Hormonal factors, such as ethinyl estradiol administration, can affect IGF-1 synthesis and degradation in skeletal muscles, potentially modulating muscle protein turnover and influencing responses to exercise and nutrient intake [18]. IGF1 is closely connected with the growth hormone (GH), which stimulates muscle growth through several mechanisms and interactions with other signaling pathways. It stimulates protein synthesis by enhancing the uptake of amino acids into muscle tissue, providing the building blocks for muscle protein synthesis. This

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process is mediated, at least in part, by the GH/IGF-1 axis. GH stimulates the liver and other tissues to produce insulin-like growth factor 1 (IGF-1), which promotes cell growth, protein synthesis, and the proliferation of satellite cells involved in muscle repair and growth [19].

GH promotes the uptake of essential nutrients, such as glucose and amino acids, into muscle cells for energy production and protein synthesis. It also affects metabolism by enhancing the breakdown of fats, providing additional energy sources for muscle growth [20]. However, pharmacological GH supplementation only increases muscle strength or size in individuals with clinical GH deficiency, and there is no evidence that exercise-induced changes in GH have the same effects in individuals with normal GH levels [21].

Testosterone is one of the most potent naturally secreted androgenic-anabolic hormones, and its biological effects include promoting muscle growth. In muscle, testosterone stimulates protein synthesis (anabolic effect) and inhibits protein degradation (anti-catabolic effect)[22–24]. Testosterone plays a crucial role in muscle growth in response to exercise and nutrition. Various studies have shed light on this topic, Vingren et al. (2010) explored the physiological aspects of testosterone in resistance exercise and training, highlighting its upstream regulatory elements [25]. They found that testosterone enhances muscle protein synthesis, stimulates satellite cell activation and proliferation, and modulates anabolic signaling pathways such as mTOR and IGF-1. These findings suggest that testosterone plays a key role in mediating the anabolic response to resistance exercise [25]. West and Phillips (2010) discussed the anabolic processes in human skeletal muscle, emphasizing the roles of growth hormone and testosterone. They concluded that testosterone acts directly on muscle tissue to promote muscle protein synthesis and hypertrophy. The interactions between testosterone, growth hormone, and anabolic signaling pathways, such as mTOR and IGF-1, contribute to the overall anabolic response in the muscle. They highlighted the importance of optimizing testosterone levels for maximizing muscle growth in response to exercise and nutrition [21]. In their work, the exercise paradigms are designed based on the assumption (not necessarily evidenced-based mechanisms) that GH and testosterone facilitate anabolic processes that lead to skeletal muscle protein accretion and hypertrophy. Moreover that exercise-induced hormonal stimulation does not enhance intracellular markers of anabolic signaling or the acute postexercise elevation of myofibrillar protein synthesis. Furthermore, they demonstrate that exercise-induced increases in GH and testosterone availability are unnecessary and do not enhance strength and hypertrophy adaptations. So they concluded that local mechanisms intrinsic to the skeletal muscle tissue performing the resistive contractions (i.e.,, weightlifting) are predominant in stimulating anabolism [21]. The regulation of satellite cells following myotrauma caused by resistance exercise is related to testosterone, which plays a critical role in activating satellite cells, which are crucial for muscle repair and growth [26]. Finally, Bhasin et al. (2001) examined the dose-response relationships of testosterone in healthy young men, and they found that testosterone administration in varying doses dose-dependently increased muscle protein synthesis rates, resulting in greater muscle mass and strength gains. These findings suggest that higher testosterone levels promote anabolic processes and contribute to muscle growth in response to exercise and nutrition [27].

1b. Factors / stimuli that determine protein accretion in muscle *in vivo*

Accretion of muscle mass depends on physiological, metabolic and hormonal factors as well as on physical activity. Conversely, it is hindered by inactivity, malnutrition, overt diseases and/or subtle, chronic pathological conditions [28–33]. The main protein-anabolic factors, at both the whole-body and skeletal muscle level, are the proteins and/or the amino acids themselves (i.e., the protein buiding blocks), as well as physical activity/exercise. In addition, energy availability, anabolic hormones (insulin, human Growth Hormone [hGH], IGF-1, β-agonists, anabolic steroids, *see also above*), adequate tissue perfusion, and, in general, a "healthy status" (i.e.,, the absence of both overt diseases and subtle pathological conditions, such as a chronic sub-inflammatory status) also condition skeletal muscle accretion. Moreover, the effects of any of these factors could be divided between "acute" (i.e.,,

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demonstrable in an acute setting or experimental conditions) and "chronic" (i.e.,, demonstrable after repeated induction or application of acute stimuli, with end-point results achieved sometime after. Protein anabolism is achieved through the stimulation of protein synthesis and/or the inhibition of protein degradation. In this narrative review, we will focus predominantly on regulating muscle protein synthesis (MPS). MPS can be determined either by measuring the incorporation of infused amino acid stable isotopes into muscle by biopsy (Figure 2) and/or through measurements of the A-V difference of labeled as well as unlabeled amino acid across a sampled district, predominantly constituted by skeletal muscle, typically a limb (either the leg or the forearm)[34] (Figure 3).

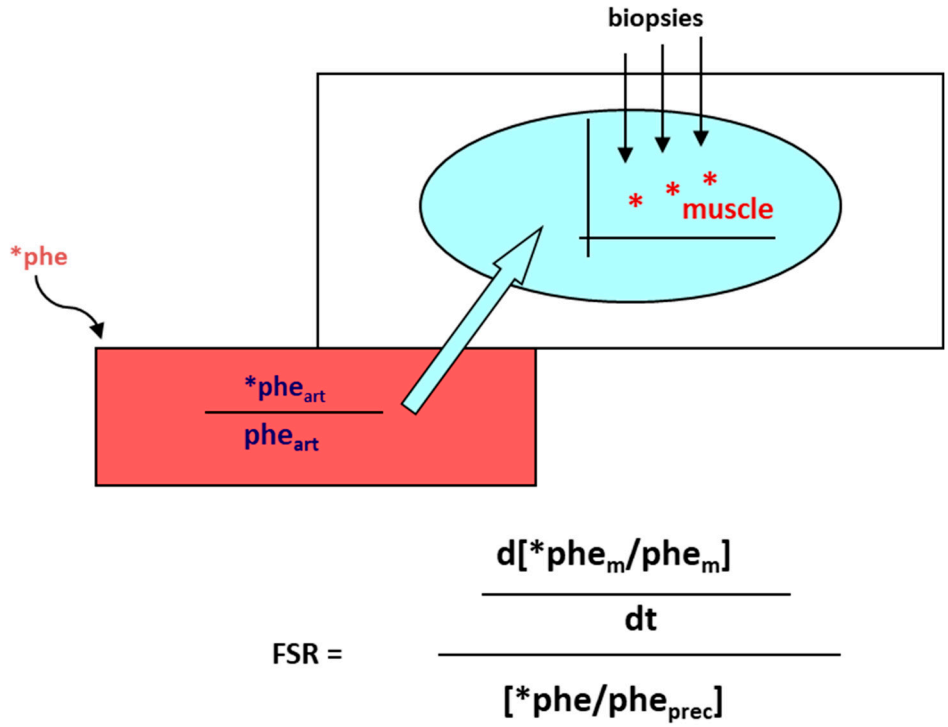

Figure 2: The figure schematically illustrates the methodology commonly employed to determine skeletal muscle protein synthesis with the combined use of amino acid tracer (in this example, phenylalanine) administration and its timed incorporation into muscle (measured by biopsy). The asterisks (*) indicates the labelled amino acid.

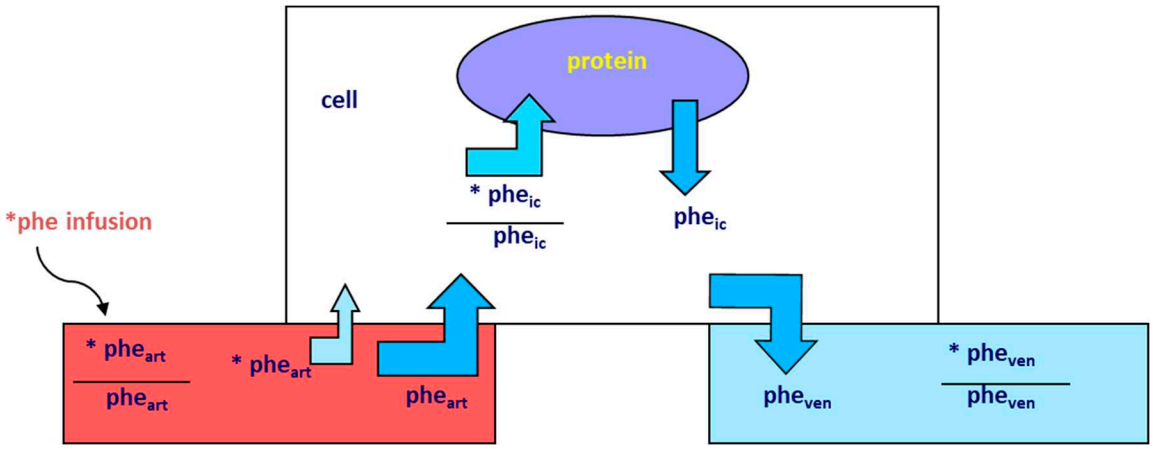

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Figure 3. Measurement of skeletal muscle protein synthesis and degradation with Arterial and Venous measurements combined with isotope infusion. The figure depicts the measurements performed in the artery and in the deep vein draining blood from the sampled muscular-rich district (i.e. the leg or the forearm). In this example, the indicator essential amino acid is phenylalanine (Phe), that is utilized by muscle only for protein synthesis, as well as it is released from muscle only from protein degradation. Asterisks (*) indicate the labelled amino acid.

2. EFFECTS OF SUBSTRATES ON SKELETAL MUSCLE PROTEIN SYNTHESIS

2a. Proteins and amino acids

Definitely, protein ingestion stimulates skeletal muscle protein synthesis (MPS) [35]. The stimulation's magnitude and duration depend both on the protein dose and its type/quality, which is closely associated with the concurrent post-ingestion rise in amino acid plasma concentrations [36,37]. Hyperaminoacidemia [38–40] specifically that of the essential amino acids (EAA) [41], among them of the branched chain ones and of leucine in particular [27,42,43]. Review largely condition tissue protein synthesis. Leucine is particularly important as a key metabolic regulator of MPS through activation of the mTOR pathway, and acutely enhances skeletal MPS both *in vitro* [42,44–47] (also above) and *in vivo* [48]. In addition to the effects of protein, other variables or factors, such as the coexistence of exercise, the pattern and/or the timing of protein administration, the age of subjects, the presence of comorbidities, etc, are important. Most reports have investigated the combined effect of protein and exercise. As an example, digiting in a PubMed search the string "Stimulation of muscle protein synthesis by protein ingestion" identified ≈600 papers.

Conversely, digiting "Stimulation of muscle protein synthesis by protein ingestion and exercise" identified ≈250 papers. Finally, the string "Stimulation of muscle protein synthesis by protein ingestion at rest" identified 60 papers. Therefore, although there is a large overlap among the selections, such an example simply underlines the predominant interest of combining protein nutrition with exercise.

In non-exercising young subjects (i.e., "at rest"), the acute administration of either a protein-containing mixed meal or a pure protein load, stimulated skeletal muscle protein synthesis [49,50]. In dose-response studies, intake of 20-g of high-quality proteins, i.e., whey protein [51] or mixed egg protein [52] was sufficient to maximally stimulate postabsorptive rates of myofibrillar MPS in resting young men over 4 hours [53]. However, in middle-aged men at rest following the ingestion of graded amounts of beef (from 57 g, i.e.,, 12 g protein; 113 g, i.e.,, 24 g protein, or 170 g, i.e., 36 g protein), the stimulation of myofibrillar MPS was the greatest with 170 g of beef [54], and apparently it did not achieve a plateau. Notably, in this study, exercise further and significantly enhanced the beef protein effect only at the highest administered dose (170 g beef). Similarly, following the administration to resting young volunteers, of a mixed meal containing both animal (beef) and vegetal proteins, at protein intakes of either 40 g or 70 g, the stimulation of skeletal muscle protein synthesis was similar at both doses, and also independent from prior resistance exercise, thus leading to the conclusion that ≈40 g mixed protein dose may attain the maximum effect on MPS [55]. The (marginal) inconsistency between the above-referenced studies could be due, besides to possible experimental variations, either to the type of the administered protein (pure whey protein, i.e., a high quality, fast absorbable protein, vs. either beef, or mixed animal and vegetal proteins), or to the complex protein matrix of "natural" proteins that could retain specific, yet unappreciated effects. The administration of free amino acids, either as a bolus ingestion of 15 g EAA [56], or of a leucine-rich EAA and carbohydrate mixture [57] increased human muscle protein synthesis. When the dose-response curves of crystalline EAA were constructed, the literature data somehow contrasted. It was initially reported that 2,5 g crystalline EAA were sufficient to elicit an increase above basal of MPS in young subjects [58]. However, in subsequent studies using intact whey protein, it was shown that the lower dose capable of eliciting a response in MPS in young muscle requires more than 10 g (=5 a EAA), and that it becomes saturated at 20-40 g EAA (Table 1).

Table 1. Protein (and) amino acid doses (in grams, g) that increase muscle protein synthesis (MPS) or Net balance (NB) in young adults.

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Type of food/protein/AA	**Subjects**	**Dose A**: Either the threshold or the lowest dose that increased MPS (or NB)	**Dose B**: Either the highest dose tested or that maximally stimulated MPS (or NB)	**Exercise status**	**Ref.**
Whey protein	Y (≈21 yr) males	>20 g	≈20-40 g	+Ex	[59]
Whey protein	Y (20-22 yr) males	10 g	40 g	-/+ Ex	[53]
Whey protein	Y adults	5-20 g	20 g	-Ex	[60]
Combined analysis of Whey Protein (n=5) and Egg (n=1) studies[1]	Y (22 yr) males	8 g	≈20 g	-/+ Ex	[61]
Milk protein + CHO	Y (27 yr)	15 g	45 g	+Ex	[62]
Milk protein concentrate	Y (22 yr) males	/	38 g	-Ex	[63]
Combined analysis of Whey Protein (n=5 studies) Egg (n=1 study)[2]	Y (22) males	8 g	≈20 g	-Ex	[61]
Egg protein	Y (22 yr) males	5-10 g	20 g	+Ex	[60]
Egg protein	Y males	5-10 g	20 g	+Ex	[52]
Whey protein Hydrolysate[3] (=AA)	Y (23 yr) males	≈10 g (as AA)	/	+Ex	[37]
Casein (micellar)[4]	Y (23 yr) males	≈10 g	/	+Ex	[37]
Soy Hydrolysate (=AA)[5]	Y (23 yr) males	≈10 g (as AA)	/	+Ex	[37]
Beef[6]	M males	>113 g	>170[7] g	-/+Ex	[64]

[1] Original data recalculated to body weight

[2] Original data recalculated to body weight

[3] That with the greatest stimulation of MPS in ref. (73)

[4] That with intermediate stimulation of MPS in ref. (73)

[5] That with the lowest stimulation of MPS in ref. (73)

[6] Beef contains ~22% of weight as protein.

[7] Further enhanced by exercise

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Mixed animal (beef) & vegetal protein	Y (≈30 yr) males	40 g	40-70 g	-/+Ex	[55]
Cristalline EAA	Y (34 yr)	15 g (as EAA)		-Ex	[56]
EAA+Leucine+CHO	Y (≈26 yr)	≈20 g (as EAA)[8]		-Ex	[65]
Mycoprotein concentrate[9]	Y (21 yr)	38 g		+Ex	[66]

Leucine, isoleucine and valine, i.e., the branched-chain amino acids (BCAA), account for about one-third of all amino acid residues in muscle protein, and have been extensively studied also for a direct regulatory role (due to leucine) on protein synthesis. Nevertheless, the demonstration of a clear cut effect of BCAA alone, on skeletal muscle hypertrophy (i.e., a long-term effect) in humans is not sound. In a meta-analysis, leucine supplementation was reported to increase the muscle protein fractional synthesis rate however without changing either body lean mass or leg lean mass [67]. The effect of glutamine on skeletal muscle in humans is uncertain and/or unwarranted [68–70]. Intravenous glutamine did not stimulate mixed muscle protein synthesis in healthy subjects [71].

The type of the administered protein is important too. Since the amount of the EAAs, and/or their relative proportions, are greater and/or more balanced in high- than in low-quality proteins, the amount and quality of the protein are relevant in the stimulation of protein synthesis. This issue is important in ageing, because the estimated recommended daily protein intake in aged people is ≈50% greater (1,2 g/kg BW) than that of young-adult subjects (0,8 g/kg BW) [72], and it could be better achieved by the intake of high quality protein, such as whey protein, albumin, egg, or, to a lesser extent, mixed milk protein, thus helping to maintain muscle mass and prevent sarcopenia.

Whey proteins (i.e., a soluble, fast-absorbable, high quality milk protein) has been largely used as a test protein. In resting young volunteers, the stimulation of MPS after consumption of 10 g EAA hydrolysate from whey protein was ≈90% greater than that with casein, and ≈20% greater than that with soy [73]. Although the effect of whey protein might be short-lived[74] because of its fast absorption and the transient rise in plasma/blood amino acid concentrations, the stimulation of muscle PS following the ingestion of isonitrogenous quantities (20 g) of either casein or whey WP was sustained over 4 hrs in both cases, that of WP however being 65 % higher [75]. Ingestion of a single dose of 38 g of mixed milk protein (i.e., including both fast- and slow-absorbable proteins) in young men, resulted in a time-dependent increase of postprandial muscle protein synthesis, detectable as soon as 60' after and lasting at least up to 5 hrs [76]. The ingestion of 30-40 g of a vegetal protein, mycoprotein, stimulated skeletal muscle protein synthesis to an extent comparable to that of an isonitrogenous omnivorous diet [66,77].

The pattern of protein administration may be important too. Diets are consumed as three main, bolus meals, plus occasional daily snacks. Alternatively, in some conditions and/or for experimental purposes, nutrition can be administered in a (sub)continuous pattern, i.e., at a near-constant rate over the day. In specific studies, the potential difference of the effects between these two patterns, concerning the protein-anabolic effects, has been investigated. Such a theoretical, experimental milieu, can be approached *in vivo* also by administering proteins with either a "fast" or "low" absorption pattern.

In young subjects, the total protein-anabolic effect of meal ingestion, measured using leucine tracers at the whole-body level, was more pronounced with a "slow" (i.e., casein) than with a "fast" (i.e., whey) protein administration [74,78]. The mechanisms leading to these effects were different,

[8] Published data are reported as 0.35 g/FFM (Lean body mass). Here they have been recalculated per mean subject assuming that FFM equals LBM (Lean Body Mass).

[9] Corresponding to a total of 70 g whole mycoprotein.

 doi:10.20944/preprints202308.0516.v1

too: the "fast" protein markedly stimulated amino acid oxidation and protein synthesis but did not change proteolysis, whereas the "slow" protein increased amino acid oxidation and protein synthesis to a lesser extent but strongly inhibited proteolysis.

Therefore, since the effects of whey proteins may be more rapidly vanishing, it would be useful to combine the effects of fast and slow proteins, irrespective of being vegetal or animal ones. In young adults, a supplement containing the vegetal, antioxidant-rich soy protein mixed with whey protein, could prolong the improvement of the AA net balance across the leg up to ≈2 h post-ingestion, compared with the 20 min attained with whey alone [79].

2b. Effects of exercise combined with nutrition on muscle protein synthesis and accretion

Exercise is a potent stimulator of muscle protein synthesis, particularly in the recovery phase [80], and it positively interacts with protein/amino acid ingestion in the stimulation of skeletal muscle anabolism. Most studies agree in reporting a powerful amplification by either protein or amino acid ingestion of the anabolic effects of concurrent exercise. An abundant supply of amino acids can also enhance amino acid transport and muscle protein synthesis following a leg resistance exercise [81].

Muscle mass accretion following resistance exercise combined with food ingestion is observed even following an adequate habitual protein intake (≥0.8 g kg^{-1} day^{-1}) [82]. A greater protein intake (1.8-3.0 g kg^{-1} day^{-1}) further augments lean body mass (i.e., protein) accretion without increasing fat mass, when compared to energy-rich, low protein intake (≈5% of energy as protein)[82]. The high-quality whey protein, combined with resistance exercise in young adults, exerted a greater effect on MPS than equivalent doses of lower-quality proteins, such as soy protein or casein, an effect, however still present up to 3-5 h post-exercise [52].

As anticipated above, a 20-g dose of whey protein was sufficient for the maximal stimulation of postabsorptive myofibrillar MPS in young men whether or not exercising [51], and it was effective up to 3-5 hr after exercise [73,83]. However, others reported that the response of muscle protein synthesis following whole-body resistance exercise is greater following 40 g than 20 g of ingested whey protein [59]. After ingesting mixed milk protein (0, 15 30, or 45 g) together with 45 g carbohydrate, the 30 g protein dose was sufficient to maximize the myosin synthesis rates during recovery from a single bout of endurance exercise in young men [62]. Therefore, a whey (milk) protein dose between 20 and 40 g seems sufficient for the maximal stimulation of MPS following exercise.

The intake of another high-quality protein, i.e., egg protein, as low as 5-10 g (approximating that contained in a single egg: ≈6.8 g) increased MPS above basal following resistance exercise, reaching a maximum with a dose of 20 g egg protein [60]. The greater potency of whey protein over other protein types is likely due to its nature of leucine-rich, high-quality protein, as well as to its rapid digestion and absorption, henceforth producing earlier and greater hyperaminoacidemia and hyperleucinemia [74,84].

The structure/form of nutrient intake (free AA vs intact protein) in respect of the timing of administration (either before, or 1 to 3 h after exercise) on the stimulation of skeletal muscle protein accretion, have been investigated too. While net amino acid uptake by skeletal muscle (measured by means of femoral arteriovenous sampling) was greater when free essential amino acids plus carbohydrates were ingested before than after resistance exercise [85],the ingestion of intact whey protein was similar irrespective of the administration time [86].

2c. Effect of other substrates

Protein synthesis is an energy-requiring process [87], therefore energy-providing substrates such as glucose and fat may affect protein turnover too. The activities of the cellular pathways controlling protein turnover are bioenergetically expensive and therefore depend on intracellular energy availability (i.e., macronutrient intake)[88].

i. **Glucose**

Glucose increased muscle protein synthesis *in vitro* [89]. Testing glucose-derived substrates separately, tissue ATP decreased during incubation with lactate and lactate+pyruvate supported protein synthesis better than pyruvate and as well as glucose. The data on the *in vivo* effects of either glucose or fat on protein metabolism, specifically on skeletal muscle, are scarce, complex and not univocal [39]. In neonatal pigs, raising glucose alone (while keeping insulin at baseline concentrations

 doi:10.20944/preprints202308.0516.v1

by means of the pancreatic clamp) increased protein synthesis in fast-twitch glycolytic muscles but not in other tissues [90]. However, enteral glucose administration did not affect either duodenal mucosal protein FSR or the activities of mucosal proteases in humans [91]. An oral glucose load in humans, and the simultaneous glucose-induced stimulation of insulin secretion, did not alter the rate of whole-body protein synthesis or breakdown [92]. Similarly, glucose ingestion added to a protein dose that maximally stimulates rates of MPS, despite greater insulin increments, did not show either additive or synergistic effects on the stimulation of MPS or the inhibition of muscle protein breakdown [93]. The co-ingestion of carbohydrates with protein did not further augment the post-exercise stimulation of muscle protein synthesis [94]. Oral glucose supplementation did not affect whole-body protein synthesis or breakdown in humans adapted to a low-protein diet, whereas it decreased leucine oxidation only in the high-protein-adapted group [79]. In humans, the acute intravenous glucose infusion decreased whole-body protein degradation, an effect possibly mediated by insulin [95]. However, animal studies support the view that glucose availability spares essential amino acids at least in the fetus. Conversely, however, the effects of hypercaloric refeeding with high-carbohydrate diets may result in increased protein turnover [49]. Hyperglycemia did not inhibit whole-body protein degradation in humans [96]. To our knowledge no data are available on the direct effects of glucose per se on skeletal muscle protein synthesis in humans. The co-ingestion of carbohydrates with protein did not further increase the post-exercise stimulation of muscle protein synthesis [94].

However, diet-induced modulation of intramuscular carbohydrate (i.e., glycogen) availability affected skeletal muscle and whole-body protein synthesis, degradation, and net balance during prolonged exercise in humans [97]. Skeletal muscle CHO stores were reduced by previous exercise in the Low-CHO group, but were replenished with a High-CHO diet for 2 days. The net leg protein balance was decreased in the Low-CHO group compared with both the pre-exercise rest and the High-CHO condition, primarily due to increased protein degradation and decreased protein synthesis late in exercise. Whole-body leucine oxidation increased above rest in the Low-CHO group only, and was higher than in the H-CHO group. Whole-body net protein balance was reduced in the Low-CHO group, largely due to decreased general protein synthesis. Overall, these observations suggest an effect of a High-CHO diet to improve the net protein balance in skeletal muscle undergoing exercise. However, it is important to consider that the exact dietary requirements for optimal muscle protein synthesis may depend on various factors such as age, sex, exercise intensity, and overall diet composition.

ii. **Lipids and ketones**

The effects of either lipid ketone infusion/administration in humans are complex. Lipid infusion in humans did not affect proteolysis [98]. In contrast, medium-chain fatty acid infusion apparently increased leucine oxidation, therefore net protein catabolism [99]. Thus, the effects on whole-body protein degradation may depend of the fatty acid length [100]. The increase of FFA decreased basal muscle protein synthesis, but not the anabolic effect of leucine [101]. Conversely, suppressing lipolysis by nicotinic acid increases protein degradation and leucine carbon oxidation in the dog, thus showing a net increase of protein catabolism [102]. When associated to dietary protein ingestion, neither acute nor short-term dietary fat overload impaired the skeletal MPS in overweight/obese men in the post-prandial phase, thus excluding a role by dietary accumulation of intramuscular lipids on the anabolic response [103]. The infusion of 3OHButyrate decreased both whole body and forearm protein turnover (using phenylalanine/tyrosine tracers), as well as phenylalanine catabolism, in post-absorptive conditions, whereas it did not modify the insulin-induced effects following an euglycemic clamp [98]. In another study, 3OHButyrate infusion did not change whole-body leucine turnover, increase leucine oxidation, or change the non- oxidative portion of leucine disposal. However, muscle PS increased, demonstrating an anabolic effect in skeletal muscle [104]. The leucine metabolite β-hydroxyl, β-methylbutyrate (HMB) was recently identified as a potentially anabolic substrate [105]. Using combined stable isotope amino acid infusion, isotope incorporation into muscle myofibrils and arterial-venous femoral sampling in humans, oral administration of 2.42 g of pure HMB increased muscle protein synthesis by ≈70%. In contrast, it decreased muscle protein degradation by ≈60%, in

 doi:10.20944/preprints202308.0516.v1

an insulin-independent manner, whereas an oral 3.42 g leucine load increased muscle protein synthesis by ≈110% [106]. HMB prevents skeletal muscle atrophy [107] and increases protein synthesis [108] through the activation of the mammalian target of rapamycin complex 1 (mTORC1) in the rat.

iii. **Other nutritional interventions**

β-alanine supplementation may increase the physical performance in middle-aged individuals [109] and improve physical performance during exercise [110]. Creatine may increase muscle mass in combination with resistance exercise, although the mechanism(s) of action remain elusive. Short-term creatine monohydrate (CrM) supplementation may exert anti-catabolic actions on selected proteins in men, but did not enhance either whole-body or mixed-muscle protein synthesis [111]. Acute metabolic studies may prove useful information for estimating the efficacy of anabolic agents [112] .

3. HORMONES AND RELATED DRUG INTERVENTIONS

3a. Insulin*:* Although insulin has an undisputed anabolic effect on tissue protein, its experimental demonstration in vivo has challenged the investigators over time, mainly because its administration induces a decrease of amino acid plasma concentration, thus obscuring its direct effect. A major advancement was the maintenance of the "amino acid "clamp" at baseline during insulin infusion or injection, also achieved when insulin was directly infused into the artery perfusing a muscle-rich organ, such as the leg or the forearm, thus inducing no system perturbation of the aminoacidemia and allowing, at the same time, to study selectively the insulin effect in the perfused limb. Using any of these techniques. Muscle protein synthesis and degradation were determined by combining amino acid isotope infusion with arterial-venous limb catheterization, often complemented by muscle biopsy in some studies. A systemic insulin infusion, with no prevention of hypoaminoacidemia, suppressed whole-body protein degradation [113,114], improved limb (either the forearm or the leg) net protein balance and the disposal of the amino acid tracer for protein synthesis. However, when insulin-induced hypoaminocidemia was prevented, insulin was shown to stimulate muscle protein synthesis [102, 103,104]. In addition, insulin enhanced the amino acid-induced stimulation of protein synthesis. In summary, the protein anabolic effect of insulin in skeletal muscle requires sufficient plasma amino acid levels, a condition that is physiologically attained following meal ingestion.

3b. Glucocorticoids: Cortisol, often referred to as a stress hormone, has both catabolic and anabolic effects, depending on the context. Physiologic cortisol concentrations and/or exposure stimulate whole-body protein breakdown and inhibits human protein synthesis [105,106]. Prednisone induces a negative whole-body protein balance by increasing protein degradation, an effect that is antagonized by growth hormone [118]. Chronic glucocorticoid treatment induces loss of lean body mass by decreasing protein synthesis and increasing degradation in skeletal muscle [119]. In humans administration of 8 mg dexamethasone daily for 4 days antagonized the anti-proteolytic effect of insulin in the forearm [120].

3c. Human Growth Hormone (hGH) and IGF-1: hGH administration increased the whole body [118] and muscle protein synthesis [121]. The insulin-like growth factor-binding proteins (IGFBPs) bind to IGFs, modulating their activity and availability. They help regulate IGFs' actions in various tissues, including muscle. When rhIGF-I was infused at a rate achieving plasma IGF-I concentrations close to those observed following rhGH treatment and yet avoiding the IGF-1 induced hypoglycemia, proteolysis and protein synthesis were not affected, even in the presence of prednisone treatment [122] However, when rhGH and rhIGF-I were administered simultaneously, nitrogen balance was remarkably improved. Thus, the exact anabolic effect of combined rhGH and rhIGF administration remains to be fully elucidated.

3d. Catecholamines: Epinephrine has both a α– and β-receptor affinity. Epinephrine infusion in humans depressed plasma amino acid concentrations, particularly of the essential ones, however without changing leucine or phenylalaine flux [123], nor it impaired the disposal of exogenous amino acids in humans [124]. However, in another study the increase of plasma epinephrine concentrations

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inhibited proteolysis and leucine oxidation in man via beta-adrenergic mechanism [125]. In the human forearm, physiological epinephrine exerted an anticatabolic action on muscle protein, however, with unknown mechanism(s) [126]. The β-agonist clenbuterol may exert anabolic effects in skeletal muscle [127]. However, its use in humans is hampered by unacceptable largely because of cardiovascular side effects [128].

3e. Estrogens: Although estrogens are female sex hormones, they are also present in smaller amounts in males. Estrogen plays a role in maintaining bone health, promoting protein synthesis and muscle growth, and influencing body composition, in both males and females. While the exact mechanisms by which estrogens affect muscle growth are still being studied, research suggests that they can, directly and indirectly, affect muscle tissue. One way estrogens may influence muscle growth is by promoting protein synthesis., interacting with receptors in muscle cells and activating signaling pathways involved in protein synthesis [129]. Decreased estrogen-associate signaling impairs mitochondrial function leading to muscle atrophy [130]. Estrogens can also indirectly affect muscle growth by modulating the production and activity of other hormones, such as growth hormone and insulin-like growth factor 1 (IGF-1), which are important for muscle development. Estrogens may regulate the release of these hormones from the pituitary gland and the liver, thereby influencing muscle growth [131]. Moreover, estrogens can influence muscle mass indirectly through their impact on body composition. Estrogens regulate fat distribution, and higher body fat levels have been associated with lower muscle mass. By influencing body composition, estrogens can indirectly affect muscle growth and maintenance [129]. Estrogen decreases in menopause may contribute to accelerated muscle loss and sarcopenia in females [132–134]. It's important to note that the role of estrogens in muscle growth is complex and can vary depending on factors such as age, sex, hormone levels, and other individual characteristics. Additionally, the research on this topic is still evolving, and different studies may have differing findings and conclusions.

3f. Androgens. Testosterone is a potent anabolic stimulus primarily through improvement in the re-utilization of amino acids released from protein degradation [135], and it will be further discussed carefully. Testosterone and progesterone, but not estradiol, stimulated muscle protein synthesis in postmenopausal women [136].

4. EXERCISE

Exercise is a powerful stimulus to promote skeletal muscle protein synthesis and net protein anabolism, involving specific metabolic and morphological adaptations in muscle [137]. Exercise produces diverse changes in amino acid metabolism and protein turnover in muscle, according to the exercise phase. Acute changes in amino acid metabolism during exercise are primarily catabolic (i.e., increased amino acid oxidation), yet exercise does not cause muscle wasting. This is because both immediate and later post-exercise phases are anabolic. Thus, regular exercise is essential for optimizing muscle growth and hypertrophy.

The type of exercise also determines the magnitudes of these processes, and exercise requires a sequence of metabolic adjustments from the catabolic period of the on-going exercise to the anabolic period of recovery. Two primary exercise types commonly associated with muscle growth are resistance and endurance. Resistance Exercise, such as weightlifting, involves using external resistance to challenge the muscles. This type of exercise is a primary intervention used to develop strength and stimulate muscle hypertrophy. Muscle mass increases constitute key components of conditioning in the outcome of various sports due to the correlation between cross-sectional muscle area and muscle strength [138,139].

Resistance exercise can be further classified into two main categories:

High-Intensity Resistance Exercise: this exercise type typically involves lifting heavy weights for a relatively low number of repetitions. It primarily targets fast-twitch muscle fibers, which have a higher potential for muscle growth. High-intensity resistance exercise promotes muscle hypertrophy by acutely causing mechanical tension and muscle damage, subsequently triggering muscle protein synthesis and adaptation [140]. High-intensity resistance exercise is a potent stimulus for skeletal

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MPS and hypertrophy in young adults [141–146] during high-intensity exercise and post-exercise recovery.

Moderate-Intensity Resistance Exercise: this exercise type involves using moderate weights for more repetitions. While the hypertrophic response may be less pronounced than high-intensity resistance exercise, moderate-intensity resistance exercise still contributes to muscle growth and can benefit endurance and functional capacity [140].

Endurance exercise, or aerobic exercise, involves continuous rhythmic movements that challenge the cardiovascular system and improve endurance. Endurance exercises include running, cycling, swimming, and brisk walking. While endurance exercise primarily focuses on cardiovascular fitness and endurance, it can also impact muscle growth, particularly in prolonged training. Endurance exercise can enhance the oxidative capacity of muscles, improve energy efficiency, and increase the density of blood vessels within the muscle tissue. These changes can contribute to improved muscle function and endurance, but the hypertrophic response regarding muscle size may be relatively limited compared to resistance exercise [147].

4a. Effects of exercise in conjunction with nutrient intake

The specific adaptation to each type of exercise can vary depending on factors such as exercise intensity, volume, frequency, and individual characteristics. Combining resistance and endurance exercises in a well-designed training program can provide comprehensive benefits for muscle growth, strength, endurance, and overall fitness. It has been shown that combined strength and endurance training in the evening may lead to larger gains in muscle mass [148]. Additionally, other factors such as nutrition, rest, and recovery play crucial roles in maximizing the benefits of exercise and promoting muscle growth. Adequate protein intake, overall caloric balance, and appropriate recovery periods are important factors to be considered when optimizing muscle growth in response to exercise. During the acute phase, the energy needs to stimulate amino acid oxidation/catabolism (together with that of glucose and fat), thus depleting intracellular amino acid pools.

In contrast, in the recovery phase, the depleted amino acid pools and energy need to be reconstituted. Since the response of muscle protein metabolism to a resistance exercise bout lasts up to 24-48 hours or more [52], such an ample post-exercise recovery phase allows a comfortable, positive anabolic interaction with food (protein) intake. Immediately following exercise, muscle protein turnover, i.e., protein synthesis, breakdown, and amino acid transport, are accelerated. Nevertheless, the net protein balance remains negative (i.e., catabolic or not above zero) unless food is ingested [81]. Therefore, food intake associated with exercise is necessary to bring muscle protein balance to be positive. Nevertheless, as it is commonly experienced, it generally takes weeks to months before training-induced changes in skeletal muscle mass become apparent. The prolonged time course for hypertrophy reflects the slow turnover rate of muscle proteins, which is about 1% per day for contractile proteins [149].

The molecular mechanism driving the anabolic effect of exercise in muscle recognizes the mammalian target of rapamycin complex 1 (mTORC1) activation as a central, although not exclusive, mechanism regulating muscle cell size and growth [150–152]. The same mTORC1 mechanism stimulates human skeletal muscle protein synthesis by essential amino acids [153]. Provision of amino acids, whether in free form [154–157] or as intact protein [158], in association with resistance exercise, increases muscle protein synthesis and net protein accretion resulting in a positive net muscle protein balance.

While dietary protein supplementation can augment the effects of resistance exercise on the increase of skeletal muscle mass and strength, it also can preserve skeletal muscle mass during periods of diet-induced energy restriction [159]. The time relationships between nutrient intake and exercise have been the object of detailed investigations.

Both pre- and/or post-exercise nutritional interventions (i.e., by carbohydrate + protein or protein alone) effectively increase body strength and improve body composition [160]. However, the size and timing of a pre-exercise meal may also affect the delay when the post-exercise protein feeding is optimally required. The post-exercise ingestion (from immediate

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to 2-h post) of high-quality protein sources stimulates robust increases in MPS. A mixed meal intake immediately after resistance exercise may effectively suppress MPB in the morning in young volunteers [161]

Without exercise, meal timing and frequency variations exhibited little effect on body composition and body weight, whereas meal frequency can favorably improve appetite and satiety.

4b. Exercise-insulin interaction

Insulin and exercise can positively interact in the stimulation of protein anabolism, in a complex fashion. Both protein synthesis and degradation rates are ≥1-fold greater following the post-exercise recovery than at rest [162]. The effects of insulin on muscle protein synthesis and degradation, either without or with exercise, are complex. Insulin-stimulated protein synthesis at rest but not in the recovery post-exercise phase. In contrast, insulin decreased protein degradation following exercise as opposed to no effect at rest. Thus, the post-exercise phase can be viewed as a (transient) insulin-resistant condition as regards protein synthesis that, in terms of the effects on net protein balance, was overwhelmed by a greater insulin-mediated suppression of proteolysis.

PART 2. THE PATHOPHYSIOLOGY OF SARCOPENIA IN AGEING: ROLE OF NUTRITION, EXERCISE, AND OTHER FACTORS

The study of the mechanism(s) leading to ageing sarcopenia captures a fast-growing interest in both the basic and clinical-experimental sciences. While basic sciences rely on a variety of in-depth molecular, *in vitro* and *in vivo* approaches, *in vivo* human studies are unique in that they translate the investigation into at the human model, in turn hampered by objective ethical/technical/investigational limitations, as outlined above (Table 1).

1. THE MOLECULAR MECHANISMS BEHIND ANABOLIC RESISTANCE WITH AGEING

Anabolic resistance refers to the reduced ability of skeletal muscle tissue to respond to common anabolic stimuli, such as dietary protein and exercise, by increasing muscle protein synthesis rates (MPS). In healthy individuals aged ~18–50 who are not sedentary and eat sufficient daily amounts of protein and energy, skeletal muscle mass remains relatively unchanged throughout daily life [163].

1a. Intracellular signaling.

mTOR kinase: Basal mTOR activation is elevated during ageing and may contribute to anabolic resistance disrupting metabolic signaling pathways [164]. Markofki and colleagues aimed to investigate the effect of age on basal muscle protein synthesis and the signaling pathways involved in muscle protein synthesis, specifically the mechanistic target of the rapamycin complex 1 (mTORC1) pathway. They recruited a large cohort of young (18-30y) and older (65-80 y) men and women to assess potential age-related differences and employed stable isotope tracer techniques and muscle biopsies to measure muscle protein synthesis rates and examine mTORC1 signaling at the basal level and in response to feeding. The study's findings demonstrated that muscle protein synthesis rates were lower in older than young individuals. Additionally, the basal activation of the mTORC1 was higher but attenuated in the older group in response to feeding. These findings suggest that age-related declines in muscle protein synthesis may be partly attributed to impairments in the mTORC1 signaling pathway. Moreover, there might be age-related differences in mTOR signaling in response to resistance exercise. Compared to their younger counterparts, older individuals experience delayed phosphorylation of mTORC1 up to 24 hours after exercise and food intake, which is necessary for promoting muscle protein synthesis [165,166].

AKT kinase: Studies suggest that anabolic resistance in ageing muscle may involve dysregulation of Akt signaling and downstream pathways [167]. Some key points regarding Akt and anabolic resistance in older people include impaired Akt activation, blunted protein synthesis response, and insulin resistance [168]. Studies have shown reduced Akt activation in response to anabolic stimuli such as resistance exercise and nutrient intake in older adults compared to younger individuals [165]. Anabolic resistance in ageing muscle is characterized by a diminished muscle

 doi:10.20944/preprints202308.0516.v1

protein synthesis response to anabolic stimuli, which can be attributed, at least in part, to impaired Akt signaling. Age-related insulin resistance can impair Akt signaling and anabolic resistance in skeletal muscle [169].

Extracellular signaling.

2a. IGF-1: The role of IGF-1 in anabolic resistance, particularly in ageing and age-related muscle loss, is a topic of ongoing research. While IGF-1 is typically associated with anabolic processes and muscle growth, anabolic resistance refers to the reduced responsiveness of skeletal muscles to anabolic stimuli, leading to impaired muscle protein synthesis and loss. Ageing is associated with a decline in circulating IGF-1 levels, which can contribute to anabolic resistance. Reduced IGF-1 availability may impair the anabolic response to exercise and nutrient intake [145]. Anabolic resistance in older adults may involve dysregulation of the IGF-1 signaling pathway, including reduced activation of the IGF-1 receptor and downstream signaling molecules, such as the Akt/mTOR pathway, leading to diminished muscle protein synthesis [170]. Insulin resistance, commonly observed in older adults, can impact IGF-1 signaling and contribute to anabolic resistance [171].

3a. GH: Several studies have explored the potential role of GH in anabolic resistance, shedding light on its potential role in combating age-related muscle loss. One review by Velloso (2008) highlighted the interactions between GH, insulin-like growth factor 1 (IGF-1), and muscle mass regulation proposing that GH may play a role in anabolic resistance and the decline in muscle mass observed with ageing [172]. Older men receiving rhGH (recombinant GH) demonstrated greater muscle strength and size gains than in the placebo group. The findings suggest that rhGH supplementation in combination with resistance exercise may have potential benefits for improving muscle strength in elderly individuals [173,174]. In a study by Cuneo et al. (1991), GH treatment was administered to elderly individuals to investigate its effects on skeletal muscle found that GH supplementation improved muscle protein synthesis and increased lean body mass, indicating a potential role for GH in overcoming anabolic resistance in older people [175]. Finally, GH treatment for 10 yr in GHD adults resulted in increased lean body and muscle mass, a less atherogenic lipid profile, reduced carotid intima-media thickness, and improved psychological well-being [176]. Clinical trials investigating the efficacy of GH for sarcopenia have produced mixed findings. While some studies have shown positive effects on muscle mass and function [177], others have yielded limited or inconsistent results. Safety concerns associated with GH supplementation also contribute to its limited use. Potential side effects such as fluid retention, joint pain, insulin resistance, and increased risk of diseases like diabetes and cardiovascular disorders raise caution about the long-term use of GH, particularly in older populations [178]. The cost and accessibility of recombinant GH pose additional challenges. GH therapy is an expensive intervention, and its high cost may limit its widespread use in clinical settings, making it less feasible as a routine treatment option for preventing or curing sarcopenia [179]. In conclusion, while recombinant GH has shown potential benefits for sarcopenia in some studies, its use in clinical practice for this condition is limited. Mixed findings, safety concerns, cost considerations, and the lack of consensus guidelines contribute to the limited use of GH in sarcopenia management. As a result, a comprehensive approach that addresses multiple aspects of sarcopenia is preferred. Future research may provide further insights into GH therapy's potential benefits and safety profile for sarcopenia.

4a Anabolic Steroids: Several studies have investigated the relationship between testosterone and anabolic resistance. Bhasin et al. (2003) examined the impact of testosterone supplementation on muscle protein synthesis in older men and found that testosterone administration effectively restored muscle protein synthesis rates in older individuals, overcoming the anabolic resistance typically observed with ageing [180]. In another study, older men with low testosterone levels who received testosterone replacement therapy showed significant improvement in muscle protein synthesis rates after resistance exercise [181]. Another study investigated the effects of short-term testosterone administration on skeletal muscle protein synthesis in older men with low testosterone levels. They

 doi:10.20944/preprints202308.0516.v1

reported that testosterone replacement therapy improved muscle protein synthesis rates, indicating that testosterone plays a crucial role in overcoming anabolic resistance in ageing muscle; testosterone coupled with resistance exercise is an effective short-term intervention to improve muscle mass/function in older non-hypogonadal men [181]. Collectively, these studies highlight the role of testosterone in combating anabolic resistance. Testosterone supplementation in individuals with low testosterone levels can restore muscle protein synthesis rates and enhance the anabolic response to exercise, counteracting the detrimental effects of anabolic resistance. It is important to note that anabolic resistance is a complex phenomenon influenced by various factors, and testosterone is just one aspect of the overall picture. Other factors, such as nutritional status, physical activity levels, and other hormonal interactions, contribute to anabolic resistance. Therefore, a comprehensive approach that addresses multiple factors is necessary to manage individuals' anabolic resistance effectively.

2: ANABOLIC RESISTANCE IN AGEING: HUMAN STUDIES

Elderly subjects exhibit many abnormalities in protein metabolism in respect to younger subjects, here concisely summarized:

1. An increased splanchnic "trapping" of the ingested substrates, henceforth reducing amino acid delivery to peripheral tissues, such as skeletal muscle;
2. A decreased amino acid utilization by muscle, and/or the requirement for a greater AA load/delivery to stimulate appropriately PS in muscle, compatible with an *anabolic-resistant* state. In other words, the skeletal muscle in ageing might be less sensitive to lower (normal) levels of amino acids than that in young adults, and may thus require more protein to acutely stimulate muscle protein synthesis above rest, to achieve the required accretion of muscle proteins;
3. A decreased energy production otherwise required to sustain the energy-expensive PS;
4. Altered protein digestion;
5. A decreases transluminal AA transport;
6. An intestinal microbiota different from that of younger people;

Any of the potential factors could explain why elderly people would require, and/or are recommended to assume, at least ≈50% more protein than either young or mature subjects [72]. In the following sections we report literature data about protein turnover in ageing, both in basal, "post-absorptive" conditions, and following nutrition, exercise and response to hormones.

2a. Basal skeletal muscle protein turnover in ageing

It was initially reported that muscle protein breakdown is elevated, by as much as ≈50%, in the elderly compared to younger adults [182] and that muscle net protein balance is negative [183]. Similarly, a decrease in the fractional synthesis rate (FSR) of skeletal muscle proteins was initially reported in elderly subjects [184–190]. A -20-30% decrease in myosin heavy chain (MHC) FSR was observed even in middle age [191]. In respect to MHC isoform expression, that of MHC-1 did not change with age, whereas the expression of MHC-IIa isoform was decreased by ≈35% in middle age (≈54 yrs) as compared to young subjects, further decreasing (by ≈50%) at older ages [191]. A ≈50% reduction of mitochondrial protein FSR was also demonstrated in middle age, not further decreasing at ≈75 yrs. In addition, a decrease of the activities of mitochondrial enzymes was observed in muscle homogenates from aged people, consistent with a decreased muscle oxidative capacity [186]. Mitochondrial ATP production measured using various substrate combinations was also lower in the elderly than in young subjects [192]. Moreover, genes and proteins related to mitochondrial shaping proteins decreased in sedentary elderly [31]. Pooled data from ≈150 healthy subjects of both sexes aged 18–89 yrs demonstrated a progressive decrease of mitochondrial DNA and mRNA abundance and mitochondrial ATP production with advancing age [190].

However, at variance with these earlier reports, more recent studies consistently failed to confirm the earlier findings of decreased basal muscle protein synthesis in elderly subjects, showing little or no differences between young and old adults [58,193–198]. These opposite, contrasting results could have been due to several previously unaccounted factors, such as the experimental methods themselves, the sample populations studied (particularly regarding the general health status, diet, habitual physical activity, or else), or other unappreciated causes, underlying the complexity, as well as the limitations, of the *in vivo* investigations. On the other hand, should basal daily muscle protein synthesis rates in the aged subjects be lower (by ≈20–30% or more), than that of younger people as

 doi:10.20944/preprints202308.0516.v1

originally reported [199], these figures would end up in a more marked, unrealistically muscle wasting than what is typically observed (estimated as 0.5-1.5% per year between 50-80 yr old subjects, or 3–8% per decade), as well as into a complete muscle loss [200], or even into *negative* numbers, when projected over years.

It should, however be recognized that basal muscle protein turnover could particularly be altered in frail, elderly subjects, being potentially associated also to a chronic, subtle, systemic inflammatory state and/or to other co-morbidities [201,202]. Indeed, inflammatory mediators, such as cytokines, particularly TNFα, may impair skeletal muscle protein FSR, by interfering with the phosphorylation of the mammalian target of the rapamycin (mTOR) pathway [203], critically involved in the regulation of mRNA translation, muscle protein synthesis and growth [204]. Therefore, it is currently accepted that basal skeletal muscle protein turnover is near-normal in healthy elderly subjects. In contrast, a different, complex picture can emerge when studying the response in ageing of skeletal muscle protein turnover to anabolic stimuli, such as substrates (i.e., mixed meals, protein, amino acid mixtures, other metabolites), exercise, anabolic hormones, or their combinations.

2b. Skeletal muscle protein turnover in ageing in response to nutrition and exercise

Utilization of oral feeding in the elderly

Elderly subjects exhibit some peculiarities in the handling of oral feeding, using the essential amino acid leucine as tracer, a *greater first-pass splanchnic uptake of ingested amino acid(s*) has been reported in elderly than in young controls, suggesting that a lower proportion of ingested amino acid reached the peripheral circulation [196]. Such a greater splanchnic extraction could limit the amino acid-mediated stimulation of muscle protein synthesis in peripheral tissues such as skeletal muscle. Sustaining splanchnic protein synthesis could indicate a "metabolic priority" during recovery from a metabolic stress in healthy elderly persons. Such a mechanism might become more relevant in older individuals suffering from chronic diseases and/or subjected to poly-medications [205]. In contrast with the above report however,, it has also been reported that oral amino acids stimulated muscle protein anabolism in the elderly, despite the higher first-pass splanchnic extraction [206].

A *protein pulse* rather than a *spread (or continued)* oral feeding stimulated at best protein accretion in the elderly [207], but not in young women, in whom the protein feeding pattern did not affect protein accretion . Also in older men, the rapidly-absorbed, whey protein stimulated postprandial muscle protein accretion more efficiently than the "slow protein" casein hydrolysate [208]. However, the whey protein effect was superior to that of casein hydrolysate too, likely due to the combination of the whey protein's fast digestion and absorption with its greater leucine content. Conversely, in younger subjects, a slowly-digested protein (casein) achieves a better anabolic effect than a rapidly-digested one [209].

Thus, fast-absorbable protein (or protein supplements), rich in well-balanced EAA, could be ideal and specific in the nutrition of aged people [210,211]. The cooking mode is also important. Well-done, more digestible meat is assimilated better than trare meat in the elderly [212].

Other protein types, such as the vegetal wheat protein (administered in the 35 g dose) increased muscle protein synthesis rates in healthy older men nearly as much as 35 g whey protein hydrolysate over 2-4 hrs [213].

Generally speaking, dietary protein supplementation can augment resistance exercise-mediated gains in skeletal muscle mass and strength and can preserve skeletal muscle mass during periods of diet-induced energy restriction [214].

Conversely, others did not show an additional effect of protein, added to that of exercise itself, on skeletal muscle hypertrophy. Verdijk et al. compared increases in skeletal muscle mass and strength following 3 months of resistance exercise training with or without protein ingestion prior to and immediately after each exercise session in elderly males who habitually consumed about 1.0 g protein/kg per day [215]. Timed protein supplementation prior to and after each exercise bout did not further increase skeletal muscle hypertrophy

2c. Anabolic response in skeletal muscle of aged people:

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The studies aiming at detecting the existence of resistance to anabolic stimuli in ageing (i.e., of "anabolic resistance") are complex and sometimes inconclusive, also due to the variability of the experimental setting. Several factors are to be considered. such as the amount, the quality, the administration pattern of the protein, the energy of the meal, etc. The same considerations should apply to amino acid mixtures. When exercise is combined with nutrition, the anabolic response can vary in intensity, timing in food administration, whether applied to the whole body or to a single leg, etc. Therefore, any conclusion drawn from individual studies should be balanced considering many concurrent variables.

A bout of aerobic exercise increased the anabolic effect of nutrient intake in older adults [216], also through an exercise-induced augmentation of the nutrient-stimulated vasodilation, resulting in an increased nutrient delivery to muscle, rather than improved insulin signaling.

Data consistent with a normal anabolic response (= no resistance)

Several studies reported a normal response of MPS to oral administration of either protein or free amino acids in the elderly. In a dose-response study performed by feeding frequent small boluses of liquid meals to healthy >60 yr old subjects, Welle and Thornton [217] reported that a comparable stimulation of MPS was attained independently from the protein loads as well as the protein contribution to total energy i.e., from low (7% corresponding to 0.6 g protein/kg/day), to moderate (14% total energy, i.e., 1.2 g protein/kg/day) or to high (28% total energy, i.e., 2.4 g protein /kg/day). They concluded that in older subjects, there was no dose-dependent effect of the amount of protein intake on MPS; therefore, the "lower" protein dose was sufficient to stimulate MPS maximally.

The lower dose tested in this study was comparable to that found in young subjects of other studies (Table 2)

Table 2. Effects of protein-rich food ingestion on Muscle Protein Synthesis (MPS) or Net Balance (NB). According to the study type, the data are grouped as: Dose-response studies in the elderly (n = 1 study). Sarcopenic vs healthy elderly subjects (n=1 study). Studies reporting similar responses in old and young subjects (=no anabolic resistance) (n = 7 studies). Studies reported a decreased/blunted/delayed response (=anabolic resistance) in old subjects compared to young controls (n=10 studies).

Type of food/protein tested	Subjects	Dose A: Either the threshold or the lowest dose that increased MPS (or NB)	Dose B: Either the highest dose tested or that maximal-ly stimulated MPS (or NB)	Exercise status	Comment	Reference
Dose-response study						
Liquid meals[10]	Healthy (62-75 yr) males and females	29 g	115 g	-/+Ex	Max effect obtained at the lowest dose. MPS increase greater after exercise.	[217]
Sarcopenic vs healthy elderly						
Leucine-enriched whey protein	Sarcopenic (81 yr) men Healthy (69 yr) men	21 g	/	-Ex	Similar increase in MPS in both groups	[218]
Similar response to controls (=no resistance)						

[10] Recalculated from original data to weight and total protein intake over the test.

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Whey protein isolate	Old (71 yr) males	10 g	40 g	-/+Ex	Exercise enhances the maximal effect at 40 g protein	[219]
Beef[11]	O (68-70 yr) vs Y (34-41 yr)	113 g	340 g	-Ex		[220,221]
CHO + WP +Leu[12]	O (75 yr) vs Y (20 yr) males	/	72 g WP+13,5 g Leu	+Ex	30 min of moderate-intensity physical activity	[222]
EAA drink	Elderly (67 yrs) males and females	15 g	/	-Ex	However, retarded albeit still sustained response in elderly , as opposed to a faster, short-lived one of the young over 3 hrs	[56]
Mixed animal (beef) and vegetal foods	Elderly (69 yrs) males and females	35 g protein	70 g protein	-Ex	≈5x greater response of MPS with the higher protein dose. However at the 35 g dose the response in O was > 5x lower than that in Y (see below)	[223]
Mixed animal (beef) and vegetal foods	Y (31 yr) males and females	40-44 g protein	66-70 g prot	-/+Ex	However at the 36 g dose the response in O was > 5x less than that in Y (see below)	[55]
Protein and CHO	O (75 yr) males / Y (21 yr) males	20 g		-Ex		[224]
Decreased/Blunted/Delayed response (=anabolic resistance)						
Combined analysis of Whey Protein (n=5 studies) Egg (n=1 study)[13]	Elderly (≈71 yr) men	8 g	≈32 g	-Ex	Delayed response in the older group	[61]
	Young (≈22 yr) men	8 g	≈20g	-Ex		
Intact whey protein	O (≥70 yr)	5-20 g	≥20-40 g	-/+Ex	In the resting elderly, the response of MPS plateaus at 20 g,	[60,219]
	Y (>23 yr)	5-20 g	20 g	-/+Ex		
Crystalline EAA	Y (28) vs	2.5 g (?)	20-40 g[15]	-/+Ex		[219,225]

[11] Beef contains -22% of weight as protein.

[12] Data recalculated for the subjects' average weight (75 kg). The table report the total of the EAA+Leu administered, that were fractionated in 6 doses every hour

[13] Original data recalculated to body weight

[15] The highest dose (40 g) was tested only in the older group.

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	O (70 yr)[14]				at a lower value than in Y (40 g). Resistance exercise can increase MPS only in O but at greater protein intake.	
EAA+Leucine	O (68-70 yr) males and females	15[16]	/	-/+Ex -/+ insuin	MPD=pre and post ex, with or without insulin	[65]
Leucine-enriched EAA	O (67) vs Y (29)	6.7 g EAA	/	-Ex	A higher leucine dose (41%) was necessary to match the increase of MPS of the O to hat of the Y	[195]

Similarly, the administration of an EAA drink (with an amino acid composition approximating their distribution in muscle protein) to both young and elderly subjects stimulated muscle protein synthesis to the same extent in both groups [226], although the response in the elderly was retarded albeit still sustained, as opposed to a faster, short-lived one of the young.

No impairment of muscle protein synthesis to protein intake was detected in elderly subjects after ingestion of large amounts of carbohydrates and proteins, or of either large (≈300 g) [221] or moderate (≈115 g) amounts of beef [220] by comparing sarcopenic (≈80 yr) and healthy (≈70 yr) older men, the ingestion of ≈20 g of a leucine-enriched whey protein load, increased muscle protein synthesis rates to the same extent in both groups [218].

In older adults following the ingestion of mixed meals containing combinations of animal (beef) and vegetal proteins, the whole-body anabolic response linearly increased with increasing protein intake, primarily due to the suppression of protein breakdown. Notably, muscle protein synthesis (i.e., one factor contributing to the net protein accretion, or balance, together with the suppression of protein degradation) was further stimulated by a protein dose (70 g) above that previously considered as "optimal" in the elderly (≈35 g/kg BW) [223], yet attaining the same maximal response as that as that of young subjects. However, at the 40 g dose, the stimulation of muscle protein synthesis in the elderly was lower than that observed in the young volunteers, therefore compatible with an anabolic resistance in the former group at a “lower” (i.e., 40 g) mixed protein dose (see also below). The above-reported protein dose(s) stimulating MPS in older subjects were however greater than the 20 g high-quality, whey protein dose producing the maximal effect on MPS in young people, either exercising or not [60]. The muscle protein synthetic response to the combined ingestion of protein and carbohydrate (i.e., an additional energy source) was not impaired in healthy older men [224]. Similarly, the co-ingestion of carbohydrate with protein and free leucine, stimulated muscle protein synthesis to the same extent in young and elderly lean men [222].

Data consistent with an impaired response (=anabolic resistance)

Other studies are however compatible with the existence of some degree(s) of anabolic resistance in the skeletal muscle of the elderly. Resistance in the eldely could be demonstrated following the administration of either protein or amino acid , as well as after hormone, exercise, or their combinations.

In the elderly, the dose of high-quality dietary protein administered as a single bolus (0.40 g protein/kg BW/meal, corresponding to ≈32 g protein/meal in an 80-kg person), required to stimulate

[14] Similar response of MPS in the two groups.

[16] Published data are reported as 0.35 g/FFM (Lean body mass). Here they have been recalculated per mean subject assuming that FFM equals LBM (Lean Body Mass).

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myofibrillar protein synthesis maximally, was greater than that required in young controls, (0.24 g protein) [61].

When crystalline EAA were administered, the literature data are somehow contrasting. It was initially reported that 2,5 g crystalline EAA were sufficient to elicit an increase above basal of MPS in both young and elderly subjects, whereas a resistance of MPS in the elderly was apparent only after 10-20 g EAAs [225]. However, in subsequent studies using intact whey protein, it was shown that the lower dose capable of eliciting a response in MPS in the elderly is greater than that of the young, and that the ageing muscle responds maximally at 20 g EAAs, a dose ≈2x lower than that of the young (40 g EAAs) [60]·in agreement also wth the data of Cuthberson [225].

In the elderly, however, MPS could however be further increased with 35-40 g intact whey protein intake. Thus, in the elderly the dose of the high quality whey protein required to attain a maximal stimulation of skeletal muscle protein synthesis was about 1-fold greater that of younger subjects [60].

Nevertheless, a ten-day administration of a supplement containing fast-digestive proteins (soluble milk proteins compared to casein alone) could overcome the muscle anabolic resistance in the elderly [211]. Furthermore, the EAA supplements that of the EAA proved to be more energy-efficient than whey protein in elderly humans [226].

In older men, skeletal muscle was also less responsive to the anabolic effects of leucine in the postprandial phase than in the young controls [227]. Also, a higher proportion of leucine within an essential amino acid mixture was required for an optimal stimulation of muscle protein synthesis in elderly than in young subjects [227].

Insulin Resistance.

Insulin-resistance on muscle protein synthesis in ageing was reported in another study showing that leg phenylalanine net balance, although not different between young and old subjects at baseline, was significantly increased in both groups with hyperinsulinemia, but to a greater extent in the young [228].

Nevertheless, increasing insulin availability through a local insulin infusion increased amino acid uptake but did not enhance muscle protein synthesis rates in healthy young and older men following the administration of a casein-based protein drink [229]. Thus, in the post-prandial phase skeletal muscle appears to be an insulin-resistant state to the stimulation of protein synthesis, without difference between young and elderly subjects (see also above).

2d. Resistance to the anabolic effects of exercise

Resistance to exercise-induced protein anabolic effects could be another factor favoring sarcopenia. Multiple pieces of evidence exist supporting this view. Following a single bout of resistance exercise in the post-absorptive state, the dose-response curve of the stimulation of protein synthesis by resistance exercise intensity reached the maximum at 60–90% 1 RM, but was) shifted to the right/low and blunted in the elderly compared to young controls, up to 1-4 hr post-exercise [230]. At the molecular level, the phosphorylation of p70s6K and 4EBP1 at 60–90% 1 RM was decreased in the older group. Phosphorylation of p70s6K 1 h post-exercise at 60–90% 1 RM predicted the rate of MPS at 1–2 h post-exercise in the young but not in the old. These data indicate that, older men exhibit a resistance to anabolic exercise of both MPS and intracellular mediators. In another study, although the acute MPS response to combined resistance exercise and EAA ingestion was comparable in young and older men, such a response was delayed with ageing, and it was associated to unresponsiveness of ERK1/2 signaling and of AMPK activation [231]. Similarly, in post-absorptive elderly subjects, following a single bout of resistance exercise, MPS increased to a lesser extent in the recovery phase than that observed in young controls [232]. At variance with the above reported however, others reported that aerobic exercise could overcome age-related (insulin) resistance of muscle protein anabolism following a leucine-enriched essential amino acid–carbohydrate mixture, through an improvement of the endothelial function and of the Akt/mammalian target of rapamycin signaling [233]. Thus, exercise combined with EAA administration could effectively counteract age-associated sarcopenia and other conditions leading to muscle wasting. No additional effect by heavy resistance

 doi:10.20944/preprints202308.0516.v1

exercise on myofibrillar protein synthesis was detected in elderly men after milk protein and carbohydrate ingestion [234].

2e. Deleterious effects of bed rest in the elderly

A condition opposite to exercise is bed rest, an undesired situation very common in hospitalized older subjects, as well as in those old persons in whom, for a variety of factors, physical activity is restrained and/or abolished, either voluntarily or not [235–238]. In older adults, bed rest significantly restrained the EAA-induced increase in MPS, through a reduction in mTORC1 signaling and amino acid transport [239]. EAA supplementation above RDA may help to preserve muscle function in the elderly during inactivity [240].

2f. The effect of complex nutritional supplements

Although natural foods rich in high-quality protein (dairy products, meat, egg) can adequately stimulate protein anabolism as efficiently as that of protein-rich supplements and/or of specifically-designed amino acid mixtures, yet the advantage of using specific nutritional supplements remains questionable [241]. In older women, the intake of 3 g of a leucine-enriched EAA supplement (LEAA) (containing 1,2 g leucine), stimulated MPS as much as 20 g WP (containing 2 g leucine). Thus the composition of EAA (rich in leucine) rather than the AA/protein amount seems to be crucial to stimulate skeletal muscle anabolism [242]. When comparing the effects of a soy & milk blend with whey protein alone, on post-exercise muscle protein synthesis, the anabolic effects were similar in older subjects [243], at variance with what was observed in the young, in whom the soy & milk blend helped to prolong the stimulatory effect on MPS from 2 hr (with whey protein alone) to 4 hr. The co-ingestion of carbohydrate and fat with an isonitrogenous nutritional supplement (21 g of leucine-enriched whey protein) did not affect nor further improve postprandial muscle protein synthesis in older men, showing that the provision of extra energy does not modulate muscle protein synthesis [244].

In a clinical trial of a 3-month supplementation period with 15 g EAA vs placebo in older women (≈70 yr), the acute anabolic response of skeletal muscle FSR to EAA supplementation was maintained over the observation period; however LBM increased only in the supplemented ones but not in the controls, whereas muscle strength was unchanged in both groups [245]. Thus, EAA supplementation may help in improving LBM and combat, with yet uncertain mechanism(s), the debilitating effects of sarcopenia

In another study, EAA supplementation with exercise in sarcopenic older women improved speed gait but not lean mass or strength . In elderly subjects supplemented with 15 g EAAs three times daily for ten days of bed rest, functional parameters were improved following inactivity [240]. The supplementation of either protein (whey and soy), leucine, or creatine did not improve the training-induced adaptations in pre-frail and frail elderly, regardless of sex. Therefore, these findings would not support using supplements to expand the effects of resistance exercise to counteract frailty-related muscle wasting dynapenia [246].

The Ingestion of casein added to a milk matrix, modulated dietary protein digestion and absorption kinetics however without affecting postprandial muscle protein synthesis in older men [214].

At variance with the above listed reports, even at low EAA ingestion, healthy older adults trained for 12 wks with progressive bouts of resistance exercise, exhibited a normal increase of muscle strength, cross-sectional area, and mixed muscle protein FSR [247], *via* the stimulation of the mTORC1 complex, thus showing that a healthy, exercise-conditioned condition may help to maintain a normal sensitivity to the anabolic effect of EAA and muscle protein accretion.

On the whole, many variables may account for the inter-study variability and the end-point message(s), that could appear somewhat confusing from the reported studies: they might be due to the specific outcomes selected, the doses and the duration of EAA supplementation the general, health-related conditions of the enrolled subjects, habitual physical activity, etc. Also some the chosen endpoints (such as the increase in MPS) may not really reflect a true improvement of muscle function.

2g. Specific effects of leucine addition

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The addition of leucine to other nutritional products may be a valid supplement to be used in the elderly, although with somehow limitated effects [248]. Older subjects consuming a leucine supplement showed a greater increase in MPS rates from baseline than did controls, but not in lean body mass or muscle function [249]. In a recent study in older men (74 y), co-ingestion of 2.5 g leucine with 20 g casein resulted in a 22% higher muscle protein synthetic rate compared with ingestion of casein alone [250].

The leucine effect seems to be dose-dependent. Leucine added as supplement (3-5 g) to either whey protein or EAA solutions in either younger or older men, showed comparable increments in skeletal muscle myofibrillar protein synthesis (MyoPS), at variance with no sustained stimulation observed in control subjects receiving only 1.8 g leucine [251,252]. However, the leucine effect in skeletal muscle of aged people might be impaired, as mTORC1 activation is defective, and sensitivity and responsiveness of muscle protein synthesis to amino acids decreased [253]. Conversely, the basal activation of mTOR seems to be higher. A combined effect of age-related impairment of muscle signaling and insufficient availability/delivery of nutrients and growth factors to the muscle, might contribute to sarcopenia. Thus, whether ageing *per se* affects mTORC1 signaling is yet uncertain, because of the common association between poor protein assumption, reduced/absent physical activity and concurrent diseases. In studies in which habitual protein intake exceeded 1.0–1.1 g/kg/day, and included a moderate/high proportion of dairy protein, prolonged leucine supplementation (2.5 g daily for six months) did not increase muscle mass or strength in healthy type 2 diabetic older adults [254].

2h. Effect of the inflammatory state

Acute and chronic inflammation retain undesired effects on skeletal muscle mass and protein metabolism. Thus, age-associated inflammation (either subtle or overt) may negatively affect the anabolic sensitivity of skeletal muscle in the elderly . Toth et al. reported strong relationship exists between MPS and circulating concentrations of several markers of immune activation [202]. At the mechanistic level, cytokines, in particularly TNF-α, may impair MPS by blunting the phosphorylation of proteins in the mammalian target of the rapamycin (mTOR) intracellular signaling pathway [255].

2j. Role of blood perfusion of skeletal muscle

Effective blood perfusion is another variable conditioning the delivery of anabolic substrates and hormones to muscle. Basal leg blood flow was greater in young than in elderly subjects, and it was stimulated in response to physiologic hyperinsulinemia in the young but not in the elderly [228]. Furthermore, a positive relationship was found between the insulin-induced changes in blood flow and the increase in muscle protein synthesis. Conversely, the stimulation of muscle protein FSR responded similarly to exogenous amino acids in healthy younger and older adults under conditions of a comparable NO-induced hyperemia he maintenance of an adequate blood flow perfusion may have a favorable impact on protein synthesis in ageing skeletal muscle [256].

2k. Optimizing nutrition-exercise interaction in the stimulation of skeletal muscle anabolism in ageing

The magnitude of the anabolic response to nutrients combined to exercise may be influenced by factors other than just the amount of a nutrient ingested or the magnitude of exercise. The daily distribution and timing of protein ingestion, the co-ingestion of different nutrients, and the type/quality of the protein or of the amino acid mixture ingested may all influence protein accretion (Table 3).

Table 3. Nutritional indications to optimize skeletal muscle mass and function, and combat anabolic resistance in ageing.

1. Consume a balanced diet rich in natural foods providing sufficient amounts of high quality protein, to sustain (skeletal) muscle protein accretion;
2. Keep / Increase protein intake at $\geq$1,5 g/kg/day, preferentially of high quality;
3. Provide $\geq$30 g protein at each main meal, equally spread into three main meals, to promote an optimal per meal stimulation of MPS;

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4. Prefer protein-rich natural foods over protein-rich supplements;
5. Avoid continuous 24h nutrition;
6. Provide sufficient energy;
7. Supplements:
a. Use them only in specific cases;
b. Use EAA supplements preferentially rich in the BCAA;
c. Add leucine either to natural protein foods or in the AA supplements
d. Consider that intake of protein supplements can proportionally lead to an involuntary reduction of protein-rich natural foods, thus partially offsetting their anabolic effect (REF).
8. When combined with exercise, assume nutrition taking into account:
a. The timing of administration
i. pre-exercise
ii. at the beginning (t=0′) (preferred)
iii. or following exercise…
b. Amount and type of nutrition?
i. Natural protein rich foods? And/or:
ii. Supplements

A number of reports have also investigated the optimal daily distribution of food protein, as well as the timing of the administration with respect to exercise performance (see also above).

In regard of the food protein distribution over the three main daily meals, it was reported that non-frail elderly subjects assume the dietary proteins more evenly than pre-frail and frail subjects, thus suggesting that the daily protein load should be assumed as ≈30 g protein (in a representative 75 kg subject) at each of the three daily mealtimes [257]. Conversely, in a subsequent randomized-controlled trial the pattern of meal-protein intake (i.e., even vs not even) over the day did not affect lean body mass, muscle strength, other functional outcomes, as well as whole body protein kinetics and MPS over 8 weeks in older adults [258].

In respect of timing of food protein food administration with exercise (i.e., before, at the beginning, i.e.,, at t = 0′, or after exercise performance), in the only published study in the elderly, the early intake of a protein supplement immediately just before (at t = 0′) each bout of resistance-type exercise in a 12-week intervention study in the elderly, sustained skeletal muscle hypertrophy with a 12-week intervention in the elderly, as opposed to no effect of supplement intake 2h after exercise [259]. Notably, protein ingestion in the evening/night before sleep increased Muscle Protein Synthesis throughout the night in healthy older men [260].

A list and the quantities of common foods providing ≈30 g of protein is reported in Table 4. The quantities of animal foods are lower than those of vegetal foods (except for soybeans and quinoa), because of their balanced content of essential amino acids. The reported quantities are only indicative, because most of these foods have not been specifically tested in controlled studies of stimulation of skeletal MPS with exercise in the elderly.

Table 4. List and quantities of common animal and vegetal foods providing ≈30 g protein, and their caloric content, useful to improve muscle anabolism in ageing with exercise.

Animal Foods	**g**	**Calories**
Cow whole milk	909	582
Ricotta from cow milk	340	498
Ricotta from buffalo milk	286	606

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Low-fat, fresh cheese	98	446
High-fat, aged cheese	89	347
Chicken egg (n = 4.4)	242	322
Beef meat	136	151
Pork meat	183	268
Chicken breast	131	131
Bresaola	94	142
Turkey breast	125	134
Seabass (filets)	141	210
Fresh salmon	163	302
Vegetal foods	**g**	**Calories**
Soybeans	100	404
Wheat meal	273	927
Corn meal	345	1248
Beans	566	752
Quinoa	234	863
Oat meal	238	917
Rice	450	1487
Lentils	475	436
Chickpeas	429	514

2i. Muscle and bones

The idea of a bone–muscle unit has emerged in the past decades. Different studies have provided valuable insights into the intricate communication between muscle and bone during anabolic resistance [261–263]. These investigations have revealed that muscle and bone are tightly interconnected tissues, with reciprocal interactions and shared regulatory mechanisms. Mechanistically, it has been established that mechanical loading, generated by muscle contractions during physical exercise, plays a pivotal role in preserving bone health. By applying forces to the bone, mechanical loading stimulates bone remodeling and fosters the maintenance of bone density and strength [264]. Conversely, disrupting this mechanical loading during periods of muscle disuse, such as immobilization or inactivity, can result in bone loss and increased susceptibility to osteoporosis [265]. Moreover, molecular mediators released by muscle cells, including growth factors, hormones, and cytokines, have emerged as crucial signaling molecules facilitating the bidirectional crosstalk between muscle and bone. These molecular messengers transmit signals and exert regulatory effects on bone cell activity, ultimately influencing bone turnover and remodeling processes. From the muscle can be released osteoinducer (IGF-1, FGF-2, IL-15, OGN, FAM5C, Tmem119, osteoactivin) and osteoinhibitor (IL-6 and myostatin) among different stimuli [266]. Consequently, interventions targeting anabolic resistance and aiming to preserve or enhance muscle mass and function, such as resistance exercise and optimizing protein intake, hold considerable potential to positively impact both muscle and bone health [267]. A comprehensive understanding of the intricate communication between muscle and bone during anabolic resistance is of paramount importance for the development of effective strategies to combat age-related muscle and bone disorders.

Closing Remarks

In conclusion, recent research has produced a huge amount of data about the molecular, nutritional, as well as lifestyle- and health-related factors that can affect the development and the worsening of sarcopenia in the eldely. These data could well be exploited in designing interventions to optimize skeletal muscle accretion and function and combat sarcopenia in the old. Further research is needed to expand our knowledge about the mechanisms and the possible treatments that could maintain muscle function and prevent age-related loss of function and falls.

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