Narrative Review

Age-related muscle anabolic resistance: inevitable or preventable?

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Age-related loss of muscle mass, strength, and performance, commonly referred to as sarcopenia, has wide-ranging detrimental effects on human health, the ramifications of which can have serious implications for both morbidity and mortality. Various interventional strategies have been proposed to counteract sarcopenia, with a particular emphasis on those employing a combination of exercise and nutrition. However, the efficacy of these interventions can be confounded by an age-related blunting of the muscle protein synthesis response to a given dose of protein/amino acids, which has been termed "anabolic resistance." While the pathophysiology of sarcopenia is undoubtedly complex, anabolic resistance is implicated in the progression of age-related muscle loss and its underlying complications. Several mechanisms have been proposed as underlying age-related impairments in the anabolic response to protein consumption. These include decreased anabolic molecular signaling activity, reduced insulin-mediated capillary recruitment (thus, reduced amino acid delivery), and increased splanchnic retention of amino acids (thus, reduced availability for muscular uptake). Obesity and sedentarism can exacerbate, or at least facilitate, anabolic resistance, mediated in part by insulin resistance and systemic inflammation. This narrative review addresses the key factors and contextual elements involved in reduction of the acute muscle protein synthesis response associated with aging and its varied consequences. Practical interventions focused on dietary protein manipulation are proposed to prevent the onset of anabolic resistance and mitigate its progression.

INTRODUCTION: IMPLICATIONS OF AN AGING POPULATION

Individuals over 60 years old are currently estimated to exceed 11% of the global population; by 2050, this figure is projected to reach 22%.[1] Individuals aged 80 years and older nearly tripled from 1990 to 2019 (54 million to 143 million). By 2050, this population is projected to triple yet again, reaching 426 million, and outnumbering adolescents and youth aged 15 to 24.[2] Alongside the rapidly expanding global demographic of older adults is the need to minimize the burden of age-related diseases to mitigate the socioeconomic consequences of ill-health in an increasingly aging society.

Frailty and sarcopenia are among the aging population's greatest health threats. These conditions are often

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referred to interchangeably due to their common ground of unintended, progressive weight loss. However, there are important distinctions between the two conditions. Frailty is a state of increased vulnerability due to age-related decline and dysfunction across multiple physiologic systems,[3] whereas sarcopenia refers to the age-related loss of skeletal muscle mass, strength, and physical performance.[4] Importantly, the decline in functional capacity associated with sarcopenic conditions suggests a clear link between sarcopenia and generalized frailty.[5,6]

Human skeletal muscle mass reaches peak levels between the second and fourth decades of life,[7] and then progressively declines thereafter under relatively sedentary conditions. On average, humans lose approximately 0.5% of their skeletal muscle mass per year after the fourth decade of life, escalating to approximately 1%–2% annually after the age of 50 and then increasing exponentially to approximately 3% annually after the age of 60.[6,8] Estimates suggest that the loss of strength is markedly greater than the loss of muscle mass.[9] Estimates of the prevalence of sarcopenia in the general population aged $\geq$60 years is approximately 10% in both men and women[10]; a range of 9.9% to 40.4% among community-dwelling elderly people has been reported, which appears to be dependent on the specific definition and cut-off criteria applied.[11] Sarcopenia prevalence in nursing homes is estimated to be 51% and 31% among men and women, respectively.[12]

The development of sarcopenia is undoubtedly complex and multifactorial, but one major contributor is the resistance of skeletal muscle to anabolic stimuli—termed anabolic resistance. This review aimed to address the key factors and contextual elements involved in the reduction of the acute muscle protein synthesis (MPS) response associated with aging and its varied consequences. Practical interventions focused on dietary protein manipulation and supplementation, as well as the importance of physical activity, will be proposed for preventing and mitigating anabolic resistance.

METHODS

Because the primary objective of this paper was to broadly and comprehensively cover the nuances of age-related anabolic resistance, a narrative approach was chosen to review the topic. Two electronic databases, PubMed and Google Scholar, were searched using a combination of the following key terms: "anabolic resistance," "muscle protein synthesis," "muscle mass," "protein intake," "protein consumption," "protein timing," "resistance training," and "strength training." Scrutiny of the reference lists of applicable studies as well as the authors' knowledge of related research further helped to identify additional relevant papers.

THE DEVELOPMENT OF ANABOLIC RESISTANCE IN AGE-RELATED MUSCLE LOSS

The metabolic mechanism for loss of muscle mass is based on the dynamic balance between MPS and muscle protein breakdown (MPB)—in particular, the net balance between the synthesis and breakdown of the myofibrillar proteins (the structural proteins responsible for changes in muscle mass). When the rate of MPS is less than MPB, ie, negative net muscle protein balance (NMPB), for a period of time, muscle mass declines. Hyperaminoacidemia from protein ingestion results in stimulation of MPS and positive NMPB. The stimulation of MPS from ingestion of a protein source is blunted with advancing age.[13,14] This decrease in sensitivity to protein ingestion means that the amount of protein necessary to stimulate the maximal response of MPS is greater in older than in younger adults.[15] Resistance exercise also stimulates MPS, and this stimulation is reduced in older muscle as well[16,17] and is associated with lower activity of anabolic signaling pathways.[13] The response of MPS to nutrition and exercise plays a larger role in NMPB than MPB does.[18] This asymmetric control system dictates that increases in NMPB are more dependent on enhancing MPS than on inhibiting MPB. Thus, the diminished MPS response to protein feeding in older individuals is thought to be a principal driver of long-term skeletal muscle loss with advancing age.[19]

The development of sarcopenia is more commonly associated with anabolic resistance to protein ingestion, but older adults also tend to be less sensitive than younger adults to the anabolic impact of insulin on muscle. The role insulin on MPS primarily is thought to be permissive.[20] However, postprandial elevations of insulin may stimulate MPS provided amino acid availability is maintained.[21,22] This anabolic effect of insulin seems to manifest indirectly via increased muscle blood flow and microvascular perfusion, leading to elevated amino acid availability to the muscle.[23] Moreover, there is evidence for a link between endothelial dysfunction and age-related anabolic resistance. The normal insulin-mediated stimulation of endothelial vasodilation is reduced with age,[24] leading to an impaired MPS response of skeletal muscle to insulin.[23] Postprandial hyperinsulinemia also contributes to positive NMPB by reducing MPB.[20] Resistance to the antiproteolytic effect of insulin seems to be blunted in older adults, possibly contributing to sarcopenia.[25] However, the contribution of the reduced impact of insulin on MPB with age to age-related muscle loss is questionable. Insulin seems to

selectively blunt the breakdown of nonmyofibrillar proteins, but not of myofibrillar proteins.[26] Thus, the resistance of skeletal muscle to anabolic stimuli (ie, hyperaminoacidemia, hyperinsulinemia, and resistance exercise) with ageing seems to be mediated primarily by reduced MPS rather than any effect on MPB.

Several mechanisms are proposed to underpin age-related impairments in the MPS response to stimuli. These mechanisms include decreased anabolic molecular signaling activity,[13] reduced insulin-mediated capillary recruitment (thus reduced amino acid delivery),[27] and increased splanchnic retention of amino acids (thus reduced availability for muscular uptake).[28] More recently, additional contributing factors have been proposed, such as insulin resistance, systemic inflammation, and decreased concentration of satellite cells.[19] An emerging body of evidence indicates a bidirectional role of the gut–muscle axis in the regulation of muscle mass and function.[29] Biological processes modulated by the gut microbiota proposed to influence anabolic resistance include reduced biosynthesis of microbe-derived amino acids, including leucine.[30] Furthermore, it is plausible that an increase in proinflammatory microbes can lead to dysbiosis and vascular dysfunction,[31] which in turn can impair perfusion and delivery of amino acids to skeletal muscle. Thus, the mechanistic regulation of age-related anabolic resistance remains to be definitively determined.

THE INFLUENCE OF NON-AGE-RELATED FACTORS ON ANABOLIC RESISTANCE

The findings of research comparing the acute MPS response to exercise and/or protein/amino acid–based nutrition in older versus younger adults are somewhat equivocal. Whereas anabolic resistance typically is considered an age-related phenomenon, it is not clear that advancing age, per se, is responsible for the development of resistance to anabolic stimuli in skeletal muscle. Other factors associated with aging (eg, increasing inactivity, low-grade inflammation, and/or increasing obesity) likely contribute to anabolic resistance. Shad et al[32] investigated this relationship in a systematic review encompassing 24 studies and illuminated methodological differences that provide potential explanations for the mixed data. The age range of the young participants was 20 years–35 years, whereas the older adults studied were 64–76 years. Among the 48 study arms of the 24 studies, 3 separate models were examined. In the model examining the effect of exercise alone, 8 of 17 study arms provided sufficient evidence of age-related anabolic resistance, characterized by significantly lower MPS in older individuals. A second model assessed the response to amino acid–based nutrition alone, and 8 of 21 study arms showed sufficient evidence of age-related muscle anabolic resistance. While the latter models indicated the presence of age-related anabolic resistance via diminished MPS in older subjects compared with younger ones, a third model that examined a combination of exercise and amino acid–based nutrition found evidence of this phenomenon in only 2 of 10 study arms. This consistent lack of anabolic resistance in the vast majority of studies examining this combination is compelling support for the likelihood that the development of anabolic resistance is not an ineluctable consequence of growing older. Moreover, this lack of certainty suggests the possibility of prevention or treatment via implementation of concurrent training and nutrition-based interventions. A trial among those in the third model that exemplifies this possibility is by Atherton et al,[33] who reported no significant differences in the anabolic response between young (24 ± 6 y) and old (70 ± 5 y) subjects. Importantly, both groups showed similar increases in myofibrillar MPS (as opposed to mixed MPS) in response to post-resistance exercise ingestion of a mixed macronutrient beverage with 4.2 g leucine.

Anabolic resistance also is instrumental in the skeletal muscle atrophy that occurs during periods of muscle disuse, regardless of age. Disuse-related atrophy of skeletal muscle (also called disuse atrophy or simple atrophy) results from volitional inactivity or immobilization as an outcome of injury, recovery from surgery, or similar scenarios that may necessitate bed rest.[19,34,35] Limb immobilization results in rapid and substantial atrophy,[36] with measurable loss of muscle reported in as little as 2 days.[37] This muscle loss is associated with reduced sensitivity of MPS to protein ingestion.[37,38] Reduced activity in the form of short-term bed rest also leads to muscle loss associated with anabolic resistance that is greater in older than in younger adults.[39] Moreover, anabolic resistance also manifests during recovery from forced immobility. Muscle regrowth is diminished in old compared with young muscle following a period of immobility,[40] likely mediated by decreased MPS. Integrated MPS, ie, including both postabsorptive and postprandial periods, was reduced with 2 weeks of decreased (70%) step count, but did not return to normal with 2 weeks of return to habitual activity in older adults.[41] Thus, forced inactivity with injuries involving limb immobilization or periods of bed rest during illnesses or hospital stays will exacerbate muscle atrophy, mediated by greater anabolic resistance of skeletal muscle.

Anabolic resistance resulting from reduced physical activity also contributes to muscle loss with age in healthy adults. Complete inactivity, such as limb immobility or bed rest, is not necessary to induce anabolic resistance. Even relative inactivity with reduced step

count induces anabolic resistance and muscle atrophy in older adults.[42] Since physical activity levels tend to drop with increasing age,[43,44] increased inactivity likely is a major contributor to age-related anabolic resistance and muscle loss. Therefore, chronological aging, per se, does not seem to be the only explanation for resistance of muscle to anabolic stimuli. Decreased habitual physical activity interspersed with periods of forced inactivity (eg, due to hospitalization, or limb immobilization) may be important key drivers of anabolic resistance leading to muscle loss.

Obesity is associated with metabolic perturbations in many tissues, including muscle. The pervasiveness of obesity in conjunction with sarcopenia in older adults is becoming an important public health issue. The global rise of obesity has occurred concurrently with the rapid global expansion of the older population, which presents a compound challenge. Data from the latest National Health and Nutrition Examination Survey (NHANES) cycle (2013–2016) estimates an obesity prevalence of 36.6% in men and 41.0% in women.[45] In addition to increasing the risk for type 2 diabetes, cardiovascular disease, respiratory disease, stroke, gallbladder disease, cancer, sleep disorders, and osteoarthritis,[46] obesity also may lead to impaired muscle metabolism and function.[47,48] As a consequence of increasing obesity rates and the aging population, there is a confluence of obesity with aging and sarcopenia. As the term implies, "sarcopenic obesity" refers to the combination of low muscle mass and function in individuals with obesity.[49]

The influence of obesity, per se, on the sensitivity of MPS to elevated amino acids is not consistent, which complicates presumptions about the relationship between these variables. Whereas obesity, independent of increasing age, has been associated with anabolic resistance to protein feeding, anabolic resistance in obese individuals is not universally reported.[47,48] Both protein feeding and a hyperinsulinemic–hyperaminoacidemic clamp have been used to determine the response of MPS to feeding in obese and overweight adults. The synthesis of mixed,[50] myofibrillar,[51] and mitochondrial[50] proteins has been reported to be reduced in obese adults compared with controls when blood amino acids were increased under clamp conditions. However, this response is not ubiquitous across studies.[52,53] Whereas this method is an important technique used to evaluate the metabolic response in muscle, the square-wave nature of elevated blood amino acids is not the same as the postprandial response to protein ingested in a meal. When amino acid availability is increased with protein feeding, the response also is inconsistent.[54–56] Similarly, the response of MPS to resistance exercise is reported to be both impaired[57] and unaffected by obesity.[58] Among the muscle protein subfractions, obesity was shown most consistently to impair the synthesis of myofibrillar proteins, but not sarcoplasmic or mitochondrial protein synthesis, in response to nutrition and resistance exercise.[47] Thus, whereas obesity has been associated with impaired muscle responses to anabolic stimuli, direct attribution is not possible at this point. Nevertheless, the relationship between obesity and sarcopenia can be viewed as a bi-directional or vicious cycle of pathological changes that simultaneously affect the myocytes and adipocytes. Li and Ma[59] recently posited that sarcopenic obesity results from the interplay of pro-inflammatory myokine secretion and dysregulated adipokine and cytokine secretion. Pathological changes within muscle include decreased IL-15 and IFG-1, alongside increased myostatin and decreased IL-6 secretion. These occur alongside increased changes in adipose tissue, including TNF-alpha, IL-1-beta, IL-6, leptin, and decreased adiponectin. The sustained effects of an energy-dense/nutrient-poor diet and a physically inactive lifestyle can exacerbate the predisposition to chronic diseases in advanced age. This sets the stage for sarcopenic obesity, where continued decreases in lean mass and increases in fat mass are accompanied by increased insulin resistance, oxidative stress, and chronic low-grade inflammation. Healthy body composition ranges are important for general awareness but are still subject to a myriad of individual factors, preventing their upper and lower limits from being viewed as strict or end-all cut-offs. Nevertheless, for the general population, Abernathy and Black[60] reported that statistically desirable body fat percent ranges for men and women are 12%–20% and 20%–30%, respectively. Similarly, Kyle et al[61] reported that healthy body fat ranges for men and women are 10.8%–21.7% and 21.7%–33.2%, respectively.

The reason for the discrepancies in reports of anabolic resistance associated with obesity is unknown but may be related to reduced physical activity levels. Obesity, as with aging, is linked with decreased levels of physical activity.[62,63] Reduced activity levels diminish the sensitivity of muscle to amino acid availability.[39] Conversely, even modest physical activity performed the day before amino acid ingestion increases the responses of MPS to feeding.[64] Thus, differences in habitual activity levels of the volunteers in the studies investigating obesity and anabolic resistance may explain the discrepant results. In support of this notion, Smeuninx et al[56] reported a clear correlation of the response of MPS to protein feeding and daily step count. Moreover, the step count of the obese volunteers with no indications of anabolic resistance reported by Kouw et al[54] was greater than is usually observed for obese individuals.[63] Therefore, anabolic resistance reported in

obese individuals may be a result of reduced activity rather than excess adiposity.

Whereas there is evidence that obesity may not lead to anabolic resistance, other attributes associated with obesity may lead to decreased sensitivity to nutrients. Additional potential obesity-related mediators of anabolic resistance include insulin resistance and systemic inflammation. Systemic inflammation seems to play a role in the dysregulation of muscle metabolism. Inflammation has been shown to be associated with a diminished response of MPS to hyperaminoacidemia in older obese adults.[51,56] Obesity also can set the stage for the development of insulin resistance via the accumulation of ectopic fat.[65] Ectopic fat storage is characterized by triglyceride accumulation in "non-adipose tissues"—the most relevant ones for this discussion are the liver and skeletal muscle, which are major sites of insulin activity. Fat accumulation in ectopic depots is considered instrumental in the development of insulin resistance and type 2 diabetes, both of which are predisposed by obesity.[66]

The findings of the third model in Shad et al[32] point to programming possibilities for circumventing age-related differences in postprandial MPS responsiveness. Specifically, leveling the field of anabolic response may be achieved by optimizing protein intake (including timing and distribution),[67] particularly when combined with regimented physical activity (particularly resistance training).[68] Additionally, Shad et al[32] hypothesized there may be an exercise volume threshold that older adults must reach to match the MPS response of younger subjects. However, this speculation is challenged by inconsistent results across studies, which might be due to differences in subjects' habitual physical activity. That is, individuals with higher levels of habitual physical activity or exercise may have less probability of exhibiting age-related anabolic resistance than their more sedentary counterparts. Habitual physical activity was objectively assessed (via accelerometry) in only 1 of the 24 studies of the meta-analysis, wherein Chevalier et al[69] reported that postprandial whole-body and muscle protein anabolic responses were preserved in active, healthy older women (73 ± 3 years). However, in more recent work (not included in Shad et al), Smeuninx et al[56] demonstrated an association between daily step-count and postprandial MPS responsiveness in older men and women. Devries et al[70] investigated the 2-week effects of resistance training during an approximately 80% reduction of daily steps in older men (70 ± 1 years). The low-load training program encompassed 3 sessions per week, consisting of 2 exercises (leg press and leg extension), 3 sets per exercise performed at 30% of 1-repetition maximum until volitional fatigue. Leg fat-free mass decreased with step-reduction but increased with step-reduction plus resistance training. Myofibrillar MPS was higher in the latter condition in both the postprandial and postabsorptive states. Recognizing the potential influence of habitual physical activity, Phillips et al[71] limited subject eligibility to individuals who had a minimum of 2 years without any resistance training or moderate- to high-intensity aerobic training. Over the course of 5 months, muscle mass was significantly increased via resistance training in younger subjects (25 ± 4 years), but not in middle-aged (50 ± 4 years) or older subjects (70 ± 3 years). Although no significant between-group differences in macronutrient intake were reported, daily protein consumption was suboptimal to maximize anabolism (<1.6 g/kg). The findings collectively indicate that resistance exercise is capable of supporting lean mass and a favorable MPS response, even in the face of step reduction. In cases where orthopedic, environmental, or other limitations prohibit older adults from being physically active through aerobic activity such as walking, a resistance-based program may be effective in mitigating losses in muscle and impairments in MPS.

Methodological considerations for future studies should include the accounting of habitual physical activity, which has the potential to positively influence the acute anabolic response to protein feeding. Furthermore, dose–response investigations of the relationship between weekly training volume (and/or intensity) and anabolic resistance could elucidate minimum effective doses of these parameters. Addressing these gray areas would be useful for populations including the chronically ill, whose limited training capabilities preclude higher volumes or loads. Finally, daily protein intakes in future investigations should be optimized when the objective is to purposely or maximally induce muscle growth or retention.

PRACTICAL APPLICATIONS

Physical activity/exercise focused on increasing or preserving both muscle mass and strength

Dynapenia (age-related strength loss)[72] has remained in the shadow of sarcopenia in terms of media attention in the lay and academic press. It is possible that the primary emphasis on preventing losses in muscle mass is an oversight, since dynapenia is a potentially stronger predictor of age-related disability and mortality.[73] Further support of this is a 5-year prospective cohort study by Scott et al,[74] who reported that in middle-aged and older community-dwelling subjects, dynapenic obesity, but not sarcopenic obesity, was associated with an increased risk of falls. Moreover, muscle mass and strength changes do not track simultaneously and are

often dissociated. Increases in muscle strength can occur in the absence of mass gains, suggesting neurological adaptations that can occur independently of muscle hypertrophy. However, Clark et al[75] recently reported a relatively even contribution of lean mass and neural excitability in the prevention of muscle weakness in older adults. Therefore, compared with preserving or increasing muscle mass, the aim to preserve strength is at least of equal importance to the aging population. The increase and/or preservation of muscle mass serves to protect metabolic function, while the pursuit of strength increase and/or maintenance can protect neuromuscular function and prolong physical independence in older adults. It is worth noting that these benefits are not merely limited to being preventive. It is never "too late" to commence countermeasures against existing age-related musculoskeletal decline. Illustrating this point is a 16-study systematic review by Lopez et al,[76] who reported that in subjects >65 years who met standard diagnostics for frailty, resistance training at a frequency of 1–6 sessions per week, 1–3 sets of 6–15 repetitions, and intensity of 30%–70% of 1-repetition maximum increased maximal strength by 6.6%–37%, muscle mass by 3.4%–7.5%, muscle power by 8.2%, and functional capacity by 4.7%–58.1%.

Total daily protein targets and within-day protein distribution

Total daily protein, and within-day protein distribution (including per-meal dosage) are both important factors for maximizing muscle anabolism in the aging population. Current position stands on the protein needs of older adults (>65 years) recommend intakes greater than recommended dietary allowance guidelines. For example, the ESPEN Expert Group[77] recommends a protein intake of at least 1.0 g/kg/day–1.2 g/kg/day for healthy older people, 1.2 g/kg/day–1.5 g/kg/day for older people who are malnourished, or have acute or chronic illness, and even higher intakes for individuals with severe illness or injury. The PROT-AGE Study Group[78] recommends an average daily intake of 1.0 g/kg/day–1.2 g/kg/day for healthy individuals, 1.2 g/kg/day–1.5 g/kg/day for those with an acute or chronic disease, and up to 2.0 g/kg/day for those with malnutrition, severe illness, or injury. Other than for those with pre-existing kidney disease,[79] there does not appear to be any imminent health risk of higher protein consumption than the aforementioned upper thresholds.[80]

Age-related muscle anabolic resistance may be overcome, and sarcopenia progression offset, by maximizing postprandial MPS through structured resistance training. A large meta-analysis by Morton et al (49 randomized controlled trials, 1863 subjects)[81] concluded that a protein intake of approximately 1.6 g/kg/day (with an upper 95% confidence interval of 2.2 g/kg/day) maximizes resistance training–induced muscle hypertrophy and strength gains in adults under eucaloric or hypercaloric conditions.[81] However, it was also found that gains in fat-free mass were reduced with increasing age.[81] This calls into question the effectiveness, and possibly the sufficiency, of the 1.6 g/kg/day benchmark when applied to older adults. It is therefore possible that this intake level should be the minimum to be maintained through older age, alongside regular resistance-type exercise, if the goal is to maximize sensitivity to anabolic stimuli and preserve muscle mass. A limitation of this meta-analysis is its exclusion of trials involving hypocaloric conditions, which could increase protein requirements for maximizing muscle retention in both clinical[82] and athletic populations.[83] A previous meta-analysis by Finger et al[84] that included 9 randomized controlled trials focused on older adults (462 subjects aged 61–79 years) found that protein supplementation in combination with resistance training was associated with gains in fat-free mass (assessed via dual X-ray absorptiometry in the majority of the trials) but not muscle mass (assessed via computed tomography in the majority of the trials) nor strength, compared with resistance training alone. The mean trial length was 22 weeks, with a mean supplemental protein intake of 0.46 g/kg/day (20.7 g). Mean baseline protein intake was not reported, leaving open questions about whether total daily protein intakes were suboptimal, despite supplementation.

A recent meta-analysis by Nunes et al,[85] the most comprehensive to date (74 randomized controlled trials, 2665 subjects), sheds further light on the state of the evidence, including its uncharted ground. The analysis was stratified into younger (< 65 years) and older subjects (≥65 years), and 3 different daily protein intake levels (<1.2 g/kg, 1.2 g/kg–1.59 g/kg, and ≥1.6 g/kg). Significant gains in muscle size and strength gain were seen at both higher protein intake levels, but in younger subjects, lean mass gain was significant only when ingesting ≥1.6 g/kg (greater lower-body strength gain was also seen at this level). In older subjects, significant lean mass gain occurred at the middle bracket of protein intake (1.2 g/kg–1.59 g/kg). However, the authors explicitly acknowledged an absence of studies examining intakes of ≥1.6 g/kg in older subjects. The analysis also excluded trials involving hypocaloric conditions targeted for weight loss, which may raise protein requirements for optimizing training effects. It was clear that protein intakes of ≥1.6 g/kg show muscle size and strength gains in subjects under the age of 65 years, but there is also a clear lack of data on such intakes in subjects who are 65 years and beyond. Furthermore,

there is an absence of trials directly comparing 1.6 g/kg with higher intakes, so the commonly presumed optimality of this dose is still speculative—especially in older adults. Nevertheless, it is worth considering that optimized protein requirements of physically active (and even more so in athletic) individuals are likely to diminish minimally, if at all, as a mere function of chronological age. As stated by Moore,[86] "Master athletes have similar muscle characteristics, physiological responses to exercise, and protein metabolism as young athletes and, therefore, are unlikely to have protein requirements that are different from their young contemporaries."

Further tactics such as strategic timing and distribution of feeding to maximize anabolism and anticatabolism may be necessary, but this remains a gray area in the literature. Protein consumed after exercise (versus at rest) has resulted in greater MPS in both young and elderly men.[87] However, the traditional focus on the post-exercise "anabolic window of opportunity" mainly reflected evidence from acute MPS studies involving comparisons of fasted subjects,[88] which limits the external validity of the concept. Furthermore, Burd et al[89] reported that resistance exercise taken to momentary muscular failure stimulated rates of myofibrillar protein synthesis above fasting rates, preserving sensitivity to protein feeding up to 24 hours into recovery. The collective evidence underscores the primacy of attaining adequate total daily protein intake, while specific timing of doses relative to training is of distantly secondary importance from an anabolism standpoint.[90]

In addition to hitting the targeted daily protein total, overcoming postprandial anabolic resistance may require consuming a sufficiently large dose of high-quality protein per meal. The PRO-AGE Study Group recommends 25 g–30 g protein per meal, containing approximately 2.5 g–2.8 g leucine.[78] However, more recent data show that this dosing range is on the low end, and it might be more accurate to issue per-meal protein recommendations on a basis proportional to body mass. Moore et al[15] retrospectively analyzed 6 of their previous studies examining myofibrillar MPS. In older subjects (71 ± 1 years), MPS reached a plateau at a per-meal dose of 0.4 g/kg. Notably, the authors caution that this is a mean value, and up to 0.6 g/kg may be required to maximize MPS in some individuals. More recently, Park et al[91] found that 70 g protein from beef patties elicited a greater MPS response than 35 g in older adults (69.3 ± 1.8 years). It is noteworthy that these results were seen in the non-trained state, raising the possibility that a resistance training bout could have potentiated an even higher dosing ceiling for maximal postprandial MPS. Holwerda et al[92] provided further insight into the topic, showing a graded anabolic dose–response to 15 g, 30 g, and 45 g milk protein supplement in the 6-hour post-exercise period following a multi-set resistance training bout. However, while whole-body net protein balance was greater in the 45 g dose, the rate of myofibrillar MPS peaked at 30 g.

The so-called "muscle full" effect denotes the finite capability of a given protein or amino acid dose to elevate MPS,[93] where elevated MPS via oral ingestion of a protein bolus peaks at approximately 120 minutes and returns to baseline levels in approximately 180 minutes, despite the persistence of elevated essential amino acids (EAA) in circulation. This finding gave rise to speculation that protein ingestion ($\geq$20 g) should be spaced sufficiently to avoid refractory effects on MPS during the "muscle full" period.[94] However, Churchward-Venne et al[95] demonstrated that although protein feeding in the resting state results in MPS levels peaking at between 1 hour and 3 hours post-ingestion, protein feeding in the resistance-trained state results in greater MPS at 3 hours–5 hours than at peak MPS in the resting state. The implications of these findings on long-term changes in muscle mass require further study. When considering the data as a whole, a protein dosing range of 0.4 g/kg–0.6 g/kg per meal seems warranted for maximizing the acute postprandial muscle anabolic response (eg, overcoming anabolic resistance). Consuming this dose for a minimum of 3–4 times per day would help ensure that the recommended total daily protein target is met.

Protein intakes that can maximize muscle growth/retention and strength ($\geq$1.6 g/kg) can involve increases of roughly 50–60% greater than what is habitually consumed among the elderly population, which can be challenging without an informed strategy. A potentially viable solution is to focus on improving the pattern of intake through the course of the day. To fortify gaps in protein distribution, it helps to know the population-level pitfalls. A typical American diet has the majority of the day's protein intake (~40–60 g protein, representing ~50% of total intake) skewed toward the dinner meal.[96] NHANES data show that lunch is the most frequently skipped meal across all age groups.[97] A skewed protein distribution towards dinner is not an exclusively American phenomenon. For example, data from Australia,[98] Japan,[99] and Europe[100] show that dinner is the highest protein-containing meal of the day. Based on these findings, older individuals should be aware of the general tendency toward protein shortcomings at breakfast and/or lunch, and either increase serving amounts, or supplement accordingly, to achieve the minimum per-meal target of 0.4 g/kg. An additional and overlooked opportunity for protein feeding is pre-sleep, particularly in the post-exercise period, where an approximately 40 g–48 g dose augments the overnight

muscle anabolic response in skeletal muscle by increasing amino acid availability to promote positive net muscle protein balance.[101–103]

It is notable that despite some support for an anabolic advantage of a more evenly spread pattern of protein feedings,[104,105] several studies have failed to demonstrate this advantage compared with a more skewed distribution.[106–109] Among these trials, those with sufficient total daily protein for targeting hypertrophy (near or beyond 1.6 g/kg) were of short duration (up to 2 wk). The 1 trial lasting 8 weeks[106] involved relatively low total daily protein intakes (1.1 g/kg), which would amount to sub-optimal protein intake per meal (<0.4 g/kg) for the purpose of maximizing acute MPS, thereby potentially compromising muscle growth over the longer term. Importantly, none of these trials involved an exercise component, let alone structured, progressive resistance training, which could potentiate the anabolic response of more evenly spread distributions of protein doses sufficient for maximizing MPS. More research is needed to better resolve the conflicting findings in this area, but from a pragmatic standpoint, "evening-out" protein intake patterns by fortifying or increasing the protein servings of typically low-protein meals would hedge the probability toward increasing total daily intake, thereby increasing the likelihood of optimizing muscle growth and retention.

Supplementation strategies

The ingestion of protein doses or daily totals that are maximally anabolic or protective against muscle loss is not always a simple or feasible task. Protein's satiating capacity has the potential to have a self-limiting effect on higher intakes. In such cases, several supplementation strategies are worth considering. Katsanos et al[110] found that supplementing 1.7 g leucine within an EAA mixture in elderly individuals (66.7 years ± 2.0 years) did not match the MPS response seen in younger adults, while 2.8 g leucine within an EAA mixture was able match this response. Bukhari et al[111] reported that the MPS response to 3 g of EAA (40% leucine) was equivalent to 20 g whey in older women, which has implications for a less satiating, yet similarly anabolic alternative to greater protein quantities. More recent work by Gwin et al[112] found greater whole-body net protein balance from ingesting a whey–EAA mixture compared with an isocaloric/isonitrogenous amount of whey (34.7 g protein each, but the whey–EAA mixture contained 5.3 g more EAA). Although the latter study did not examine older adults, these results provide a plausible basis for application to older populations with suboptimal protein intakes. In further support of this point, a recent meta-analysis by Cheng et al[113] found that in low-protein-consuming (~10–15% of total energy), frail, sarcopenic, dependent elderly individuals, including those with acute or chronic conditions, supplementation with EAA outperformed high-protein oral nutritional supplements and protein-rich foods for improving fat-free mass, muscle strength, and physical function—although all of the aforementioned treatments provided benefit. Importantly, this effect was seen despite the absence of resistance training. However, it bears emphasis that this meta-analysis included patients with acute disease, musculoskeletal injury, and in post-surgical states. It is possible that improved protein and/or amino acid–based nutrition would serve to alleviate the severity of malnourishment and muscular weakness or dysfunction. These results may not necessarily translate to the prevention of sarcopenia in normally functioning older adults.

In scenarios that preclude optimal total daily protein intakes, it is reasonable to consider a full complement of EAAs as a superior supplementation strategy to merely the branched-chain amino acids. However, Casperson et al[114] reported that in older sedentary adults consuming approximately the recommended dietary allowance for protein (0.8 g/kg/day), 2 weeks of leucine supplementation (4 g/meal, 3 meals/d) resulted in greater postprandial MPS from the same low-protein meal used in the pre-trial test. It was thus concluded that leucine supplementation could serve as an energetically efficient means of improving MPS in the face of low protein intakes.

Creatine supplementation warrants consideration due to the vast and consistent evidence for its role in enhancing muscle size, strength, and power. Creatine (typically in the monohydrate form) dosed at approximately 3 g/day–5 g/day after a week-long loading phase of approximately 20 g/day–25 g/day increases intramuscular creatine levels and forms phosphocreatine, which serves as a buffer to resynthesize ATP, ultimately increasing the capacity to perform maximal effort anaerobic exercise. Rawson et al[115] compared the effects of a short-term creatine loading phase (20 g/d for 5 d) on young (20 ± 32 years) and old men (63 ± 83 years) and reported a slightly but significantly greater increase in muscle phosphocreatine in young compared with old subjects (27.6 ± 0.5 mmol kg and 25.7 ± 0.8 mmol kg, respectively). Despite this, the preponderance of evidence shows anabolic and ergogenic benefits of creatine supplementation in older subjects. A meta-analysis by Devries and Phillips[116] examined the effects of creatine on older subjects (63.6 ± 5.9 years, n = 357) in studies lasting at least 6 weeks, and concluded that creatine enhanced gains in muscle mass, strength, and functional performance over resistance training alone. A subsequent meta-analysis by Chilibeck et al[117] with nearly

double the number of subjects (57–70 years, n = 721) included studies ranging 7–52 weeks and found similar results: creatine supplementation increased muscle mass and strength in older resistance trainees.

Vitamin D is commonly known for its role in bone health, but it might also play a direct role in muscle function and metabolism, as evidenced by the presence of a vitamin D receptor in skeletal muscle,[118] whose expression declines with age.[119] Vitamin D deficiency is considered a highly prevalent public health problem, affecting up to an estimated 1 billion people globally.[120] Vitamin D deficiency is associated with muscle fiber atrophy,[121] while treatment with vitamin D has resulted in hypertrophy of type II fibers in human muscle.[122] A meta-analysis by Kalyani et al[123] found that vitamin D therapy (200 IU–1000 IU) resulted in 14% fewer falls than calcium or placebo in subjects aged 71 years–92 years. Importantly, all included studies involved subjects who had vitamin D insufficiency (≤30 ng/ml) at baseline, so these results cannot necessarily be generalized to those with adequate vitamin D status. Rosendahl-Riise et al[124] conducted a meta-analysis of the effects of vitamin D supplementation on muscle strength and mobility in community-dwelling elderly subjects. No significant improvement in handgrip strength was seen, along with a slight decrement in mobility. In 6 of the 15 studies, serum 25-hydroxyvitamin D (25(OH)D) exceeded the sufficiency threshold, and was below this threshold in 8 studies; 1 study did not report baseline levels. Antoniak et al[125] conducted a meta-analysis that examined the combined effects of resistance exercise training and vitamin D3 supplementation (400 IU/d–1920 IU/d) on musculoskeletal health in older adults (mean age 72.8 years; n = 792). An additive effect of vitamin D3 supplementation (beyond resistance training alone) was seen for the improvement of lower limb muscle strength in older adults. Interestingly, the strength benefit of D3 supplementation was not limited to subjects with insufficient baseline levels, as seen in a previous meta-analysis by Stockton et al.[126] A recent 12-month trial by Shea et al[127] found that increasing serum 25(OH)D to an average of >32 ng/ml did not affect lower-body power, strength, or lean mass in older community-dwelling adults (≥60 years). The lack of effect was seen despite low baseline 25(OH)D levels (mean value of 20.2 ng/ml). A lack of resistance training may explain these null findings. Based on the collective evidence, resistance training appears to be critical for facilitating the benefits of supplemental vitamin D to muscular function. The US recommended dietary allowance for vitamin D for individuals aged 14 years–70 years is 600 IU/day and increases to 800 IU/day at 71 years and up.[128] The Endocrine Society recommends a minimum blood 25(OH)D level of 30 ng/ml, but also suggests that 40 ng/ml may be a superior minimum target.[129] In addition, the Endocrine Society recommends a supplemental vitamin D3 intake of 1500 IU–2000 IU for adults aged 19 and above, in order to increase the likelihood of attaining a minimum 25(OH)D level of 30 ng/ml.

Another nutrient worthy of discussion is omega-3 polyunsaturated fatty acids (n-3 PUFA) – specifically, eicosapentanoic acid (EPA) and docosahexanoic acid (DHA), present in marine oils. Franceschi et al[130] coined the term "inflamm-aging," in reference to their proposed model of the aging that results from a decreased ability of the immune system to neutralize physiological stressors, and a concurrent increase in pro-inflammatory status—indicated by chronically elevated levels of inflammatory cytokines such as C-reactive protein (CRT), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNFα). The anti-inflammatory effects of n-3 PUFA intake across a broad range of clinical populations is well established.[131–133] However, a more direct connection of n-3 PUFA as an intervention for anabolic resistance is their anabolic and ergogenic potential. Smith et al[134] found that 8 weeks of n-3 PUFA supplementation (4 g/d consisting of 1.86 g EPA and 1.50 g DHA) in older subjects (71 ± 2 years) with anabolic resistance enhanced the hyperaminoacidemia–hyperinsulinemia–induced increase in MPS, as well as activation of the mTOR-p70S6K signaling pathway. A recent meta-analysis by Huang et al[135] examined the effects of n-3 PUFA supplementation on muscle mass, muscle strength, and muscle performance in elderly subjects (>60 years, n = 692), and concluded that n-3 PUFA supplementation increases muscle mass (>2 g/d resulted in a mean gain of 0.67 kg) and walking speed, particularly in trials exceeding 6 months. However, of the 10 studies in the meta-analysis, only 5 studies reported data regarding their training protocols, which casts a shade of caution on the findings. Another notable limitation was the lack of baseline assessment of nutritional status.

Beta-hydroxy-beta-methylbutyrate (HMB), a downstream metabolite of leucine, has also shown potential for mitigating age-related muscle loss, although the data are mixed on the topic. A meta-analysis by Courel-Ibáñez et al[136] reported that HMB had minimal benefits on body composition, strength, and physical performance in older individuals (50 years–80 years, n = 384). These disappointing findings were echoed in a recent meta-analysis by Jakubowski et al,[137] who found that HMB lacks efficacy for body composition and strength outcomes in younger adults (18 years–45 years). In contrast, a more broadly encompassing meta-analysis by Bear et al[138] (36 years–87 years, n = 2137) reported modest increases in muscle mass

Table 1 **Synopsis of recommendations for nutrient intake and supplementation in an older population to stave off anabolic resistance**

Population	Recommendation	Source
Daily protein requirements of older individuals (> 65 y)		
Healthy individuals in eucaloric conditions	1.0 g/kg–1.5 g/kg	Bauer et al, 2013 Deutz et al, 2014
Individuals with acute or chronic illness	1.2 g/kg–1.5 g/kg	Bauer et al, 2013 Deutz et al, 2014
Malnutrition, or severe illness or injury	Up to 2.0 g/kg	Deutz et al, 2014
Per-meal protein requirements of older individuals		
General	At least 25 g–30 g, yielding 2.5 g–2.8 g leucine	Bauer et al, 2013
Individuals seeking to maximize muscle growth or retention	0.4 g/kg–0.6 g/kg	Moore et al, 2015
Daily protein requirements not specifically directed toward older individuals, but likely apply to them[a]		
Physically active or athletic individuals seeking to maximize muscle growth in eucaloric or hypercaloric conditions	1.6 g/kg–2.2 g/kg	Morton et al, 2018 Nunes et al, 2022
Highly trained athletes seeking to maximize muscle growth or retention in hypocaloric conditions	1.6 g/kg–2.4 g/kg	Hector and Phillips, 2018
Supplementation options		
General, especially individuals consuming suboptimal total daily protein	EAA (or leucine alone) containing leucine dosed at 2 g/meal–4 g/meal	Gwin et al, 2021; Katsanos, et al, 2006
General, recreational and competitive performance athletes aiming for muscle size, strength/power increases	Creatine: 3 g/d–5 g/d	Devries and Phillips, 2014 Chilibeck et al, 2017
General	Vitamin D3: 1500 IU/d–2000 IU/d	Holick et al, 2011; Antoniak et al, 2017
General	n-3 PUFA: ∼2 g/d–3 g/d (combined EPA and DHA)	Huang et al, 2020 Smith et al, 2011

[a] There is a relative paucity of research examining protein requirements of older individuals in the referenced populations, limiting the ability to draw strong inferences on the topic. *Abbreviations:* DHA, docosahexanoic acid; EAA, essential amino acids; EPA, eicosapentanoic acid; IU, international units; n-3 PUFA, omega-3 polyunsaturated fatty acids.

and robust increases in strength. However, only 3 of the 15 studies used HMB as a single supplement (and not combined with other compounds). Of greater relevance to the present discussion, the 2 meta-analyses limited to adults aged 65 and over[139,140] drew positive conclusions about HMB's ability to preserve muscle mass and strength in elderly subjects who are frail, sarcopenic, or bed-ridden. Doses in the latter 2 meta-analyses were 2 g/day–3 g/day. In younger individuals, HMB does not seem to have benefit when daily protein intake is sufficient ($\geq$1.6 g/kg)[141]; whether this holds true in older individuals requires further study. As the current evidence stands, the inconsistent efficacy of HMB does not warrant its inclusion in Table 1.

CONCLUDING PERSPECTIVES

Individuals aged 65 and older are the fastest-growing population in the world. This carries profound implications for the global optimization of healthy aging. Increased susceptibility to anabolic resistance appears to be an inevitable consequence of advanced chronological age. However, susceptibility does not automatically equate to inevitability. The collective evidence indicates that age-related muscle anabolic resistance is exacerbated by lifestyle/habits that are largely under human control. Mitigation of age-related MPS impairment has been observed when protein/amino acid–based nutrition and exercise are properly programmed.[19,32,142] Nevertheless, it should be cautioned that age-related skeletal muscle loss is a multifactorial problem that is not always preventable by simply increasing protein intake and optimizing protein distribution. These tactics are not always feasible across older populations. Anabolic resistance in clinical populations might not be resolved by merely maximizing MPS. Impairments in protein digestion, absorption, amino acid uptake/utilization, macro- and microvascular blood flow, impaired anabolic signaling, accelerated muscle protein breakdown (especially in chronically or critically ill patients), obesity, ectopic fat accumulation, and altered gut microbiota can potentially antagonize protein feeding–based countermeasures. Despite inevitable degrees of uncertainty, evidence indicates that the onset and progression of anabolic resistance can be significantly delayed or mitigated by lifestyle habits that simultaneously facilitate exercise, body fat level, and protein/nutrient intake (and/or supplementation) conducive to this goal.

Expert panel recommendations for adequate total daily protein intake for older adults range from 1.0 g/

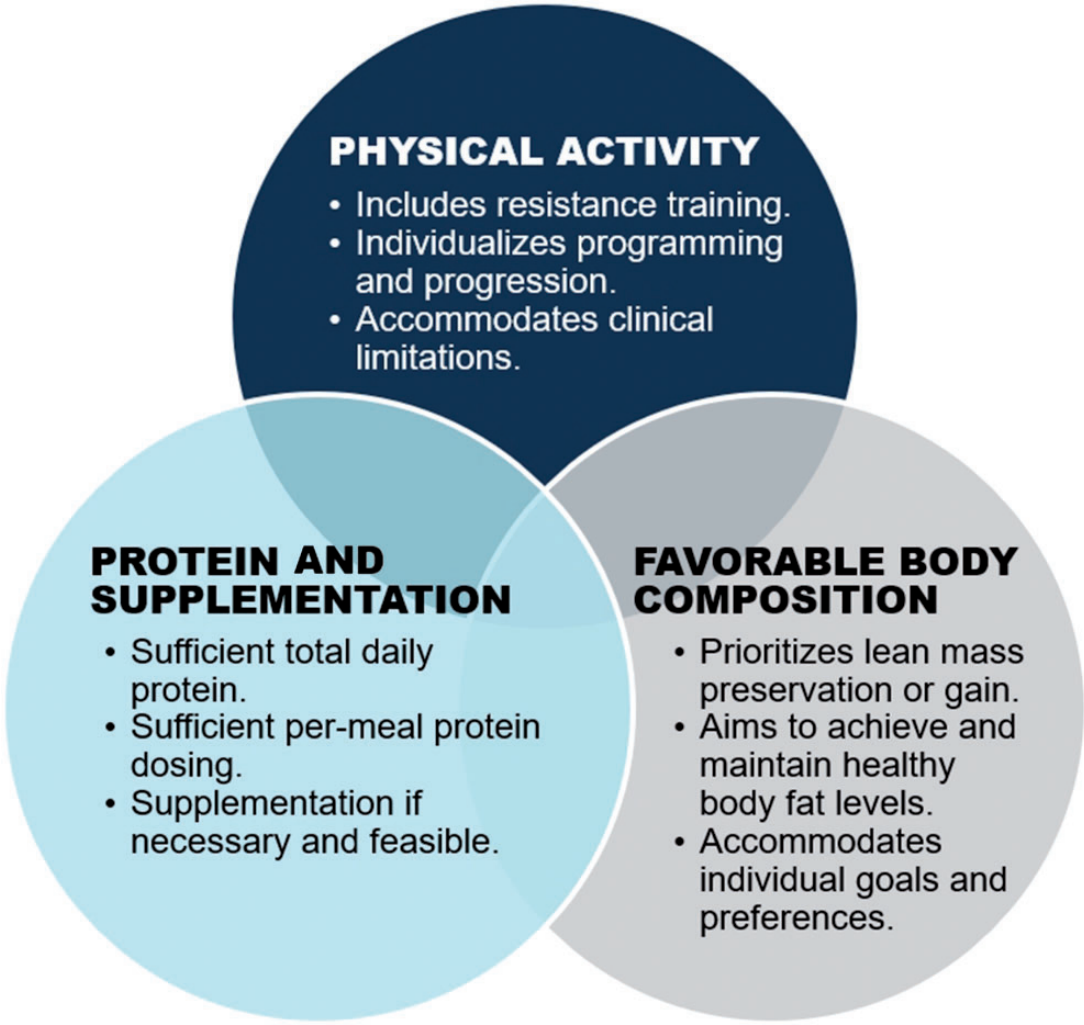

Figure 1 **Conceptual framework of the integral tactics for preventing and mitigating anabolic resistance.**

kg/day to 1.5 g/kg/day.[77,78] However, 1.6 g/kg/day–2.2 g/kg/day would seem to be a superior target for maximizing muscle anabolism in combination with structured exercise[81] while covering elevated needs during acute or chronic illness[77,78]—thereby potentially minimizing catabolism with advancing age. Per-meal protein intake is optimized at approximately 0.4 g/kg–0.6 g/kg in older individuals,[15,91] consumed at least 3–4 times through the span of the day to reach these recommended daily totals. Hypocaloric conditions compromise lean mass preservation and necessitate higher protein intakes.[143] A recent review by Hector and Phillips[83] examined the needs of elite athletes in hypocaloric conditions. Dietary caloric restriction in this population includes "making weight" for weight class–based sports, improving power-to-weight ratio, and improving body composition in aesthetic sports. It was concluded that an appropriate range of protein intake for athletes in hypocaloric conditions is 1.6 g/kg–2.4 g/kg. In addition to purposefully hypocaloric interventions to alleviate obesity, older individuals may also be prone to sustaining inadvertently hypocaloric conditions. For example, Yeung et al[144] found that more than half of the individuals referred to geriatric outpatient mobility clinics had energy and/or protein deficits. Supplementation is a viable tactic in the battle to preserve anabolic capacity. Potentially beneficial agents for this purpose that have been prolifically investigated include leucine, EAA, creatine, vitamin D, n-3 PUFA, and HMB. Table 1 provides a synopsis of recommendations for nutrient intake and supplementation in an older population to stave off anabolic resistance.[15,77,78,81,83,85,110,112,116,117,125,129,134,135]

In addition to its synergy with hyperaminoacidemia for maximizing net protein balance,[145] resistance training is more effective than endurance training at stimulating myofibrillar protein synthesis.[146] A physically active lifestyle that regularly challenges the musculoskeletal system should include a focus on maintaining favorable levels of lean and fat mass. Obesity can

exacerbate anabolic resistance,[49,56,57] so the effectiveness of nutrition and exercise for mitigating anabolic resistance is enhanced when keeping adiposity under control.

Figure 1 provides the conceptual framework of the integral tactics for preventing and mitigating anabolic resistance. Ideally, all 3 interlocking components would operate concurrently to optimize outcomes.

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REFERENCES

1. Kanasi E, Ayilavarapu S, Jones J. The aging population: demographics and the biology of aging. *Periodontol 2000.* 2016;72:13–18.
2. United Nations, Department of Economic and Social Affairs, Population Division. World Population Prospects 2019: Highlights (ST/ESA/SER.A/423). Available at: https://population.un.org/wpp/Publications/Files/WPP2019_Highlights.pdf. Updated 2019. Accessed May 2, 2022.
3. Xue QL. The frailty syndrome: definition and natural history. *Clin Geriatr Med.* 2011;27:1–15.
4. Cruz-Jentoft AJ, Bahat G, Bauer J, et al.; Writing Group for the European Working Group on Sarcopenia in Older People 2 (EWGSOP2), and the Extended Group for EWGSOP2. Sarcopenia: revised European consensus on definition and diagnosis. *Age Ageing.* 2019;48:16–31.
5. Suetta C, Haddock B, Alcazar J, et al. The Copenhagen Sarcopenia Study: lean mass, strength, power, and physical function in a Danish cohort aged 20–93 years. *J Cachexia Sarcopenia Muscle.* 2019;10:1316–1329.
6. Waters DL, Baumgartner RN, Garry PJ, et al. Advantages of dietary, exercise-related, and therapeutic interventions to prevent and treat sarcopenia in adult patients: an update. *Clin Interv Aging.* 2010;5:259–270.
7. Burton LC, Shapiro S, German PS. Determinants of physical activity initiation and maintenance among community-dwelling older persons. *Prev Med.* 1999;29:422–430.
8. Zacker RJ. Health-related implications and management of sarcopenia. *JAAPA.* 2006;19:24–29.
9. Reid KF, Pasha E, Doros G, et al. Longitudinal decline of lower extremity muscle power in healthy and mobility-limited older adults: influence of muscle mass, strength, composition, neuromuscular activation and single fiber contractile properties. *Eur J Appl Physiol.* 2014;114:29–39.
10. Shafiee G, Keshtkar A, Soltani A, et al. Prevalence of sarcopenia in the world: a systematic review and meta- analysis of general population studies. *J Diabetes Metab Disord.* 2017;16:21.
11. Mayhew AJ, Amog K, Phillips S, et al. The prevalence of sarcopenia in community-dwelling older adults, an exploration of differences between studies and within definitions: a systematic review and meta-analyses. *Age Ageing.* 2019;48:48–56.
12. Papadopoulou SK, Tsintavis P, Potsaki P, et al. Differences in the prevalence of sarcopenia in community-dwelling, nursing home and hospitalized individuals. a systematic review and meta-analysis. *J Nutr Health Aging.* 2020;24:83–90.
13. Cuthbertson D, Smith K, Babraj J, et al. Anabolic signaling deficits underlie amino acid resistance of wasting, aging muscle. *FASEB J.* 2005;19:422–424.
14. Volpi E, Mittendorfer B, Rasmussen BB, et al. The response of muscle protein anabolism to combined hyperaminoacidemia and glucose-induced hyperinsulinemia is impaired in the elderly. *J Clin Endocrinol Metab.* 2000;85:4481–4490.
15. Moore DR, Churchward-Venne TA, Witard O, et al. Protein ingestion to stimulate myofibrillar protein synthesis requires greater relative protein intakes in healthy older versus younger men. *J Gerontol A Biol Sci Med Sci.* 2015;70:57–62.
16. Fry CS, Drummond MJ, Glynn EL, et al. Aging impairs contraction-induced human skeletal muscle mTORC1 signaling and protein synthesis. *Skelet Muscle.* 2011;1:11.
17. Kumar V, Selby A, Rankin D, et al. Age-related differences in the dose–response relationship of muscle protein synthesis to resistance exercise in young and old men. *J Physiol.* 2009;587:211–217.
18. Tipton KD, Hamilton DL, Gallagher IJ. Assessing the role of muscle protein breakdown in response to nutrition and exercise in humans. *Sports Med.* 2018;48:53–64.
19. Morton RW, Traylor DA, Weijs PJM, et al. Defining anabolic resistance: implications for delivery of clinical care nutrition. *Curr Opin Crit Care.* 2018;24:124–130.
20. Abdulla H, Smith K, Atherton PJ, et al. Role of insulin in the regulation of human skeletal muscle protein synthesis and breakdown: a systematic review and meta-analysis. *Diabetologia.* 2016;59:44–55.
21. Fujita S, Rasmussen BB, Cadenas JG, et al. Effect of insulin on human skeletal muscle protein synthesis is modulated by insulin-induced changes in muscle blood flow and amino acid availability. *Am J Physiol Endocrinol Metab.* 2006;291:E745–54.
22. Biolo G, Declan Fleming RY, Wolfe RR. Physiologic hyperinsulinemia stimulates protein synthesis and enhances transport of selected amino acids in human skeletal muscle. *J Clin Invest.* 1995;95:811–819.
23. Rasmussen BB, Fujita S, Wolfe RR, et al. Insulin resistance of muscle protein metabolism in aging. *FASEB J.* 2006;20:768–769.
24. Meneilly GS, Elliot T, Bryer-Ash M, et al. Insulin-mediated increase in blood flow is impaired in the elderly. *J Clin Endocrinol Metab.* 1995;80:1899–1903.
25. Wilkes EA, Selby AL, Atherton PJ, et al. Blunting of insulin inhibition of proteolysis in legs of older subjects may contribute to age-related sarcopenia. *Am J Clin Nutr.* 2009;90:1343–1350.
26. Möller-Loswick AC, Zachrisson H, Hyltander A, et al. Insulin selectively attenuates breakdown of nonmyofibrillar proteins in peripheral tissues of normal men. *Am J Physiol.* 1994;266:E645–52.
27. Fujita S, Glynn EL, Timmerman KL, et al. Supraphysiological hyperinsulinaemia is necessary to stimulate skeletal muscle protein anabolism in older adults: evidence of a true age-related insulin resistance of muscle protein metabolism. *Diabetologia.* 2009;52:1889–1898.
28. Boirie Y, Gachon P, Beaufrère B. Splanchnic and whole-body leucine kinetics in young and elderly men. *Am J Clin Nutr.* 1997;65:489–495.
29. Watson MD, Cross BL, Grosicki GJ. Evidence for the contribution of gut microbiota to age-related anabolic resistance. *Nutrients.* 2021;13:706.
30. Raj T, Dileep U, Vaz M, et al. Intestinal microbial contribution to metabolic leucine input in adult men. *J Nutr.* 2008;138:2217–2221.
31. Zeng MY, Inohara N, Nuñez G. Mechanisms of inflammation-driven bacterial dysbiosis in the gut. *Mucosal Immunol.* 2017;10:18–26.
32. Shad BJ, Thompson JL, Breen L. Does the muscle protein synthetic response to exercise and amino acid-based nutrition diminish with advancing age? A systematic review. *Am J Physiol Endocrinol Metab.* 2016;311:E803–E817.
33. Atherton PJ, Kumar V, Selby AL, et al. Enriching a protein drink with leucine augments muscle protein synthesis after resistance exercise in young and older men. *Clin Nutr.* 2017;36:888–895.
34. Tipton KD. Nutritional support for exercise-induced injuries. *Sports Med.* 2015;45(suppl 1):S93–104.
35. Wall BT, Dirks ML, van Loon LJ. Skeletal muscle atrophy during short-term disuse: implications for age-related sarcopenia. *Ageing Res Rev.* 2013;12:898–906.
36. Wall BT, Dirks ML, Snijders T, et al. Substantial skeletal muscle loss occurs during only 5 days of disuse. *Acta Physiol.* 2014;210:600–611.
37. Kilroe SP, Fulford J, Jackman SR, et al. Temporal muscle-specific disuse atrophy during one week of leg immobilization. *Med Sci Sports Exerc.* 2020;52:944–954.
38. Wall BT, Dirks ML, Snijders T, et al. Short-term muscle disuse lowers myofibrillar protein synthesis rates and induces anabolic resistance to protein ingestion. *Am J Physiol Endocrinol Metab.* 2016;310:E137–E147.
39. Biolo G, Pišot R, Mazzucco S, et al. Anabolic resistance assessed by oral stable isotope ingestion following bed rest in young and older adult volunteers: relationships with changes in muscle mass. *Clin Nutr.* 2017;36:1420–1426.
40. Suetta C, Frandsen U, Mackey AL, et al. Ageing is associated with diminished muscle re-growth and myogenic precursor cell expansion early after immobility-induced atrophy in human skeletal muscle. *J Physiol.* 2013;591:3789–3804.
41. McGlory C, von Allmen MT, Stokes T, et al. Failed recovery of glycemic control and myofibrillar protein synthesis with 2 wk of physical inactivity in overweight, prediabetic older adults. *J Gerontol A Biol Sci Med Sci.* 2018;73:1070–1077.

42. Breen L, Stokes KA, Churchward-Venne TA, et al. Two weeks of reduced activity decreases leg lean mass and induces "anabolic resistance" of myofibrillar protein synthesis in healthy elderly. *J Clin Endocrinol Metab*. 2013;98:2604–2612.
43. Harvey JA, Chastin SF, Skelton DA. How sedentary are older people? A systematic review of the amount of sedentary behavior. *J Aging Phys Act*. 2015;23:471–487.
44. Westerterp KR, Meijer EP. Physical activity and parameters of aging: a physiological perspective. *J Gerontol A Biol Sci Med Sci*. 2001;56(Spec No 2):7–12.
45. Inoue Y, Qin B, Poti J, et al. Epidemiology of obesity in adults: latest trends. *Curr Obes Rep*. 2018;7:276–288.
46. Apovian CM. Obesity: definition, comorbidities, causes, and burden. *Am J Manag Care*. 2016;22:S176–S185.
47. Beals JW, Burd NA, Moore DR, et al. Obesity alters the muscle protein synthetic response to nutrition and exercise. *Front Nutr*. 2019;6:87.
48. Morgan PT, Smeuninx B, Breen L. Exploring the impact of obesity on skeletal muscle function in older age. *Front Nutr*. 2020;7:569904.
49. Barazzoni R, Bischoff S, Boirie Y, et al. Sarcopenic obesity: time to meet the challenge. *Obes Facts*. 2018;11:294–305.
50. Guillet C, Delcourt I, Rance M, et al. Changes in basal and insulin and amino acid response of whole body and skeletal muscle proteins in obese men. *J Clin Endocrinol Metab*. 2009;94:3044–3050.
51. Murton AJ, Marimuthu K, Mallinson JE, et al. Obesity appears to be associated with altered muscle protein synthetic and breakdown responses to increased nutrient delivery in older men, but not reduced muscle mass or contractile function. *Diabetes*. 2015;64:3160–3171.
52. Tran L, Kras KA, Hoffman N, et al. Lower fasted-state but greater increase in muscle protein synthesis in response to elevated plasma amino acids in obesity. *Obesity (Silver Spring)*. 2018;26:1179–1187.
53. Chevalier S, Burgos SA, Morais JA, et al. Protein and glucose metabolic responses to hyperinsulinemia, hyperglycemia, and hyperaminoacidemia in obese men. *Obesity (Silver Spring)*. 2015;23:351–358.
54. Kouw IWK, van Dijk JW, Horstman AMH, et al. Basal and postprandial myofibrillar protein synthesis rates do not differ between lean and obese middle-aged men. *J Nutr*. 2019;149:1533–1542.
55. Beals JW, Sukiennik RA, Nallabelli J, et al. Anabolic sensitivity of postprandial muscle protein synthesis to the ingestion of a protein-dense food is reduced in overweight and obese young adults. *Am J Clin Nutr*. 2016;104:1014–1022.
56. Smeuninx B, Mckendry J, Wilson D, et al. Age-related anabolic resistance of myofibrillar protein synthesis is exacerbated in obese inactive individuals. *J Clin Endocrinol Metab*. 2017;102:3535–3545.
57. Beals JW, Skinner SK, McKenna CF, et al. Altered anabolic signalling and reduced stimulation of myofibrillar protein synthesis after feeding and resistance exercise in people with obesity. *J Physiol*. 2018;596:5119–5133.
58. Hulston CJ, Woods RM, Dewhurst-Trigg R, et al. Resistance exercise stimulates mixed muscle protein synthesis in lean and obese young adults. *Physiol Rep*. 2018;6:e13799.
59. Ji T, Li Y, Ma L. Sarcopenic obesity: an emerging public health problem. *Aging Dis*. 2022;13:379–388.
60. Abernathy RP, Black DR. Healthy body weights: an alternative perspective. *Am J Clin Nutr*. 1996;63:448S–451S.
61. Kyle UG, Schutz Y, Dupertuis YM, et al. Body composition interpretation. Contributions of the fat-free mass index and the body fat mass index. *Nutrition*. 2003;19:597–604.
62. Hansen BH, Holme I, Anderssen SA, et al. Patterns of objectively measured physical activity in normal weight, overweight, and obese individuals (20–85 years): a cross-sectional study. *PLoS One*. 2013;8:e53044.
63. Tudor-Locke C, Brashear MM, Johnson WD, et al. Accelerometer profiles of physical activity and inactivity in normal weight, overweight, and obese U.S. men and women. *Int J Behav Nutr Phys Act*. 2010;7:60.
64. Timmerman KL, Dhanani S, Glynn EL, et al. A moderate acute increase in physical activity enhances nutritive flow and the muscle protein anabolic response to mixed nutrient intake in older adults. *Am J Clin Nutr*. 2012;95:1403–1412.
65. Snel M, Jonker JT, Schoones J, et al. Ectopic fat and insulin resistance: pathophysiology and effect of diet and lifestyle interventions. *Int J Endocrinol*. 2012;2012:983814.
66. Yki-Järvinen H. Ectopic fat accumulation: an important cause of insulin resistance in humans. *J R Soc Med*. 2002;95(suppl 42):39–45.
67. Traylor DA, Gorissen SHM, Phillips SM. Perspective: protein requirements and optimal intakes in aging: are we ready to recommend more than the recommended daily allowance? *Adv Nutr*. 2018;9:171–182.
68. Endo Y, Nourmahnad A, Sinha I. Optimizing skeletal muscle anabolic response to resistance training in aging. *Front Physiol*. 2020;11:874.
69. Chevalier S, Goulet ED, Burgos SA, et al. Protein anabolic responses to a fed steady state in healthy aging. *J Gerontol A Biol Sci Med Sci*. 2011;66:681–688.
70. Devries MC, Breen L, Von Allmen M, et al. Low-load resistance training during step-reduction attenuates declines in muscle mass and strength and enhances anabolic sensitivity in older men. *Physiol Rep*. 2015;3:e12493.
71. Phillips BE, Williams JP, Greenhaff PL, et al. Physiological adaptations to resistance exercise as a function of age. *JCI Insight*. 2017;2:e95581.
72. Clark BC, Manini TM. Sarcopenia =/= dynapenia. *J Gerontol A Biol Sci Med Sci*. 2008;63:829–834.
73. Clark BC, Manini TM. Functional consequences of sarcopenia and dynapenia in the elderly. *Curr Opin Clin Nutr Metab Care*. 2010;13:271–276.
74. Scott D, Sanders KM, Aitken D, et al. Sarcopenic obesity and dynapenic obesity: 5-year associations with falls risk in middle-aged and older adults. *Obesity (Silver Spring)*. 2014;22:1568–1574.
75. Clark LA, Manini TM, Wages NP, et al. Reduced neural excitability and activation contribute to clinically meaningful weakness in older adults. *J Gerontol A Biol Sci Med Sci*. 2021;76:692–702.
76. Lopez P, Pinto RS, Radaelli R, et al. Benefits of resistance training in physically frail elderly: a systematic review. *Aging Clin Exp Res*. 2018;30:889–899.
77. Deutz NE, Bauer JM, Barazzoni R, et al. Protein intake and exercise for optimal muscle function with aging: recommendations from the ESPEN Expert Group. *Clin Nutr*. 2014;33:929–936.
78. Bauer J, Biolo G, Cederholm T, et al. Evidence-based recommendations for optimal dietary protein intake in older people: a position paper from the PROT-AGE Study Group. *J Am Med Dir Assoc*. 2013;14:542–559.
79. Ko GJ, Obi Y, Tortorici AR, et al. Dietary protein intake and chronic kidney disease. *Curr Opin Clin Nutr Metab Care*. 2017;20:77–85.
80. Jäger R, Kerksick CM, Campbell BI, et al. International society of sports nutrition position stand: protein and exercise. *J Int Soc Sports Nutr*. 2017;14:20.
81. Morton RW, Murphy KT, McKellar SR, et al. A systematic review, meta-analysis and meta-regression of the effect of protein supplementation on resistance training–induced gains in muscle mass and strength in healthy adults. *Br J Sports Med*. 2018;52:376–384.
82. Dickerson RN. Protein requirements during hypocaloric nutrition for the older patient with critical illness and obesity: an approach to clinical practice. *Nutr Clin Pract*. 2020;35:617–626.
83. Hector AJ, Phillips SM. Protein recommendations for weight loss in elite athletes: a focus on body composition and performance. *Int J Sport Nutr Exerc Metab*. 2018;28:170–177.
84. Finger D, Goltz FR, Umpierre D, et al. Effects of protein supplementation in older adults undergoing resistance training: a systematic review and meta-analysis. *Sports Med*. 2015;45:245–255.
85. Nunes EA, Colenso-Semple L, McKellar SR, et al. Systematic review and meta-analysis of protein intake to support muscle mass and function in healthy adults. *J Cachexia Sarcopenia Muscle*. 2022;13:795–810.
86. Moore DR. Protein requirements for master athletes: just older versions of their younger selves. *Sports Med*. 2021;51:13–30.
87. Pennings B, Koopman R, Beelen M, et al. Exercising before protein intake allows for greater use of dietary protein–derived amino acids for de novo muscle protein synthesis in both young and elderly men. *Am J Clin Nutr*. 2011;93:322–331.
88. Schoenfeld BJ, Aragon AA. Is there a postworkout anabolic window of opportunity for nutrient consumption? Clearing up controversies. *J Orthop Sports Phys Ther*. 2018;48:911–914.
89. Burd NA, West DW, Moore DR, et al. Enhanced amino acid sensitivity of myofibrillar protein synthesis persists for up to 24 h after resistance exercise in young men. *J Nutr*. 2011;141:568–573.
90. Schoenfeld BJ, Aragon AA, Krieger JW. The effect of protein timing on muscle strength and hypertrophy: a meta-analysis. *J Int Soc Sports Nutr*. 2013;10:53.
91. Park S, Jang J, Choi MD, et al. The anabolic response to dietary protein is not limited by the maximal stimulation of protein synthesis in healthy older adults: a randomized crossover trial. *Nutrients*. 2020;12:3276.
92. Holwerda AM, Paulussen KJM, Overkamp M, et al. Dose-dependent increases in whole-body net protein balance and dietary protein–derived amino acid incorporation into myofibrillar protein during recovery from resistance exercise in older men. *J Nutr*. 2019;149:221–230.
93. Atherton PJ, Etheridge T, Watt PW, et al. Muscle full effect after oral protein: time-dependent concordance and discordance between human muscle protein synthesis and mTORC1 signaling. *Am J Clin Nutr*. 2010;92:1080–1088.
94. Mitchell WK, Phillips BE, Hill I, et al. Human skeletal muscle is refractory to the anabolic effects of leucine during the postprandial muscle-full period in older men. *Clin Sci (Lond)*. 2017;131:2643–2653.
95. Churchward-Venne TA, Burd NA, Mitchell CJ, et al. Supplementation of a suboptimal protein dose with leucine or essential amino acids: effects on myofibrillar protein synthesis at rest and following resistance exercise in men. *J Physiol*. 2012;590:2751–2765.
96. Baum JI, Kim IY, Wolfe RR. Protein consumption and the elderly: what is the optimal level of intake? *Nutrients*. 2016;8:359.
97. Krok-Schoen JL, Jonnalagadda SS, Luo M, et al. Nutrient intakes from meals and snacks differ with age in middle-aged and older Americans. *Nutrients*. 2019;11:1301.
98. Sui Z, Raubenheimer D, Rangan A. Exploratory analysis of meal composition in Australia: meat and accompanying foods. *Public Health Nutr*. 2017;20:2157–2165.
99. Yasuda J, Asako M, Arimitsu T, et al. Association of protein intake in three meals with muscle mass in healthy young subjects: a cross-sectional study. *Nutrients*. 2019;11:612.
100. Tieland M, Borgonjen-Van den Berg KJ, Van Loon LJ, et al. Dietary protein intake in Dutch elderly people: a focus on protein sources. *Nutrients*. 2015;7:9697–9706.

101. Holwerda AM, Trommelen J, Kouw IWK, et al. Exercise plus presleep protein ingestion increases overnight muscle connective tissue protein synthesis rates in healthy older men. *Int J Sport Nutr Exerc Metab*. 2021;31:217–226.
102. Kim J. Pre-sleep casein protein ingestion: new paradigm in post-exercise recovery nutrition. *Phys Act Nutr*. 2020;24:6–10.
103. Snijders T, Trommelen J, Kouw IWK, et al. The impact of pre-sleep protein ingestion on the skeletal muscle adaptive response to exercise in humans: an update. *Front Nutr*. 2019;6:17.
104. Norton C, Toomey C, McCormack WG, et al. Protein supplementation at breakfast and lunch for 24 weeks beyond habitual intakes increases whole-body lean tissue mass in healthy older adults. *J Nutr*. 2016;146:65–69.
105. Mamerow MM, Mettler JA, English KL, et al. Dietary protein distribution positively influences 24-h muscle protein synthesis in healthy adults. *J Nutr*. 2014;144:876–880.
106. Kim IY, Schutzler S, Schrader AM, et al. Protein intake distribution pattern does not affect anabolic response, lean body mass, muscle strength or function over 8 weeks in older adults: a randomized-controlled trial. *Clin Nutr*. 2018;37:488–493.
107. Kim IY, Schutzler S, Schrader A, et al. Quantity of dietary protein intake, but not pattern of intake, affects net protein balance primarily through differences in protein synthesis in older adults. *Am J Physiol Endocrinol Metab*. 2015;308:E21–E28.
108. Arnal MA, Mosoni L, Boirie Y, et al. Protein feeding pattern does not affect protein retention in young women. *J Nutr*. 2000;130:1700–1704.
109. Arnal MA, Mosoni L, Boirie Y, et al. Protein pulse feeding improves protein retention in elderly women. *Am J Clin Nutr*. 1999;69:1202–1208.
110. Katsanos CS, Kobayashi H, Sheffield-Moore M, et al. A high proportion of leucine is required for optimal stimulation of the rate of muscle protein synthesis by essential amino acids in the elderly. *Am J Physiol Endocrinol Metab*. 2006;291:E381–E387.
111. Bukhari SS, Phillips BE, Wilkinson DJ, et al. Intake of low-dose leucine-rich essential amino acids stimulates muscle anabolism equivalently to bolus whey protein in older women at rest and after exercise. *Am J Physiol Endocrinol Metab*. 2015;308:E1056–E1065.
112. Gwin JA, Church DD, Hatch-McChesney A, et al. Essential amino acid–enriched whey enhances post-exercise whole-body protein balance during energy deficit more than iso-nitrogenous whey or a mixed-macronutrient meal: a randomized, crossover study. *J Int Soc Sports Nutr*. 2021;18:4.
113. Cheng H, Kong J, Underwood C, et al. Systematic review and meta-analysis of the effect of protein and amino acid supplements in older adults with acute or chronic conditions. *Br J Nutr*. 2018;119:527–542.
114. Casperson SL, Sheffield-Moore M, Hewlings SJ, et al. Leucine supplementation chronically improves muscle protein synthesis in older adults consuming the RDA for protein. *Clin Nutr*. 2012;31:512–519.
115. Rawson ES, Clarkson PM, Price TB, et al. Differential response of muscle phosphocreatine to creatine supplementation in young and old subjects. *Acta Physiol Scand*. 2002;174:57–65.
116. Devries MC, Phillips SM. Supplementation during resistance training in older adults—a meta-analysis. *Med Sci Sports Exerc*. 2014;46:1194–1203.
117. Chilibeck PD, Kaviani M, Candow DG, et al. Effect of creatine supplementation during resistance training on lean tissue mass and muscular strength in older adults: a meta-analysis. *Open Access J Sports Med*. 2017;8:213–226.
118. Simpson RU, Thomas GA, Arnold AJ. Identification of 1,25-dihydroxyvitamin D3 receptors and activities in muscle. *J Biol Chem*. 1985;260:8882–8891.
119. Bischoff-Ferrari HA, Borchers M, Gudat F, et al. Vitamin D receptor expression in human muscle tissue decreases with age. *J Bone Miner Res*. 2004;19:265–269.
120. Sizar O, Khare S, Goyal A, et al. Vitamin D Deficiency. Treasure Island, FL: StatPearls Publishing LLC; 2022.
121. Bang W-S, Lee D-H, Kim K-T, et al. Relationships between vitamin D and paraspinal muscle: human data and experimental rat model analysis. *Spine J*. 2018;18:1053–1061.
122. van der Meijden K, Bravenboer N, Dirks NF, et al. Effects of 1,25(OH)2 D3 and 25(OH)D3 on C2C12 myoblast proliferation, differentiation, and myotube hypertrophy. *J Cell Physiol*. 2016;231:2517–2528.
123. Kalyani RR, Stein B, Valiyil R, et al. Vitamin D treatment for the prevention of falls in older adults: systematic review and meta-analysis. *J Am Geriatr Soc*. 2010;58:1299–1310.
124. Rosendahl-Riise H, Spielau U, Ranhoff AH, et al. Vitamin D supplementation and its influence on muscle strength and mobility in community-dwelling older persons: a systematic review and meta-analysis. *J Hum Nutr Diet*. 2017;30:3–15.
125. Antoniak AE, Greig CA. The effect of combined resistance exercise training and vitamin D(3) supplementation on musculoskeletal health and function in older adults: a systematic review and meta-analysis. *BMJ Open*. 2017;7:e014619.
126. Stockton KA, Mengersen K, Paratz JD, et al. Effect of vitamin D supplementation on muscle strength: a systematic review and meta-analysis. *Osteoporos Int*. 2010;22:859–871.
127. Shea MK, Fielding RA, Dawson-Hughes B. The effect of vitamin D supplementation on lower-extremity power and function in older adults: a randomized controlled trial. *Am J Clin Nutr*. 2019;109:369–379.
128. Ross AC, Taylor CL, Yaktine AL, et al. Institute of Medicine, Food and Nutrition Board. Dietary Reference Intakes for Calcium and Vitamin D. Washington, DC: National Academy Press; 2010.
129. Holick MF, Binkley NC, Bischoff-Ferrari HA, et al.; Endocrine Society. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab*. 2011;96:1911–1930.
130. Franceschi C, Bonafè M, Valensin S, et al. Inflamm-aging. An evolutionary perspective on immunosenescence. *Ann N Y Acad Sci*. 2000;908:244–254.
131. Gioxari A, Kaliora AC, Marantidou F, et al. Intake of ω-3 polyunsaturated fatty acids in patients with rheumatoid arthritis: a systematic review and meta-analysis. *Nutrition*. 2018;45:114–124.e4.
132. Natto ZS, Yaghmoor W, Alshaeri HK, et al. Omega-3 fatty acids effects on inflammatory biomarkers and lipid profiles among diabetic and cardiovascular disease patients: a systematic review and meta-analysis. *Sci Rep*. 2019;9:18867–18019.
133. Goldberg RJ, Katz J. A meta-analysis of the analgesic effects of omega-3 polyunsaturated fatty acid supplementation for inflammatory joint pain. *Pain*. 2007;129:210–223.
134. Smith GI, Atherton P, Reeds DN, et al. Dietary omega-3 fatty acid supplementation increases the rate of muscle protein synthesis in older adults: a randomized controlled trial. *Am J Clin Nutr*. 2011;93:402–412.
135. Huang YH, Chiu WC, Hsu YP, et al. Effects of omega-3 fatty acids on muscle mass, muscle strength and muscle performance among the elderly: a meta-analysis. *Nutrients*. 2020;12:3739.
136. Courel-Ibáñez J, Vetrovsky T, Dadova K, et al. Health benefits of β-Hydroxy-β-Methylbutyrate (HMB) supplementation in addition to physical exercise in older adults: a systematic review with meta-analysis. *Nutrients*. 2019;11:2082.
137. Jakubowski JS, Nunes EA, Teixeira FJ, et al. Supplementation with the Leucine Metabolite β-hydroxy-β-methylbutyrate (HMB) does not improve resistance exercise–induced changes in body composition or strength in young subjects: a systematic review and meta-analysis. *Nutrients*. 2020;12:1523.
138. Bear DE, Langan A, Dimidi E, et al. β-Hydroxy-β-methylbutyrate and its impact on skeletal muscle mass and physical function in clinical practice: a systematic review and meta-analysis. *Am J Clin Nutr*. 2019;109:1119–1132.
139. Oktaviana J, Zanker J, Vogrin S, et al. The effect of β-hydroxy-β-methylbutyrate (HMB) on sarcopenia and functional frailty in older persons: a systematic review. *J Nutr Health Aging*. 2019;23:145–150.
140. Wu H, Xia Y, Jiang J, et al. Effect of beta-hydroxy-beta-methylbutyrate supplementation on muscle loss in older adults: a systematic review and meta-analysis. *Arch Gerontol Geriatr*. 2015;61:168–175.
141. Holland BM, Roberts BM, Krieger JW, et al. Does HMB enhance body composition in athletes? A systematic review and meta-analysis. *J Strength Cond Res*. 2022;36:585–592.
142. Moro T, Brightwell CR, Deer RR, et al. Muscle protein anabolic resistance to essential amino acids does not occur in healthy older adults before or after resistance exercise training. *J Nutr*. 2018;148:900–909.
143. Mettler S, Mitchell N, Tipton KD. Increased protein intake reduces lean body mass loss during weight loss in athletes. *Med Sci Sports Exerc*. 2010;42:326–337.
144. Yeung SSY, Trappenburg MC, Meskers CGM, et al. Inadequate energy and protein intake in geriatric outpatients with mobility problems. *Nutr Res*. 2020;84:33–41.
145. Biolo G, Tipton KD, Klein S, et al. An abundant supply of amino acids enhances the metabolic effect of exercise on muscle protein. *Am J Physiol*. 1997;273:E122–E129.
146. Wilkinson SB, Phillips SM, Atherton PJ, et al. Differential effects of resistance and endurance exercise in the fed state on signalling molecule phosphorylation and protein synthesis in human muscle. *J Physiol*. 2008;586:3701–3717.