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REVIEWS: CURRENT TOPICS

Dietary implications on mechanisms of sarcopenia: roles of protein, amino acids and antioxidants

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Abstract

Sarcopenia, the age-related loss of muscle mass and strength, is a fundamental cause of frailty, functional decline and disability. In the year 2000, $18.5 billion in health care costs were directly attributable to sarcopenia. This economic burden will increase dramatically as the elderly population grows over the next decade. The primary causes of sarcopenia include a sedentary lifestyle and malnutrition. While resistance training appears to be a promising intervention, older individuals exhibit a blunted hypertrophic response to exercise stimuli. It has been posited that this decrement in regenerative capacity may be due to the loss of postprandial anabolism as well as an increase in reactive oxygen species. As such, a combination of resistance training and nutritional interventions may be a promising candidate in combating sarcopenia. Nevertheless, the mechanisms by which the manipulation of dietary variables may improve the sarcopenic condition are not well understood. To address this gap in extant knowledge, this review will examine the effects of protein, amino acid and/or antioxidant intake on sarcopenia both at rest and following resistance training exercise.

Keywords: Aging; Skeletal muscle; Nutritional intervention; Resistance exercise training; Regenerative capacity; Protein metabolism; Essential amino acids; Anabolic resistance; Leucine; Oxidative stress

1. Introduction

The human body undergoes remarkable changes during the aging process. One significant alteration is the gradual loss of skeletal muscle after the fifth decade of life, known as sarcopenia [1]. Sarcopenia is a multifactorial age-related disease associated with sedentary lifestyles [2], malnutrition [3] and loss of anabolic and anticatabolic responsiveness to changes in extracellular amino acid concentrations [4], as well as an increase in abnormal reactive oxygen species (ROS) [5]. Sarcopenia is characterized by selective atrophy and loss of type II myofibers [6,7], which are associated with a decrement in strength [8], a greater potential of disability and functional impairment in activities of daily living [2,9], insulin resistance [10], an increased incidence of falls and hip fractures [11–13]. The functional limitations and impairments due to sarcopenia reduce quality of life and compromise functional independence throughout senescence [2]. As the number of individuals aged 65 years or older increase from 13% of the United States population in 2000 to 20% in 2030 [14], a parallel increase of $2 to $6 billion in hip fracture expenditures is projected to occur [11]. Therefore, an understanding of the factors that may delay or possibly reverse sarcopenia is critical for improving the quality of life in elderly populations as well as for potentially decreasing the estimated health care expenditures due to the aging population.

While its etiology and underlying mechanisms are complicated, the progression of sarcopenia may be primarily driven by a failing compensatory effort within skeletal muscles to stave off degenerative/deteriorative processes. The catabolic alterations engendered by these degenerative processes impair the myogenic mechanisms responsible for maintenance of muscle mass and muscle protein turnover [6,15,16]. It is well established that interventions, which have increased physical activity through long-term resistance exercise training (RET), are particularly effective in combating sarcopenia through their capacity to enhance strength, power and mobility [17,18], and induce varying

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degrees of skeletal muscle hypertrophy [6,7]. However, our current research reveals blunted cellular and molecular responses [19,20] of myofiber hypertrophy to RET in the elderly as compared to the young. It is conceivable that the decrement in the ability to combat catabolic processes and the attenuated regenerative response to exercise evidenced in the elderly may be indicative of a general lack of compensation for the alterations in nutritional needs that accompany advanced age. Fortunately, an extensive body of research has begun to elucidate the nutritional mechanisms that underlie sarcopenia. Therefore, the purpose of this review was to enhance our understanding of possible dietary alterations that may counter sarcopenia and the mechanisms by which they are currently thought to operate. The paper is divided into three main sections including (1) optimizing protein and amino acid feedings in the elderly; (2) the effects of amino acid intake on impaired regenerative capacity in response to exercise; and (3) the role of oxidative stress in age-related muscle loss and the possible therapeutic effects of dietary supplements with antioxidant properties on countering this phenomenon.

2. Optimizing protein and amino acid feedings in the elderly

There is a substantial body of research suggesting that over 80% of the stimulatory effect on protein synthesis observed after a meal can be attributed to amino acids [21–23]. In addition to their role in protein synthesis, amino acids also play a role in the regulation of protein breakdown [24–26]. Ultimately, these nutrients are provided by a combination of total protein intake and supplementation of individual amino acids. The following three subsections will discuss possible nutritional mechanisms relative to protein and amino acid consumption, which are posited to explain a portion of the variance of sarcopenia. These include an overall inadequate protein intake in aging populations, resistance to protein synthesis following amino acid feedings alone or in combination with carbohydrates and a blunted ability to lower protein degradation after the consumption of a meal.

2.1. Protein requirements in aging populations

A combined analysis of several key nitrogen balance studies in aging populations (56–80 years of age) has indicated greater protein needs for the elderly (1.14 g kg^{-1} day^{-1}) relative to the young (0.8 g kg^{-1} day^{-1}) [27]. This research [27] provides critical information in terms of inadequate protein intake as a possible mechanism underlying sarcopenia, particularly because protein intake has been reported as inversely proportional to age [28]. It is estimated that 50% of the elderly consume less than 1.14 g kg^{-1} day^{-1} of protein, while 25% consume less than the recommended daily allowance (RDA). Furthermore, older individuals whose consumption levels approach the RDA are at greater risk for disease than those consuming more than 1.2 g kg^{-1} day^{-1} of protein [28].

To date, the gold standard for assessing the effects of RDA protein consumption on aging muscle is through the 'adaptation' and 'accommodation' paradigm. Contextually, 'adaptation' can be defined as a steady state following a change in protein metabolism in response to a change in protein intake, which may enhance or not compromise physiological function [29]. In contrast, 'accommodation' takes place via non-steady-state metabolic changes in response to a decreased protein intake that occurs to conserve protein, but only through compromise or loss in physiological function [29]. With the use of this paradigm to assess the effects of consuming the RDA for protein in older individuals, the results of a 14-week study [29] indicated that nitrogen excretion continually decreased from Weeks 2 to 14 (21%), suggesting that subjects had not yet reached a steady state. These decrements in nitrogen excretion (21%) were significantly correlated (r=0.83) with decreased mid-thigh muscle cross-sectional area (−1.7 cm^2), suggesting that the RDA for protein leads to metabolic accommodation. More recent research examining the mechanisms explaining accommodation [30] suggests that inadequate protein intake is associated with up-regulation of transcripts related to the negative control of proliferation and with down-regulation of transcripts associated with muscle stem cell (i.e., satellite cell) proliferation and myosin light and heavy chain formation.

2.2. Anabolic resistance to amino acid feedings in the elderly

Skeletal muscle mass is relatively constant during young to middle-aged adulthood [31], suggesting that the net accretion of proteins during absorptive states (i.e., postprandial, following feeding) is equally balanced by net catabolism during fasting states [8]. However, in the elderly sarcopenic population, changes in protein turnover result in a net protein balance which favors a slow but continual state of net breakdown [4,8,32,33]. The following subsections address the mechanisms which underlie these age-related changes in protein turnover.

2.2.1. Age-related changes in protein synthesis

No differences exist in protein balance in the elderly relative to the young postabsorptive [8] or following administration of either 30 g of beef protein or 15 g of essential amino acids (EAAs) [34,35]. However, when given roughly half this amount of EAAs (6.7 g), the overall protein synthetic response is blunted in the elderly relative to the young [4,33]. This anabolic resistance is currently thought to result from a decrease in leucine sensitivity and may be countered by increasing the proportion of this amino acid in the diet [4]. For example, when a 6.7-g bolus of EAAs enriched with leucine (46% leucine compared to the normal 26% leucine found in whey) is given to the elderly, protein synthesis is fully restored [4].

It appears that a number of factors are responsible for the observed anabolic resistance (Fig. 1). Initially, the elderly appear to demonstrate declines in both the capacity (RNA/total protein) and efficiency (muscle protein synthesis/RNA) for skeletal muscle protein synthesis [36].

Changes in the efficiency of protein synthesis are thought to be mediated through impairments in mammalian target of rapamycin (mTOR)-dependent increases in translation initiation [37]. mTOR is regulated by Ras homologue enriched in brain (RHEB) guanosine triphosphate phosphatase (GTPase), which is active when bound to GTP [38]. It is suggested that RHEB binds directly to mTOR, and the strength of this association is proportional to mTOR's kinase activity [38]. RHEB is positively regulated by amino acids, insulin-like growth factor-1 (IGF-1) and is inhibited by AMP-activated protein kinase (AMPK) [4]. IGF-1 and AMPK are thought to control RHEB by inhibiting and promoting the formation of tuberous sclerosis complex 1 and 2 (TSC1/2), respectively [39]. TSC2 is a GTPase-activating protein for RHEB, functioning to lower its capacity to stimulate mTOR kinase activity, while TSC1 prevents TSC2 ubiquitination.

Total mTOR and p70S6K concentrations in older skeletal muscle were recently shown to be approximately 50% lower than those in the young [36]. Moreover, the phosphorylative status of these kinases is blunted in the elderly relative to the young for any given bolus of amino acids. This phenomenon is thought to be at least partly mediated by a fourfold greater concentration of the muscle-wasting signaling protein nuclear factor kappa beta (NF-κβ) [36] and a five-fold greater activity of AMPK in old vs. young rats [40]. Additionally, both the circulating and intramuscular levels of IGF-1 are depressed in elderly relative to young participants [6].

In summary, anabolic resistance to amino acid feedings appears to be explained by an assortment of factors,

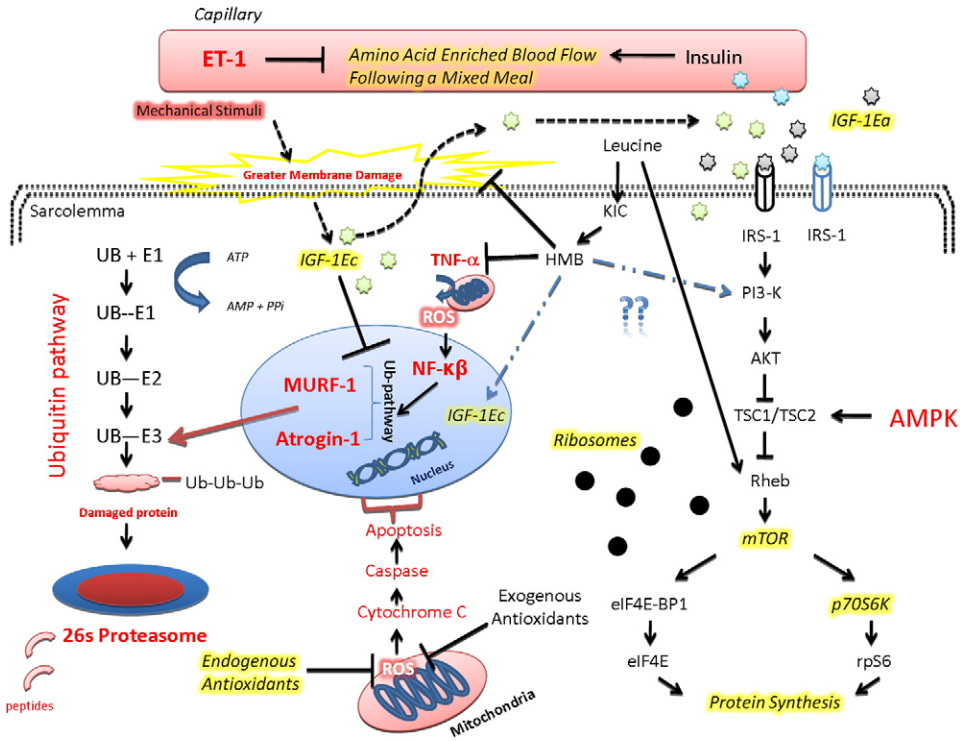

Fig. 1. Potential mechanisms by which nutritional interventions may influence protein metabolism and apoptosis in sarcopenic muscles. The figure demonstrates a decrease in factors that positively influence protein balance (italicized and highlighted) and an increase in factors that negatively influence protein balance and apoptosis (red font). Positive regulators of protein synthesis include mechanical stimuli, which increase IGF-1 Ea (blue stars) and Ec (green stars) as well as insulin (blue stars), both of which activate mTOR through phosphoinositol-3 kinase (PI3K), V-akt murine thymoma viral oncogene homolog (AKT)-dependent inhibition of the formation of tuberous sclerosis complex (TSC) 1 and 2. mTOR increases protein synthesis through hyperphosphorylating eukaryotic initiation factor binding protein 1 (eIF4E-BP1), thereby freeing up eIF-4E to initiate translation, and by phosphorylating ribosomal protein S6 (rpS6) kinase (p70S6K). Negative regulators of protein synthesis include adenosine monophosphate kinase (AMPK), which enhances formation of the TSC1/TSC2 complex, and endothelin-1 (ET-1), which antagonizes insulin-mediated increases in blood flow. The ubiquitin (Ub) pathway increases protein degradation via tagging the substrate to be degraded with multiple Ub molecules via the successive action of Ub-activating enzyme (E1), Ub-conjugating enzyme (E2) and Ub ligase (E3). The Ub pathway is positively influenced by tumor necrosis factor alpha (TNF-α), which increases Ub-pathway expression via an NF-κβ-dependent mechanism. Negative regulators of protein breakdown include IGF-1Ea and C. Leucine has dual effects on protein balance via a Ras homologue enriched in brain (Rheb)-dependent increase in protein synthesis, as well as by potentially inhibiting Ub-pathway expression via its conversion to β-hydroxy-β-methylbutyrate (HMB). Apoptosis is positively affected by ROS-mediated activation of the caspase pathway and is inhibited by both endogenous and exogenous antioxidants. Arrows (↑; solid — well established; dashed — not well established) indicate stimulation, and blocked lines (T) indicate inhibition.

including a decreased capacity and efficiency for protein synthesis, the latter of which is likely explained by decreased mTOR and p70S6K, and a concomitant decrease and increase in positive (e.g., IGF-1) and negative regulators (e.g., AMPK) of this pathway, respectively (Fig. 1).

2.2.2. Age-related impairment in protein synthesis following a mixed meal

Conditions which demonstrate impaired insulin action (e.g., type II diabetes) or low insulin concentrations (e.g., type I diabetes) are associated with muscle wasting and impaired protein metabolism [41]. The incidence of insulin resistance increases with age, likely explaining, at least in part, the changes in muscle tissue with age [42]. Insulin's effects on protein synthesis are mediated in part by a nitric oxide (NO)-dependent increase in skeletal muscle blood flow [42]. Thus, young individuals increase skeletal muscle protein synthesis following a meal containing both the insulin secretagogue glucose and amino acids compared to amino acids alone [32]. Paradoxically, the elderly demonstrate decreases in both blood flow and protein synthesis following the addition of glucose to amino acid feedings [43]. Impaired vasodilatory responses in the elderly may be explained by greater vascular endothelin-1 (ET-1) concentrations relative to younger subjects [42]. Moreover, this powerful vasoconstrictor and antagonist of endothelial NO synthase is inversely related to skeletal muscle blood flow (r=0.7) following insulin administration [42].

One possible intervention to enhance protein synthesis in the elderly is through incorporation of aerobic exercise, which has been demonstrated to decrease ET-1 concentrations independent of age [42]. Furthermore, 45 min of moderate intensity treadmill running in the elderly (70 years) was able to simultaneously decrease ET-1 and restore insulin-induced increases in protein synthesis [42]. These findings may indicate that the elderly can enhance their capacity to utilize nutrients through aerobic exercise.

2.2.3. Age-related changes in protein breakdown

The elderly appear to have slightly elevated proteolysis in a fasting state relative to the young [8]. The ubiquitin-proteasome-proteolytic pathway (Ub pathway) appears to be predominantly responsible for the degradation of skeletal muscle proteins [44]. Briefly, the system is thought to work through (a) activating Ub molecule(s) through the Ub-activating enzyme (E1); (b) covalent attachment via Ub-conjugating enzyme (E2) of the ubiquitin molecule(s) to the protein targeted for termination; and (c) transfer by Ub-protein ligase (E3) from E2 to a target proteasome complex (26-proteasome complex) for degradation [45] (Fig. 1). Recently, Combaret et al. [46] reported an inhibition of the Ub pathway after feeding in young (8 months), but not older (22-month), rats. These impairments in protein breakdown were associated with increased mRNA expression of Ub, the 26s proteasome complex, E2 ligase and tumor necrosis factor-α (TNF-α). The mechanisms of increased expression of the Ub pathway appear to be due to the general increase in TNF-α signaling [47] (Fig. 1) which activates NF-κβ after binding to the type 1 TNF-α receptor [48]. This is important, as nuclear accumulation of NF-κβ appears to be a critical step in the transcription of components necessary for the Ub pathway [49] (Fig. 1).

Short-term supplementation of meals with 5% leucine has been demonstrated to completely reverse age-related increases in proteasome activities and rates of substrate ubiquitination, while chronic (10 days) supplementation fully blunted the increased mRNA expression of the Ub pathway seen in older rats [46]. Leucine's effects on proteolysis are maximized at 10–20 times (5–10 mM L^{-1}) the concentration required for leucine to maximally stimulate protein synthesis. Thus, it is probable that these effects are partly mediated by leucine's conversion to β-hydroxy-β-methylbutyrate (HMB) which has been demonstrated to decrease total proteolysis and inhibit expression of NF-κβ [44]. Moreover, Baier et al. [50] found that an HMB-containing supplement compared to a placebo without exercise increased limb circumference, grip strength, overall protein balance and lean body mass (LBM) in 76 elderly men and women (76 years).

Overall, there is evidence of slightly elevated proteolysis at rest and an impaired ability to depress this process following meal consumption in the elderly. It is likely that these age-related changes are explained by greater expression of the Ub pathway. Dietetic implications include increasing the overall proportion of EAAs (particularly leucine) in the diets of aging populations, as well as potentially supplementing diets with the leucine metabolite HMB.

3. Effects of amino acid intake on impaired regenerative capacity in the elderly

3.1. Impaired regenerative capacity in aged muscle following resistance training

Although RET is a promising strategy for countering sarcopenia, our current research demonstrates that older adults (60–75 years) exhibit blunted cellular and molecular responses [19,20] of myofiber hypertrophy to RET, as compared to young individuals (20–35 years). These results may be partially explained by a greater susceptibility to load-induced myofiber damage and/or an attenuated regenerative capacity [51] in response to consecutive resistance exercise bouts. Our findings are supported by current evidence indicating limited myofiber plasticity at the single-muscle fiber level in older males (>80 years) in response to RET [52].

3.1.1. Age-related attenuations in mitogenic and myogenic capacity

The mechanisms of repair, regeneration and hypertrophy of myofibers are largely dependent on activation of muscle

stem cells (i.e., satellite cells) [6,53–55]. One of the mechanisms by which satellite cells operate is to increase myofiber nuclei [6]. According to the myonuclear domain theory, each myonucleus is responsible for the transcriptional regulation of a limited region of cytoplasm [6]. We have expanded this concept by proposing a theoretical myonuclear ceiling in which a myonuclear domain (fiber area per myonucleus) can only expand to an area of ~2000 μm^2 prior to the requisite addition of nuclei for continued growth [6]. In our previous human study, employing the direct quantification of the myonuclear domain and number of satellite cells in 26 young (27.0±1 years, 50% women) and 26 older (63.7±1 years, 50% women) adults following 16-week RET, we found that differences in myofiber hypertrophic responses between young and older adults were partly driven by young men who were able to increase nuclei per myofiber by 19%, with no significant changes found in older men [20].

The ability of satellite cells to break quiescence is dependent on Notch-1 signaling [53–56]. Following injury, satellite cells in young skeletal muscle increase their expression of both activated Notch-1 and its ligand, Delta-1, leading to complete or nearly complete regeneration [53]. Notch-1 activation in aged muscle following injury is blunted, and the regenerative process either fails or is incomplete [53–56]. This blunted response is stimulated when Notch-1 activation is inhibited in young mice, while forced activation of Notch-1 in old mice restores recovery to youthful levels [53]. Notch-1 appears to signal satellite cell proliferation by decreasing the expression of the cyclic-dependent kinase inhibitors (CDKs) p15, p16, p21 and p27 in satellite cells by inhibiting the binding of pSmad3 to their promoter regions [56]. In aging muscle, at least one of these CDKs is generally elevated following injury over a 5-day sampling period, as is the association of pSmad-3 with their promoter regions. Moreover, these responses are inhibited by activation of the Notch-1 signaling pathway [56]. Thus, it appears that impaired regenerative drive in old muscle is at least partly explained by an imbalance between Notch-1 (decreased in aged muscle) and pSmad-3 (elevated in aged muscle) signaling [56].

The activation and incorporation of satellite cells are also modulated by endogenous anabolic factors such as myogenic regulatory factors (MRFs: MyoD, myf-5, MRF4 and myogenin) and locally expressed autocrine/paracrine growth factors (e.g., IGF-1Ea and Ec) [20]. Sarcopenic muscle appears to exert a failing compensatory effort during gradually progressive degeneration and denervation processes [20]. We have provided support for a diminished compensatory capability by demonstrating higher basal mRNA expression of stress-sensitive MRFs in older individuals [20]. At rest, myogenin protein expression was 44% higher in older compared with young adults, and MRF4 tended to be higher in older compared to young women [7,19,20]. However, there was a greater increase in myogenin mRNA in young compared to older participants following RET. In summary, aging muscle appears to attempt to compensate for deteriorative processes by increasing the expression of factors associated with regeneration at rest. Following an actual muscle damaging stimulus, compared to the young, the elderly display a blunted capacity to effectively elevate these factors to optimize the regenerative response.

Our findings have also demonstrated a load-induced increase in expression of the muscle IGF-1 isoforms including IGF-1Ea (systemic) and IGF-1Ec (muscle specific). IGF-1Ec, also known as mechano-growth factor (MGF), is locally expressed in skeletal muscles in response to the mechanical load and is known to be a major player in signaling pathways leading to satellite cell activation for initiation of the regeneration process of damaged myofibers [20]. Concurrent with a blunted RET-induced myofiber hypertrophic response in aged human muscles, we also observed an attenuated MGF mRNA response in aged muscles following acute and chronic RET (Fig. 1).

Myostatin, a member of the transforming growth factor-beta (TGF-β) superfamily, is considered an inhibitor of satellite cell proliferation and differentiation and thus impairs muscle regeneration and growth [57,58]. It has been hypothesized that it is related to sarcopenic processes due to its roles in skeletal muscle. Although the underlying mechanisms are not well understood, myostatin has been shown to interact with Smad2 and Smad3, and it is thought that this signaling pathway modulates the expression of MyoD [58]. Moreover, a recent study suggested that Forkhead Box O1 (FoxO1) and Smad2 transcription factors control the differentiation of C_2C_{12} myoblasts by regulating myostatin mRNA and its promoters [58], while a similar model found that stimulation of myostatin increased cyclin D1 degradation, inducing cell-cycle arrest [59]. Although our previous research did not demonstrate any direct interaction between myostatin and the regulators of cell-cycle progression in young and aged muscles, we did observe a decrease in myostatin and $p27^{kip}$ with a concomitant increase in IGF-1 isoforms and cyclin D1 mRNA expression following RET [5,19]. Interestingly, following an acute loading bout, older individuals experienced a blunted response to lower myostatin mRNA levels, while cyclin B1, the positive cell-cycle regulator, increased only in young participants, which may at least partially explain the impaired regenerative capacity observed in untrained aged muscles compared to young muscles [19]. Intriguingly, however, long-term RET has ameliorated some of these age-related attenuations [5].

3.1.2. Age-related attenuations in load-induced protein synthesis/breakdown

Our data (under review) and others also clearly demonstrate a blunted protein synthetic response following muscle overload in the elderly [60], while greater muscle tissue damage leads to a prolonged period of muscle protein degradation relative to the young [40]. Diminished load-

mediated anabolic responses, including reduced responses in activation of downstream targets of Akt/mTOR signaling and phosphorylation of extracellular signal-regulated kinase in aged muscles to an acute external mechanical stimulus, have been demonstrated [61]. These blunted protein synthetic responses appear to be involved in the greater activity of phosphorylated AMPK in the elderly [40], while increased proteolytic activity in this context would relate to a loss of membrane integrity and/or increased levels of circulating inflammatory and catabolic cytokines (e.g., TNF-α) [62].

These findings clearly demonstrate that myofiber regenerative capacity is compromised in aged muscles. However, current data suggest that certain nutritional interventions such as high-protein intake or an increased intake of the branched chain amino acid (BCAA) leucine with RET may help to reverse impaired regenerative drive in sarcopenic muscle by enhancing anabolic pathways and inhibiting catabolic pathways [63]. In general, milk-based products range from a 10% (milk isolate, casein) to 12% (whey) leucine content, while meat and egg-based products contain approximately 8–9% leucine [64]. In contrast, plant-based products such as wheat protein fall below 8% leucine, suggesting they are not optimal for stimulation of skeletal muscle protein synthesis. Furthermore, if any nutrients possess the capacity to provide protective effects that sustain sarcolemmal integrity [65], they would be expected to partially or completely reverse the observed age-related deficits in net anabolism following RET. It has been documented that oral postexercise amino acid supplementation induces a synergistic effect on the contraction-induced escalation of muscle protein synthesis following an acute resistance exercise bout [66] and ultimately enhances hypertrophy in response to RET [67,68].

3.2. The importance of adequate energy intake in the elderly during RET

As previously stated, older individuals have to fight against an impaired sensitivity towards stimulating protein synthesis from nutritional intake, as well as a reduced response with regard to protein turnover following exercise [69]. It is possible that inadequate substrate to support full regeneration underlies age-related impairments in hypertrophy. A foundational work by Singh et al. [70] demonstrated the importance of adequate energy intake in frail elderly during RET. This research group investigated the effects of a 360-cal nutritional supplement composed of 60% carbohydrates, 17% soy protein and 23% fat alone or with a 10-week, 3 days week^{-1} RET program in 100 frail elderly men and women (72–98 years) who consumed slightly less than the RDA in energy intake. They found that the RET plus supplement group gained more than double the strength as compared to the RET-only group. Only the RET plus supplement group showed an increase in type II fiber area (10.1±9.0%). Furthermore, the changes in strength were associated with alterations in caloric consumption following supplementation (r=0.67), while changes in type II fiber area were correlated with baseline energy intake (r=0.64). Thus, it appears that a nutritional supplement (i.e., increased caloric intake) can enhance strength and muscle tissue growth in hypocaloric aging populations. Interestingly, the additional caloric intake did not alter the levels of RET-mediated muscle IGF-1 and myosin expression, suggesting no synergetic effects of the additional caloric intake on regenerative muscle capacity in the frail elderly during RET [70]. Further research suggests that undernourished frail elderly men and women who are not engaged in RET lower their daily caloric intake subsequent to a 360-cal supplement, resulting in no change in net caloric intake [71,72]. However, when supplementation is combined with RET, total caloric intake and weight increase [71,72], suggesting that RET may change satiation and appetite dynamics in the elderly. Finally, it is noteworthy that the addition of a high-protein supplement can increase muscle size and strength during RET [3] in frail elderly when a baseline diet providing the RDA in energy and protein intake is clamped.

3.2.1. The ability of increased protein supplementation to increase regeneration during RET in the elderly

A number of investigations have evaluated the effects of high-protein supplementation with and without RET on healthy and frail elderly populations [3,73,74]. For example, a 560-cal high-protein supplement increased mid-thigh muscle cross-sectional area to a greater extent than a nonsupplemented group when combined with RET in a population of healthy elderly men (61 to 72 years) [73]. The supplemented group consumed an average of 118±10 vs. 72±11 g of protein compared to the nonsupplemented group. Changes in mid-thigh muscle area were correlated with both a change in energy (r=0.7) and protein (r=0.63) intake during the 12-week program, suggesting that a high-protein supplement can enhance muscle tissue accretion in healthy participants consuming near the RDA in energy and protein. In addition, Bos et al. [3] investigated the effects of a drink containing 30 g of protein and 50 g of carbohydrate in frail undernourished elderly participant's ability to regenerate following admission to the hospital with a reported minimum of 5% bodyweight lost. Their findings suggested that raising protein intake to a moderately high range while in a eucaloric state via the use of a supplement in elderly patients was able to increase fat-free mass by 1.3 kg compared to 0.1 kg in the nonsupplemented group. Moreover, these changes were explained by an increase in fasting protein synthesis, of which 46% of the variance was explained by increased protein intake following supplementation.

The ability of EAA supplementation to reverse the blunted protein synthetic response to RET in the elderly was demonstrated by comparing RET with and without EAA supplementation. When analyzing the effects of RET alone, it was recently found that while the elderly were able to

increase protein synthesis in a dose-dependent manner to rising training intensities, these responses were blunted relative to the young [75]. The elderly are also resistant to the anabolic properties of amino acids in small boluses [4,33]. However, when large boluses of amino acids are consumed (15 g), the elderly are able to stimulate protein synthesis similar to the young [34]. A recent study found that a similar 15-g bolus of EAAs administered 1 h after RET performed at 70% of the 1 RM (repetition maximum) was able to equalize protein synthesis over a 5-h period in young and elderly participants [69]. Moreover, unlike RET alone, the phosphorylative status of all indices of the mTOR pathway increased from 1 to 6 h, with no differences observed between groups. Interestingly, however, the kinetics of protein metabolism were altered as the young increased protein synthesis from 1 to 3 h, with no apparent response in the elderly, while at 3–6 h protein synthesis had surpassed the young, equalizing the overall accretion of muscle protein. This latter result is most likely explained by a significant increase in AMPK phosphorylation status in the old at 1 and 3 h, but not in the young.

Intriguingly, a number of studies have not found benefits from protein supplementation [74,76,77]. For example, 12 g of EAAs combined with 70 g of dextrose administered immediately following RET did not affect muscle strength or size in elderly men in a 12-week program [76], and Welle et al. [77] found that a high-carbohydrate meal containing 10–15% protein did not enhance adaptations to a chronic RET program either. As discussed previously, in contrast to young individuals, the elderly appear to display a blunted protein synthetic response when insulin is greatly increased, most likely as a factor of impaired blood flow [42]. Thus, it is conceivable that meals containing high glycemic index carbohydrates such as the 70 g of dextrose discussed above resulted in large increases in insulin levels, subsequently decreasing skeletal muscle hypertrophy. This contention is supported by a study conducted by Esmarck et al. [67], who found that a protein supplement with only 7 g of carbohydrates administered immediately following RET was able to enhance the mean fiber area and cross-sectional area of the vastus lateralis and quadriceps femoris in a population of elderly adults.

3.3. Protein quality and its effect on skeletal muscle regeneration in the elderly following RET

The quality of a protein is generally defined as the capacity of the protein source to deliver EAAs to the individual [78]. In general, meat-based products contain higher EAAs than vegetable-based proteins. In this context, Campbell et al. [78] investigated the effects of an omnivorous diet compared to a lacto-ovo vegetarian diet on muscle strength and size in elderly males (51–69 years) participating in a 12-week RET program. The omnivorous diet increased LBM by 2.4 kg and decreased body fat by 1.4%. In contrast, the lacto-ovo-vegetarian diet resulted in a slight loss of LBM (−1.2 kg) and an increase in 1% body fat. These findings are in agreement with results obtained by Pannemans et al. [79], who reported that elderly women experienced a greater inhibition of protein degradation and had a higher net protein balance when consuming a diet high in animal protein (15.1% of energy; 5.0% from animal protein), as compared to those consuming a diet high in vegetable protein (14.5% of energy; 5.1% from vegetable protein).

There are a number of possible explanations for these results. First, the evidence clearly demonstrates that net protein balance increases proportionally to extracellular levels of EAAs [80]. Therefore, diets higher in EAA content would be predicted to elicit an overall greater stimulus on protein balance. A second explanation concerns the efficiency of EAA uptake by splanchnic tissue relative to the nonessential amino acids. Splanchnic uptake accounts for up to a 90% extraction rate of individual amino acids [80], effectively reducing their availability in peripheral tissues. BCAAs are unique in their ability to escape splanchnic uptake. As an illustration, after consumption of beef, more than half of the amino acids released from splanchnic tissues are BCAAs, even though beef is composed of only 20% BCAAs [81]. A further analysis of the total activity of branched-chain aminotransferase and branched-chain alpha-keto acid dehydrogenase, the enzymes responsible for the first two steps in BCAA degradation, indicates that more than 50% of the capacity to degrade BCAAs lies within the skeletal muscle tissue, with the liver accounting for only 20% of the degradation capacity. These findings suggest that, of the 20 amino acids, BCAAs have the greatest opportunity to interact with peripheral tissues, making them candidates for the regulation of both the structure and function of skeletal muscle tissue. Therefore, protein sources with lower EAA and particularly BCAA content will likely be taken up by splanchnic tissues with greater efficiency than protein with a higher content of these amino acids. For example, Martinez et al. [82] found that a legume-based diet caused severe atrophy of the gastrocnemius muscle compared to a casein-based diet in rats. These findings were attributed to lowered muscular protein synthesis in the legume-based condition, with a subsequent higher rate of liver protein synthesis.

As discussed, a growing body of evidence suggests that the EAA content of a diet affects protein accretion in elderly men and women. Because meat-based diets are higher in EAA content, the elderly are suggested to consume a diet rich in lean sources of meat-based products or to consume EAA supplements in combination with RET.

3.4. Effects of the leucine metabolite, HMB, on sarcopenic muscle during RET

Within the last decade, the importance of long-term EAA intake has been recognized as an important component in the prevention and treatment of sarcopenia [4,46,83–86]. Exogenous amino acids can stimulate net muscle protein

synthesis in sarcopenic muscle simply by increasing amino acid availability [86]. As previously explained among the EAAs, leucine has been found to be a crucial component within an amino acid complex to combat sarcopenia. The underlying mechanisms of leucine's effects can be explained by recent evidence by Han et al. [63], who demonstrated that a 30-min leucine treatment of primary myogenic muscle stem cells (satellite cells) resulted in favorable responses related to protein synthesis and myogenesis, such as increases in the phosphorylation of mTOR (+83%), p70S6K (+191%) and 4EBP-1. Interestingly, the effects of leucine treatment were similar to those of IGF-1 treatment [63], which is one of the well-known stimulators of skeletal muscle growth and satellite cell proliferation.

In addition, leucine appears to decrease muscle wasting in a number of conditions, and it mediates these effects via its conversion to HMB, as previously indicated [87]. The mechanisms of HMB activity appear to be important for aging populations that demonstrate greater load-induced myofiber damage [88], protein degradation and an attenuation of protein synthesis following exercise relative to the young [88]. Upon more detailed investigation, intramuscular HMB concentrations were suggested to provide readily available substrate for the synthesis of cholesterol, facilitating formation and stabilization of the sarcolemma [87,89]. This is thought to occur via HMB's conversion to 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA), which increases cholesterol formation by raising the activity of its rate-limiting enzyme HMG-CoA reductase. Aside from its structural properties, HMB has been demonstrated to signal the simultaneous increase and decrease in protein synthesis and proteolysis in both aging [50] and clinically cachexic conditions [44]. As previously discussed, HMB appears to at least partly exert its effects on proteolysis via depressing NF-κβ expression, while its effects on protein synthesis are mTOR dependent, the latter of which occurs through either direct stimulation of the mTOR pathway or through HMB's capacity to increase the expression of growth factors (e.g., IGF-1) [37,90].

Given its protective effects on the sarcolemma [65] and capacity to subsequently enhance and depress anabolic and catabolic pathways [91], HMB would be a good candidate as a dietary supplement to partially reverse deficits in net anabolism in sarcopenic muscle following RET. This is supported by data demonstrating that HMB combined with resistance training in an elderly group led to larger gains in lower (13% vs. 7%) and upper (13% vs. 11%) body strength than supplementation with a placebo. However, further studies are necessary to determine its optimal dosage and duration in the elderly population with and without RET.

4. The role of oxidative stress in age-related muscle loss

It is currently thought that sarcopenia is at least partly explained by the up-regulation of oxidative metabolism and the ensuing increase in abnormal ROS [92]. Accumulation of ROS can induce higher rates of cellular damage to important substances such as deoxyribonucleic acid (DNA), proteins and lipid-containing structures [93–95].

Mitochondria are the center of aerobic metabolism and are consequently a major source of ROS production [96]. As such, these structures are a target for oxidative damage. During the aging process, increased oxidative stress leads to the modification of mitochondrial DNA (mtDNA), which can prevent protein synthesis and ATP production, and result in increases in necrosis and apoptosis [97,98]. The mitochondria can induce apoptosis via cysteine-dependent aspartate-specific protease (caspase) activation [99]. Moreover, it was demonstrated that the progressive decline in muscle weight with advancing age was associated with a simultaneous increase in apoptotic DNA fragmentation, which was correlated to rising levels of cytosolic and nuclear levels of active caspase-9 in old and senescent rats [100]. In addition to mediating apoptotic processes, ROS production may affect skeletal muscle morphology and function. Specifically, the sarcopenic phenotype is characterized by chronic low-grade inflammation, resting muscle damage (e.g., extensive Z-band streaming, or zigzagging) and, in some cases, complete disarray of the myofibril architecture [70]. The mechanism may be tied to increased ROS production in aging muscle, which appears to be related to muscle damage.

ROS have been reported to be related to muscle damage and appear to modulate skeletal muscle contraction by acting on the functional status of Ca^{2+} channels [62]. Calcium is one of the major factors involved in the increase of ROS production in the mitochondria. In fact, Ca^{2+} uptake by the sarcoplasmic reticulum is reduced during aging, and this reduction serves to increase both intracellular and mitochondrial Ca^{2+}, leading to ROS generation, muscle protein oxidation and necrosis. Accumulation of Ca^{2+} in the mitochondria can serve as an impetus for opening of the permeability transition pore (PTP), which plays a critical role in mediating cell death [101]. In particular, the opening of PTP leads to damage of the outer mitochondrial membrane by increasing solutes in the mitochondrial matrix and intermembrane space [102]. The resultant dysfunctional mitochondria accelerate ROS production, eventually leading to myofiber death [99]. The impaired integrity of the outer membrane may also be explained by an increase in the ratio of pro-apoptotic (e.g., Bax, Bid) to anti-apoptotic (e.g., Bcl-2) members of the BcL-2 family. An increase in oxidative stress or other apoptotic stimuli triggers Bid to stimulate a conformational change in Bax, permitting its entry into the outer mitochondrial membrane and thereby increasing mitochondrial permeability to apoptotic factors [100,103–105]. However, this process is inhibited by BcL-2. A number of studies suggest an increase in the ratio of Bax and Bid with age in both resting and overloaded muscles [103–105]. Moreover, both the increase in Bax and Bid were associated with an increase in oxidative stress.

An additional and plausible theory for age-related impairments in muscle tissue considers the oxidative stress of satellite cells. Renault et al. [106] reported that a single bout of oxidative stress induced a loss of viability, a shorter lifespan and a substantial decrease in the proliferative capacity of satellite cells. They also demonstrated a significant decrease in the number of satellite cells in aging muscle, making it plausible that a reduction in myofiber number with age is a factor of an impaired ability to regenerate when the need arises. The increase in ROS production with age is compounded by the finding that satellite cell antioxidant capacity decreases during later years of life.

4.1. Effects of antioxidant supplementation on antioxidant systems in aged muscle

The human body is equipped with intricate antioxidant defense systems that continually manage oxidative stress. Antioxidant systems are supported by enzymatic and nonenzymatic antioxidants that work in concert to counteract ROS [107]. The major enzymatic antioxidants include superoxide dismutase [108], glutathione peroxidase (GPx), catalase (CAT) and glutathione reductase [109]. These enzymes work to improve or maintain an antioxidant balance, and to prevent oxidative damage by scavenging or preventing transformation of ROS to intracellular molecules and inhibiting their conversion to more deleterious forms. Manganese SOD located in the mitochondria and cytosolic SOD can catalyze the conversion of superoxide anions into hydrogen peroxide [110]. GPx is located in the same area as SOD isoforms, while superoxide dismutase and CAT are located in the cytosol and mitochondria of the heart where they work to convert hydrogen peroxide and organic hydroperoxide into water. However, ROS overwhelms the endogenous antioxidant defense system during the aging process, causing detrimental modifications of myofiber cellular proteins, lipids and DNA [93,111,112].

Endogenous nonenzymatic antioxidants that enhance antioxidant systems include vitamin C, E, glutathione, carotenoids, flavonoids and ubiquinones. Moreover, several trace minerals exist, including copper, iron, manganese, selenium and zinc [113–116]. These components play important roles by contributing to the antioxidant system as cofactors for antioxidant enzymes. Nunes et al. [117] reported significantly increased caspase-like activation in chicks fed a vitamin E and selenium-deficient diet. They also demonstrated that caspase-like activation and cell death in skeletal muscle are induced by oxidative stress or an antioxidant-deficient diet. Rafique et al. [118] accumulated data during a 48-week vitamin E-deficient diet in 21-day-old rats. They found significantly elevated lipid peroxidation levels, decreased cytochrome oxidase activity and an age-related decreased rate of reduced nicotinamide adenine dinucleotide coenzyme Q1 reductase activity in the gastrocnemius muscle.

The antioxidant defense system can be augmented with appropriate dietary levels of antioxidants and/or supplementation. A number of studies have been conducted in an attempt to improve antioxidant systems via exogenous administration of antioxidant supplementation [119–123]. Rebrin et al. [124] investigated the effects of supplementation with a combination of antioxidants on oxidative stress in skeletal muscle tissue homogenates and mitochondria in senescence-accelerated mice. Mice were fed vitamin E and C as well as a blend of bioflavonoids, polyphenols and carotenoids for 10 months. Antioxidant supplementation resulted in significantly elevated activities of glutathione and a reductive shift in the glutathione redox state in muscle homogenates and mitochondria compared to the control group. However, Sumien et al. [125] found that increasing skeletal muscle mitochondria α-tochopherol concentrations by 100% after supplementing 21-month-old mice with α-tocopheryl acetate for 13 weeks only tended to reduce oxidative stress. As the above findings were not statistically significant, it is possible that antioxidant supplementation can be optimized by a combination of exogenous antioxidants. For example, glutathione supplementation lowered mitochondrial DNA damage when combined with either vitamin C or E, but not when it was administered alone [126].

In addition to improving the indices of oxidative stress, a very recent paper [127] indicated that antioxidant supplementation could reverse the classic anabolic resistance to leucine discussed previously. In particular, supplementation with a mixture containing rutin, vitamin E, vitamin A, zinc and selenium in 20-month-old rats restored their reduced ability to stimulate protein synthesis following leucine administration. The mechanism behind these changes remains to be found as it was independent of changes in oxidative damage to p70S6K. However, it is conceivable that 7 weeks of antioxidant supplementation lowered inflammation or improved the energetic state of the cells, which would subsequently lower AMPK activity. Altogether, the above studies suggest that a diet supplemented with a combination of antioxidants can increase antioxidant defense, lower oxidative damage and improve protein balance during senescence.

Antioxidants are found in varying amounts in foods such as vegetables, fruits, nuts and spices. Vegetables richest in antioxidants include beans, artichokes and russet potatoes. Fruits richest in antioxidants are cranberries, blueberries and blackberries [128]. Nuts highest in antioxidants include pecans, walnuts and hazelnuts, while ground cloves, cinnamon and oregano appear to contain the greatest antioxidant status among spices [128].

5. Summary and conclusions

The purpose of this manuscript was to review plausible dietary mechanisms that may underlie the sarcopenic

phenotype. Evidence was presented that the elderly demonstrate a progressive loss of lean mass when consuming the RDA for protein (0.8 g kg^{-1} day^{-1}) and appear to ultimately require greater protein, particularly BCAA intake, per meal and per day relative to the young to achieve nitrogen balance. The elderly appear to lack the ability to increase protein synthesis and inhibit degradation following a standard RDA meal. However, these decrements in protein turnover are apparently restored after supplementation with leucine, an effect thought to be partly mediated by the amino acid's conversion to HMB.

While RET has been demonstrated to increase skeletal muscle mass in the elderly, our research strongly suggests that these responses are blunted relative to the young. The lack of responsiveness to changes in mechanical load is likely explained by impaired sarcolemmal integrity, a heightened susceptibility to muscle damage and subsequent protein breakdown, as well as a decrease in the ability to elevate protein synthesis following exercise. Increasing one's intake of high-quality protein (animal-derived products) may enhance the regenerative drive in aging muscle by concomitantly increasing protein synthesis and lowering protein degradation. Moreover, the leucine metabolite HMB was implicated as a possible derivative capable of lowering muscle damage and speeding reparative processes.

The final topic discussed was the role played by ROS in apoptosis and protein balance. An increase in ROS and a decrease in endogenous antioxidant defense mechanisms are observed with age. Data were presented that an increase in the administration of antioxidants lowered the indices of oxidative damage and apoptosis in aging populations. Moreover, recent research suggests that antioxidant administration may also reverse anabolic resistance to leucine feedings by an unknown mechanism.

In conclusion, sarcopenia appears to be mediated by a number of factors that seem to indicate changing nutritional needs with age. These changing requirements suggest the need to increase the proportion of high-quality proteins and the importance of consuming a diet rich in antioxidants. Although the importance of exercise was not the emphasis of this review, dietary interventions combined with a proper RET regimen may be the most efficient means of combating sarcopenia.

References

[1] Melton 3rd LJ, Khosla S, Crowson CS, O'Connor MK, O'Fallon MW, Riggs BL. Epidemiology of sarcopenia. J Am Geriatr Soc 2000;48:625–30.

[2] Janssen I, Heymsfield SB, Ross R. Low relative skeletal muscle mass (sarcopenia) in older persons is associated with functional impairment and physical disability. J Am Geriatr Soc 2002;50:889–96.

[3] Bos C, Benamouzig R, Bruhat A, Roux C, Mahe S, Valensi P, et al. Short-term protein and energy supplementation activates nitrogen kinetics and accretion in poorly nourished elderly subjects. Am J Clin Nutr 2000;71:1129–37.

[4] Katsanos CS, Kobayashi H, Sheffield-Moore M, Aarsland A, Wolfe RR. A high proportion of leucine is required for optimal stimulation of the rate of muscle protein synthesis by essential amino acids in the elderly. Am J Physiol Endocrinol Metab 2006;291:E381–7.

[5] Kim JS, Petrella JK, Cross JM, Bamman MM. Load-mediated downregulation of myostatin mRNA is not sufficient to promote myofiber hypertrophy in humans: a cluster analysis. J Appl Physiol 2007;103:1488–95.

[6] Petrella JK, Kim JS, Cross JM, Kosek DJ, Bamman MM. Efficacy of myonuclear addition may explain differential myofiber growth among resistance-trained young and older men and women. Am J Physiol Endocrinol Metab 2006;291:E937–46.

[7] Kosek DJ, Kim JS, Petrella JK, Cross JM, Bamman MM. Efficacy of 3 days/wk resistance training on myofiber hypertrophy and myogenic mechanisms in young vs. older adults. J Appl Physiol 2006;101: 531–44.

[8] Volpi E, Sheffield-Moore M, Rasmussen BB, Wolfe RR. Basal muscle amino acid kinetics and protein synthesis in healthy young and older men. JAMA 2001;286:1206–12.

[9] Avlund KSM, Davidsen M, Lovborg B, Rantanen T. Maximal isometric muscle strength and functional ability in daily activities among 75-year-old men and women. Scand J Med Sci Sports 1994;4:32–40.

[10] Langlois JA, Visser M, Davidovic LS, Maggi S, Li G, Harris TB. Hip fracture risk in older white men is associated with change in body weight from age 50 years to old age. Arch Intern Med 1998;158: 990–6.

[11] Kostka T. Quadriceps maximal power and optimal shortening velocity in 335 men aged 23-88 years. Eur J Appl Physiol 2005;95: 140–5.

[12] Frontera WR, Hughes VA, Lutz KJ, Evans WJ. A cross-sectional study of muscle strength and mass in 45- to 78-yr-old men and women. J Appl Physiol 1991;71:644–50.

[13] Lindle RS, Metter EJ, Lynch NA, Fleg JL, Fozard JL, Tobin J, et al. Age and gender comparisons of muscle strength in 654 women and men aged 20–93 yr. J Appl Physiol 1997;83:1581–7.

[14] Ferretti G, Antonutto G, Denis C, Hoppeler H, Minetti AE, Narici MV, et al. The interplay of central and peripheral factors in limiting maximal O_2 consumption in man after prolonged bed rest. J Physiol 1997;501:677–86.

[15] Bamman MM, Petrella JK, Kim JS, Mayhew DL, Cross JM. Cluster analysis tests the importance of myogenic gene expression during myofiber hypertrophy in humans. J Appl Physiol 2007;102:2232–9.

[16] Welle S, Totterman S, Thornton C. Effect of age on muscle hypertrophy induced by resistance training. J Gerontol A Biol Sci Med Sci 1996;51:M270–5.

[17] Kim JS, Hinchcliff KW, Yamaguchi M, Beard LA, Markert CD, Devor ST. Exercise training increases oxidative capacity and attenuates exercise-induced ultrastructural damage in skeletal muscle of aged horses. J Appl Physiol 2005;98:334–42.

[18] Petrella JK, Kim JS, Tuggle SC, Bamman MM. Contributions of force and velocity to improved power with progressive resistance training in young and older adults. Eur J Appl Physiol 2007;99: 343–51.

[19] Kim JS, Cross JM, Bamman MM. Impact of resistance loading on myostatin expression and cell cycle regulation in young and older men and women. Am J Physiol Endocrinol Metab 2005.

[20] Kim JS, Kosek DJ, Petrella JK, Cross JM, Bamman MM. Resting and load-induced levels of myogenic gene transcripts differ between older adults with demonstrable sarcopenia and young men and women. J Appl Physiol 2005;99:2149–58.

[21] Rennie MJ, Bohe J, Wolfe RR. Latency, duration and dose response relationships of amino acid effects on human muscle protein synthesis. J Nutr 2002;132:3225S–7S.

[22] Borsheim E, Tipton KD, Wolf SE, Wolfe RR. Essential amino acids and muscle protein recovery from resistance exercise. Am J Physiol Endocrinol Metab 2002;283:E648–57.

[23] Volpi E, Kobayashi H, Sheffield-Moore M, Mittendorfer B, Wolfe RR. Essential amino acids are primarily responsible for the amino

acid stimulation of muscle protein anabolism in healthy elderly adults. Am J Clin Nutr 2003;78:250–8.
[24] Ikizler TA, Pupim LB, Brouillette JR, Levenhagen DK, Farmer K, Hakim RM, et al. Hemodialysis stimulates muscle and whole body protein loss and alters substrate oxidation. Am J Physiol Endocrinol Metab 2002;282:E107–16.
[25] Boirie Y, Dangin M, Gachon P, Vasson MP, Maubois JL, Beaufrere B. Slow and fast dietary proteins differently modulate postprandial protein accretion. Proc Natl Acad Sci U S A 1997;94:14930–5.
[26] Zanchi NE, Nicastro H, Lancha Jr AH. Potential antiproteolytic effects of L-leucine: observations of in vitro and in vivo studies. Nutr Metab (Lond) 2008;5:20.
[27] Campbell WW, Crim MC, Dallal GE, Young VR, Evans WJ. Increased protein requirements in elderly people: new data and retrospective reassessments. Am J Clin Nutr 1994;60:501–9.
[28] Rousset S, Patureau Mirand P, Brandolini M, Martin JF, Boirie Y. Daily protein intakes and eating patterns in young and elderly French. Br J Nutr 2003;90:1107–15.
[29] Campbell WW, Trappe TA, Wolfe RR, Evans WJ. The recommended dietary allowance for protein may not be adequate for older people to maintain skeletal muscle. J Gerontol A Biol Sci Med Sci 2001;56: M373–80.
[30] Thalacker-Mercer AE, Fleet JC, Craig BA, Carnell NS, Campbell WW. Inadequate protein intake affects skeletal muscle transcript profiles in older humans. Am J Clin Nutr 2007;85:1344–52.
[31] Larsson L, Grimby G, Karlsson J. Muscle strength and speed of movement in relation to age and muscle morphology. J Appl Physiol 1979;46:451–6.
[32] Volpi E, Nazemi R, Fujita S. Muscle tissue changes with aging. Curr Opin Clin Nutr Metab Care 2004;7:405–10.
[33] Katsanos CS, Kobayashi H, Sheffield-Moore M, Aarsland A, Wolfe RR. Aging is associated with diminished accretion of muscle proteins after the ingestion of a small bolus of essential amino acids. Am J Clin Nutr 2005;82:1065–73.
[34] Paddon-Jones D, Sheffield-Moore M, Zhang XJ, Volpi E, Wolf SE, Aarsland A, et al. Amino acid ingestion improves muscle protein synthesis in the young and elderly. Am J Physiol Endocrinol Metab 2004;286:E321–8.
[35] Symons TB, Schutzler SE, Cocke TL, Chinkes DL, Wolfe RR, Paddon-Jones D. Aging does not impair the anabolic response to a protein-rich meal. Am J Clin Nutr 2007;86:451–6.
[36] Cuthbertson D, Smith K, Babraj J, Leese G, Waddell T, Atherton P, et al. Anabolic signaling deficits underlie amino acid resistance of wasting, aging muscle. Faseb J 2005;19:422–4.
[37] Eley HL, Russell ST, Baxter JH, Mukerji P, Tisdale MJ. Signaling pathways initiated by beta-hydroxy-beta-methylbutyrate to attenuate the depression of protein synthesis in skeletal muscle in response to cachectic stimuli. Am J Physiol Endocrinol Metab 2007;293: E923–31.
[38] Long X, Ortiz-Vega S, Lin Y, Avruch J. Rheb binding to mammalian target of rapamycin (mTOR) is regulated by amino acid sufficiency. J Biol Chem 2005;280:23433–6.
[39] Potter CJ, Pedraza LG, Xu T. Akt regulates growth by directly phosphorylating Tsc2. Nat Cell Biol 2002;4:658–65.
[40] Thomson DM, Gordon SE. Diminished overload-induced hypertrophy in aged fast-twitch skeletal muscle is associated with AMPK hyperphosphorylation. J Appl Physiol 2005;98:557–64.
[41] Fedele MJ, Hernandez JM, Lang CH, Vary TC, Kimball SR, Jefferson LS, et al. Severe diabetes prohibits elevations in muscle protein synthesis after acute resistance exercise in rats. J Appl Physiol 2000;88:102–8.
[42] Fujita S, Rasmussen BB, Cadenas JG, Drummond MJ, Glynn EL, Sattler FR, et al. Aerobic exercise overcomes the age-related insulin resistance of muscle protein metabolism by improving endothelial function and Akt/mammalian target of rapamycin signaling. Diabetes 2007;56:1615–22.
[43] Volpi E, Mittendorfer B, Rasmussen BB, Wolfe RR. The response of muscle protein anabolism to combined hyperaminoacidemia and glucose-induced hyperinsulinemia is impaired in the elderly. J Clin Endocrinol Metab 2000;85:4481–90.
[44] Smith HJ, Mukerji P, Tisdale MJ. Attenuation of proteasome-induced proteolysis in skeletal muscle by {beta}-hydroxy-{beta}-methylbutyrate in cancer-induced muscle loss. Cancer Res 2005;65:277–83.
[45] Pickart CM. Mechanisms underlying ubiquitination. Annu Rev Biochem 2001;70:503–33.
[46] Combaret L, Dardevet D, Rieu I, Pouch MN, Bechet D, Taillandier D, et al. A leucine-supplemented diet restores the defective postprandial inhibition of proteasome-dependent proteolysis in aged rat skeletal muscle. J Physiol 2005;569:489–99.
[47] Clavel S, Coldefy AS, Kurkdjian E, Salles J, Margaritis I, Derijard B. Atrophy-related ubiquitin ligases, atrogin-1 and MuRF1 are up-regulated in aged rat tibialis anterior muscle. Mech Ageing Dev 2006;127:794–801.
[48] Reid MB, Li YP. Tumor necrosis factor-alpha and muscle wasting: a cellular perspective. Respir Res 2001;2:269–72.
[49] Wyke SM, Tisdale MJ. NF-kappaB mediates proteolysis-inducing factor induced protein degradation and expression of the ubiquitin–proteasome system in skeletal muscle. Br J Cancer 2005;92:711–21.
[50] Baier S, Johannsen D, Abumrad N, Rathmacher JA, Nissen S, Flakoll P. Year-long changes in protein metabolism in elderly men and women supplemented with a nutrition cocktail of beta-hydroxy-beta-methylbutyrate (HMB), L-arginine, and L-lysine. JPEN J Parenter Enteral Nutr 2009;33:71–82.
[51] Collins CA, Zammit PS, Ruiz AP, Morgan JE, Partridge TA. A population of myogenic stem cells that survives skeletal muscle aging. Stem Cells 2007;25:885–94.
[52] Slivka D, Raue U, Hollon C, Minchev K, Trappe S. Single muscle fiber adaptations to resistance training in old (>80 yr) men: evidence for limited skeletal muscle plasticity. Am J Physiol Regul Integr Comp Physiol 2008;295:R273–80.
[53] Conboy IM, Conboy MJ, Smythe GM, Rando TA. Notch-mediated restoration of regenerative potential to aged muscle. Science 2003;302:1575–7.
[54] Conboy IM, Conboy MJ, Wagers AJ, Girma ER, Weissman IL, Rando TA. Rejuvenation of aged progenitor cells by exposure to a young systemic environment. Nature 2005;433:760–4.
[55] Conboy IM, Rando TA. The regulation of Notch signaling controls satellite cell activation and cell fate determination in postnatal myogenesis. Dev Cell 2002;3:397–409.
[56] Carlson ME, Hsu M, Conboy IM. Imbalance between pSmad3 and Notch induces CDK inhibitors in old muscle stem cells. Nature 2008;454:528–32.
[57] Langley B, Thomas M, Bishop A, Sharma M, Gilmour S, Kambadur R. Myostatin inhibits myoblast differentiation by down-regulating MyoD expression. J Biol Chem 2002;277:49831–40.
[58] Allen DL, Unterman TG. Regulation of myostatin expression and myoblast differentiation by FoxO and SMAD transcription factors. Am J Physiol Cell Physiol 2007;292:C188–99.
[59] Yang W, Zhang Y, Li Y, Wu Z, Zhu D. Myostatin induces cyclin D1 degradation to cause cell cycle arrest through a phosphatidylinositol 3-kinase/AKT/GSK-3 beta pathway and is antagonized by insulin-like growth factor 1. J Biol Chem 2007;282:3799–808.
[60] Funai K, Parkington JD, Carambula S, Fielding RA. Age-associated decrease in contraction-induced activation of downstream targets of Akt/mTor signaling in skeletal muscle. Am J Physiol Regul Integr Comp Physiol 2006;290:R1080–6.
[61] Parkington JD, LeBrasseur NK, Siebert AP, Fielding RA. Contraction-mediated mTOR, p70S6k, and ERK1/2 phosphorylation in aged skeletal muscle. J Appl Physiol 2004;97:243–8.
[62] Reid MB, Li YP. Cytokines and oxidative signalling in skeletal muscle. Acta Physiol Scand 2001;171:225–32.
[63] Han B, Tong J, Zhu MJ, Ma C, Du M. Insulin-like growth factor-1 (IGF-1) and leucine activate pig myogenic satellite cells through

mammalian target of rapamycin (mTOR) pathway. Mol Reprod Dev 2008;75:810–7.
[64] Norton L, Wilson GJ. Optimal protein intake to maximize muscle protein synthesis. AgroFood Industry Hi-Tech 2009;2:54–67.
[65] Nissen S, Sharp R, Ray M, Rathmacher JA, Rice D, Fuller Jr JC, et al. Effect of leucine metabolite beta-hydroxy-beta-methylbutyrate on muscle metabolism during resistance-exercise training. J Appl Physiol 1996;81:2095–104.
[66] Tipton KD, Ferrando AA, Phillips SM, Doyle Jr D, Wolfe RR. Postexercise net protein synthesis in human muscle from orally administered amino acids. Am J Physiol 1999;276:E628–34.
[67] Esmarck B, Andersen JL, Olsen S, Richter EA, Mizuno M, Kjaer M. Timing of postexercise protein intake is important for muscle hypertrophy with resistance training in elderly humans. J Physiol 2001;535:301–11.
[68] Thomson DM, Gordon SE. Impaired overload-induced muscle growth is associated with diminished translational signalling in aged rat fast-twitch skeletal muscle. J Physiol 2006;574:291–305.
[69] Drummond MJ, Dreyer HC, Pennings B, Fry CS, Dhanani S, Dillon EL, et al. Skeletal muscle protein anabolic response to resistance exercise and essential amino acids is delayed with aging. J Appl Physiol 2008;104:1452–61.
[70] Singh MA, Ding W, Manfredi TJ, Solares GS, O'Neill EF, Clements KM, et al. Insulin-like growth factor I in skeletal muscle after weight-lifting exercise in frail elders. Am J Physiol 1999;277: E135–43.
[71] Fiatarone MA, O'Neill EF, Ryan ND, Clements KM, Solares GR, Nelson ME, et al. Exercise training and nutritional supplementation for physical frailty in very elderly people. N Engl J Med 1994;330: 1769–75.
[72] Fiatarone Singh MA, Bernstein MA, Ryan AD, O'Neill EF, Clements KM, Evans WJ. The effect of oral nutritional supplements on habitual dietary quality and quantity in frail elders. J Nutr Health Aging 2000; 4:5–12.
[73] Meredith CN, Frontera WR, O'Reilly KP, Evans WJ. Body composition in elderly men: effect of dietary modification during strength training. J Am Geriatr Soc 1992;40:155–62.
[74] Walrand S, Short KR, Bigelow ML, Sweatt AJ, Hutson SM, Nair KS. Functional impact of high protein intake on healthy elderly people. Am J Physiol Endocrinol Metab 2008;295:E921–8.
[75] Kumar V, Selby A, Rankin D, Patel R, Atherton P, Hildebrandt W, Williams J, Smith K, Seynnes O, et al. Age-related differences in dose response of muscle protein synthesis to resistance exercise in young and old men. J Physiol 2008.
[76] Godard MP, Williamson DL, Trappe SW. Oral amino-acid provision does not affect muscle strength or size gains in older men. Med Sci Sports Exerc 2002;34:1126–31.
[77] Welle S, Thornton CA. High-protein meals do not enhance myofibrillar synthesis after resistance exercise in 62- to 75-yr-old men and women. Am J Physiol 1998;274:E677–83.
[78] Campbell WW, Barton Jr ML, Cyr-Campbell D, Davey SL, Beard JL, Parise G, et al. Effects of an omnivorous diet compared with a lactoovovegetarian diet on resistance-training-induced changes in body composition and skeletal muscle in older men. Am J Clin Nutr 1999;70:1032–9.
[79] Pannemans DL, Wagenmakers AJ, Westerterp KR, Schaafsma G, Halliday D. Effect of protein source and quantity on protein metabolism in elderly women. Am J Clin Nutr 1998;68:1228–35.
[80] Tipton KD, Gurkin BE, Matin S, Wolfe RR. Nonessential amino acids are not necessary to stimulate net muscle protein synthesis in healthy volunteers. J Nutr Biochem 1999;10:89–95.
[81] Wahren J, Felig P, Hagenfeldt L. Effect of protein ingestion on splanchnic and leg metabolism in normal man and in patients with diabetes mellitus. J Clin Invest 1976;57:987–99.
[82] Martinez JA, Goena M, Santidrian S, Larralde J. Response of muscle, liver and whole-body protein turnover to two different sources of protein in growing rats. Ann Nutr Metab 1987;31:146–53.
[83] Vukovich MD, Sharp RL, Kesl LD, Schaulis DL, King DS. Effects of a low-dose amino acid supplement on adaptations to cycling training in untrained individuals. Int J Sport Nutr 1997;7:298–309.
[84] Flakoll P, Sharp R, Baier S, Levenhagen D, Carr C, Nissen S. Effect of beta-hydroxy-beta-methylbutyrate, arginine, and lysine supplementation on strength, functionality, body composition, and protein metabolism in elderly women. Nutrition 2004;20:445–51.
[85] Fujita S, Volpi E. Amino acids and muscle loss with aging. J Nutr 2006;136:277S–80S.
[86] Volpi E, Ferrando AA, Yeckel CW, Tipton KD, Wolfe RR. Exogenous amino acids stimulate net muscle protein synthesis in the elderly. J Clin Invest 1998;101:2000–7.
[87] Wilson GJ, Wilson JM, Manninen AH. Effects of beta-hydroxy-beta-methylbutyrate (HMB) on exercise performance and body composition across varying levels of age, sex, and training experience. A review Nutr Metab (Lond) 2008;5:1.
[88] Zerba E, Komorowski TE, Faulkner JA. Free radical injury to skeletal muscles of young, adult, and old mice. Am J Physiol 1990;258: C429–35.
[89] Nissen S, Sharp RL, Panton L, Vukovich M, Trappe S, Fuller Jr JC. Beta-hydroxy-beta-methylbutyrate (HMB) supplementation in humans is safe and may decrease cardiovascular risk factors. J Nutr 2000;130:1937–45.
[90] Kornasio R, Riederer I, Butler-Browne G, Mouly V, Uni Z, Halevy O. Beta-hydroxy-beta-methylbutyrate (HMB) stimulates myogenic cell proliferation, differentiation and survival via the MAPK/ERK and PI3K/Akt pathways. Biochim Biophys Acta 2009.
[91] Smith HJ, Wyke SM, Tisdale MJ. Mechanism of the attenuation of proteolysis-inducing factor stimulated protein degradation in muscle by beta-hydroxy-beta-methylbutyrate. Cancer Res 2004;64:8731–5.
[92] Mosoni L, Breuille D, Buffiere C, Obled C, Mirand PP. Age-related changes in glutathione availability and skeletal muscle carbonyl content in healthy rats. Exp Gerontol 2004;39:203–10.
[93] Fulle S, Protasi F, Di Tano G, Pietrangelo T, Beltramin A, Boncompagni S, et al. The contribution of reactive oxygen species to sarcopenia and muscle ageing. Exp Gerontol 2004;39:17–24.
[94] Wei YH, Lee HC. Oxidative stress, mitochondrial DNA mutation, and impairment of antioxidant enzymes in aging. Exp Biol Med (Maywood) 2002;227:671–82.
[95] Fibach E, Rachmilewitz E. The role of oxidative stress in hemolytic anemia. Curr Mol Med 2008;8:609–19.
[96] Chen Q, Vazquez EJ, Moghaddas S, Hoppel CL, Lesnefsky EJ. Production of reactive oxygen species by mitochondria: central role of complex III. J Biol Chem 2003;278:36027–31.
[97] Ishii N. Role of oxidative stress from mitochondria on aging and cancer. Cornea 2007;26:S3–9.
[98] Dirks A, Leeuwenburgh C. Apoptosis in skeletal muscle with aging. Am J Physiol Regul Integr Comp Physiol 2002;282:R519–27.
[99] Hajnoczky G, Csordas G, Das S, Garcia-Perez C, Saotome M, Sinha Roy S, et al. Mitochondrial calcium signalling and cell death: approaches for assessing the role of mitochondrial Ca^{2+} uptake in apoptosis. Cell Calcium 2006;40:553–60.
[100] Marzetti E, Wohlgemuth SE, Lees HA, Chung HY, Giovannini S, Leeuwenburgh C. Age-related activation of mitochondrial caspase-independent apoptotic signaling in rat gastrocnemius muscle. Mech Ageing Dev 2008;129:542–9.
[101] Basso E, Fante L, Fowlkes J, Petronilli V, Forte MA, Bernardi P. Properties of the permeability transition pore in mitochondria devoid of cyclophilin D. J Biol Chem 2005;280:18558–61.
[102] Green DR, Kroemer G. The pathophysiology of mitochondrial cell death. Science 2004;305:626–9.
[103] Alway SE, Degens H, Krishnamurthy G, Smith CA. Potential role for Id myogenic repressors in apoptosis and attenuation of hypertrophy in muscles of aged rats. Am J Physiol Cell Physiol 2002;283:C66–76.
[104] Chung L, Ng YC. Age-related alterations in expression of apoptosis regulatory proteins and heat shock proteins in rat skeletal muscle. Biochim Biophys Acta 2006;1762:103–9.

[105] Song W, Kwak HB, Lawler JM. Exercise training attenuates age-induced changes in apoptotic signaling in rat skeletal muscle. Antioxid Redox Signal 2006;8:517–28.
[106] Renault V, Thornell LE, Butler-Browne G, Mouly V. Human skeletal muscle satellite cells: aging, oxidative stress and the mitotic clock. Exp Gerontol 2002;37:1229–36.
[107] Mates JM, Perez-Gomez C, Nunez de Castro I. Antioxidant enzymes and human diseases. Clin Biochem 1999;32:595–603.
[108] Sodergard R, Backstrom T, Shanbhag V, Carstensen H. Calculation of free and bound fractions of testosterone and estradiol-17 beta to human plasma proteins at body temperature. J Steroid Biochem 1982;16:801–10.
[109] Mates JM, Sanchez-Jimenez F. Antioxidant enzymes and their implications in pathophysiologic processes. Front Biosci 1999;4: D339–45.
[110] Zelko IN, Mariani TJ, Folz RJ. Superoxide dismutase multigene family: a comparison of the CuZn-SOD (SOD1), Mn-SOD (SOD2), and EC-SOD (SOD3) gene structures, evolution, and expression. Free Radic Biol Med 2002;33:337–49.
[111] Choksi KB, Nuss JE, Boylston WH, Rabek JP, Papaconstantinou J. Age-related increases in oxidatively damaged proteins of mouse kidney mitochondrial electron transport chain complexes. Free Radic Biol Med 2007;43:1423–38.
[112] Rowe LA, Degtyareva N, Doetsch PW. DNA damage-induced reactive oxygen species (ROS) stress response in *Saccharomyces cerevisiae*. Free Radic Biol Med 2008;45:1167–77.
[113] Roussel AM, Kerkeni A, Zouari N, Mahjoub S, Matheau JM, Anderson RA. Antioxidant effects of zinc supplementation in Tunisians with type 2 diabetes mellitus. J Am Coll Nutr 2003;22:316–21.
[114] Galecki P, Kedziora J, Florkowski A, Galecka E. Lipid peroxidation and copper–zinc superoxide dismutase activity in patients treated with fluoxetine during the first episode of depression. Psychiatr Pol 2007;41:615–24.
[115] Kamp F, Donangelo CM. Supplementing young women with both zinc and iron protects zinc-related antioxidant indicators previously impaired by iron supplementation. J Nutr 2008;138:2186–9.
[116] Nazifi S, Mansourian M, Nikahval B, Razavi SM. The relationship between serum level of thyroid hormones, trace elements and antioxidant enzymes in dromedary camel (*Camelus dromedarius*). Trop Anim Health Prod 2009;41:129–34.
[117] Nunes VA, Gozzo AJ, Juliano MA, Cesar MC, Sampaio MU, Sampaio CA, et al. Antioxidant dietary deficiency induces caspase activation in chick skeletal muscle cells. Braz J Med Biol Res 2003;36:1047–53.
[118] Rafique R, Schapira AH, Coper JM. Mitochondrial respiratory chain dysfunction in ageing; influence of vitamin E deficiency. Free Radic Res 2004;38:157–65.
[119] Gupta AD, Dhundasi SA, Ambekar JG, Das KK. Effect of L-ascorbic acid on antioxidant defense system in testes of albino rats exposed to nickel sulfate. J Basic Clin Physiol Pharmacol 2007;18:255–66.
[120] Kadirvel R, Sundaram K, Mani S, Samuel S, Elango N, Panneerselvam C. Supplementation of ascorbic acid and alpha-tocopherol prevents arsenic-induced protein oxidation and DNA damage induced by arsenic in rats. Hum Exp Toxicol 2007;26:939–46.
[121] Judge AR, Selsby JT, Dodd SL. Antioxidants attenuate oxidative damage in rat skeletal muscle during mild ischaemia. Exp Physiol 2008;93:479–85.
[122] Rodrigo R, Prat H, Passalacqua W, Araya J, Bachler JP. Decrease in oxidative stress through supplementation of vitamins C and E is associated with a reduction in blood pressure in patients with essential hypertension. Clin Sci (Lond) 2008;114:625–34.
[123] Washio K, Inagaki M, Tsuji M, Morio Y, Akiyama S, Gotoh H, et al. Oral vitamin C supplementation in hemodialysis patients and its effect on the plasma level of oxidized ascorbic acid and Cu/Zn superoxide dismutase, an oxidative stress marker. Nephron Clin Pract 2008;109:c49–54.
[124] Rebrin I, Zicker S, Wedekind KJ, Paetau-Robinson I, Packer L, Sohal RS. Effect of antioxidant-enriched diets on glutathione redox status in tissue homogenates and mitochondria of the senescence-accelerated mouse. Free Radic Biol Med 2005;39:549–57.
[125] Sumien N, Forster MJ, Sohal RS. Supplementation with vitamin E fails to attenuate oxidative damage in aged mice. Exp Gerontol 2003;38:699–704.
[126] Lee S, Pagoria D, Raigrodski A, Geurtsen W. Effects of combinations of ROS scavengers on oxidative DNA damage caused by visible-light-activated camphorquinone/*N*,*N*-dimethyl-*p*-toluidine. J Biomed Mater Res B Appl Biomater 2007;83:391–9.
[127] Marzani B, Balage M, Venien A, Astruc T, Papet I, Dardevet D, et al. Antioxidant supplementation restores defective leucine stimulation of protein synthesis in skeletal muscle from old rats. J Nutr 2008;138:2205–11.
[128] Wu X, Beecher GR, Holden JM, Haytowitz DB, Gebhardt SE, Prior RL. Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. J Agric Food Chem 2004;52:4026–37.