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A role for nutritional intervention in addressing the aging neuromuscular junction

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Abbreviations

AChRs; acetylcholine receptors

DHA; docosahexaenoic acid

EPA; eicosapentaenoic acid

GH; growth hormone

HMB; beta-hydroxy-beta-methylbutyrate

IGF-1; insulin-like growth factor 1

MFGM; milk fat globule membrane

mTOR; mammalian/mechanistic target of rapamycin

MU; motor unit

NMJ; neuromuscular junction

PUFA; polyunsaturated fatty acid

ROS; reactive oxygen species

VDR; vitamin D receptor

WM; white matter

Abstract

The purpose of this review is to discuss the structural and physiological changes that underlie age-related neuromuscular dysfunction and to summarize current evidence on the potential role of nutritional interventions on neuromuscular dysfunction-associated pathways. Age-related neuromuscular deficits are known to coincide with distinct changes in the central and peripheral nervous system, in the neuromuscular system, and systemically. Although many features contribute to the age-related decline in neuromuscular function, a comprehensive understanding of their integration and temporal relationship is needed. Nonetheless, many nutrients and ingredients show promise in modulating neuromuscular output by counteracting the age-related changes that coincide with neuromuscular dysfunction. In particular, dietary supplements, such as vitamin D, omega-3 fatty acids, beta-hydroxy-beta-methylbutyrate (HMB), creatine, and dietary phospholipids, demonstrate potential in ameliorating age-related neuromuscular dysfunction. However, current evidence seldom directly assesses neuromuscular outcomes and is not always in the context of aging. Additional clinical research studies are needed to confirm the benefits of dietary supplements on neuromuscular function, as well as to define the appropriate population, dosage, and duration for intervention.

Keywords: dietary supplement; diet; motor function; sarcopenia; dynapenia; motor unit

1. Introduction

Normal, healthy aging in humans is accompanied by a decline in physical and neurocognitive abilities, which encompass a decrease in muscle function, motor performance, interdependent cognitive-motor control, and many aspects of executive function [1-3]. Although physical abilities tend to decline more rapidly and to a greater extent in aging, the neurocognitive decline is still common, yet difficult to manage. Moreover, the underlying physiological causes of this neurocognitive decline accentuates, and may even initiate, the age-related decline in physical performance. Consequently, this general decline can adversely affect functional activities of daily life that can lead to an increased risk of injury (e.g., accidental fall) and functional dependence. In fact, the number of older adults requiring long-term care due to functional dependence is projected to quadruple by 2050 [4]. This concern affects men and women alike, as the timing and rate of age-related declines in muscle strength and neuromuscular function are similar in both genders [5].

Given that physical performance is determined by the output from the neuromuscular system, both neural and musculoskeletal properties are key contributors to the age-related decline in physical abilities. The neuromuscular junction (NMJ) is thought to play a crucial role as it demonstrates distinct age-related deterioration involving both neural and muscular aspects. Furthermore, characteristics of this age-related degeneration at the NMJ have revealed potential underlying mechanisms to target with specific nutritional factors.

In regards to treatment strategies, emphasizing certain lifestyle factors, like physical activity and proper nutrition, play critical roles in normal healthy aging. With increasing evidence of dietary influences on healthy functional living in aging [6], specific dietary nutrients have been shown to positively affect cognitive and musculoskeletal function in older adults [7, 8]. Consequently, the aim of this review is to examine the effects of dietary supplements that promote healthy neuromuscular aging by potentially counteracting age-related changes that contribute to neuromuscular dysfunction.

This review summarizes the structural and physiological changes that not only affect the aging NMJ but also coincide with the age-related decline in neuromuscular function. In particular, changes in the central and peripheral nervous system, the neuromuscular system, and the system as a whole with features related to neurodegeneration, musculoskeletal alterations, the decline in anabolic hormones, mitochondrial dysfunction, oxidative stress, and inflammation are discussed. Lastly, the review covers both nutrients and ingredients - such as vitamin D, omega-3 fatty acids, beta-hydroxy-beta-methylbutyrate (HMB), creatine, and dietary phospholipids - that can positively affect neuromuscular output and therefore may be beneficial in counteracting the age-related changes contributing to neuromuscular dysfunction.

The studies included in this review were identified by a literature search conducted in multiple databases (Embase, MEDLINE, and PubMed) using the following descriptors in associations: neuromuscular OR neuromuscular junction OR physical function, AND aging OR aged OR dysfunction, AND humans OR animal models, AND nutrition OR nutrients OR nutritional ingredients. Review articles and meta-analyses, as well as those resulting from reverse search,

were selected. Although several nutrients and ingredients appeared beneficial in preventing or attenuating the age-related decline in muscle and physical function, the focus of this examination was only on those that have specifically demonstrated the capability to influence the aging neuromuscular junction or its functional output. Thus, studies were further identified by a PubMed database search using "neuromuscular" AND specific nutrients or dietary supplements previously identified.

2. Age-related neuromuscular degeneration

Although many features coincide with the age-related decline in neuromuscular function depicted in Fig. 1, a comprehensive understanding of their cause and integrative influence is lacking. Age-related muscular atrophy occurs along with a decrease in muscular strength, power, and function. However, muscle atrophy is not the only factor contributing to loss of strength and function, as a 5-year longitudinal study that recruited well-functioning men and women (n = 1678) in their 70's has demonstrated that, in those who lost or remained at a constant weight, the age-related loss in muscle strength was 2-5 times faster than the observed loss of muscle, as measured by maximal isokinetic knee extension and mid-thigh cross sectional area, respectively [9]. In fact, those that gained weight still lost muscle strength - albeit less than those that lost weight - regardless of the small increases in muscle, suggesting a loss in muscle quality or activation. Across all groups, an average decrease in strength of ~15% across the 5 years was observed. This loss in strength is similar to the findings of a smaller (n = 358) but lengthier prospective cohort study [5], in which generally healthy, independent living men and women recruited at 50, 60, and 70 years of age lost on average 22 to 31% of their grip strength

across a 10-year period. Interestingly, although grip strength decreased similarly across the age groups, balance and gait speed had the greatest deterioration after 60 and 70 years of age, respectively. Altogether, these findings provide useful information for the optimal ages (i.e., by 50, 60, and 70 years of age, respectively) to intervene with targeted lifestyle and exercise strategies focused on muscle strength, balance, and gait. Lastly, aside from plausible muscle quality decrements with aging, increasing evidence exists that age-related neural deficits contribute to the loss of strength and functional performance via a decrease in information processing, force generation, movement speed, motor control, gait, balance, coordination, and response speed [10, 11].

Given that both neurocognitive and musculoskeletal deficits play a role in the decline in physical abilities with age, a growing concern has contributed to a greater exploring of the NMJ's integrity and its role in physical decline [12]. Considerable evidence of structural and functional changes at the NMJ are implicated in the age-related muscular performance deficits. Although the integration and temporal relationship of these contributing factors are not fully understood, recent studies have suggested that the age-related neuromuscular dysfunction precedes, and may be a requisite to initiate, the loss of muscle mass and function [e.g., 13]. Here, factors contributing to NMJ dysfunction are discussed, beginning with a top-down approach followed by a more systemic consideration.

2.1. Central and peripheral nervous system

In normal, healthy aging, there are many neuroanatomical and neurophysiological changes that are associated with cognitive deficits; however, their involvement in age-related performance deficits are less clear. Interestingly, cognition, itself, is strongly associated with physical performance in older adults, such that those experiencing greater cognitive decline also suffer from more prominent gait deficits [14]. In this section, the numerous brain-related changes are not reviewed (see [15] for a detailed review), as many studies do not assess relationships between precise neuroanatomy and motor performance. However, it is plausible that the neurobiology that influences brain atrophy in select regions, namely sensorimotor regions, contributes to age-related motor performance deficits. Furthermore, maximal activation of muscle by the nervous system is certainly influenced by the excitability of cortical neurons and the synchronicity of firing spinal motor neurons [16].

A substantial distributed loss of white matter volume occurs throughout the brain in normal aging. White matter (WM) is primarily composed of myelinated axons and, as its name suggests, appears white due to the lipid content of myelin. WM loss is also accompanied by an age-related decline in WM integrity, which is associated with lower scores on muscular strength, fine motor coordination, processing speed, reaction time, gait, and balance [17]. Moreover, age-related loss in WM is not only confined to the brain but extends to the peripheral nervous system. In the aging spinal cord, a reduced number and diameter of myelinated motor axons exist in the ventral roots, specifically with a greater loss of large-diameter axons [18], that presumably contribute to the reduced nerve conduction velocity seen in aging [19].

2.2. Neuromuscular system

Well-characterized age-related changes occur in the neuromuscular junction (NMJ), which consists of the pre-synaptic motor nerve terminal, the synaptic cleft (basal lamina), and the post-synaptic motor endplate (i.e., the muscle membrane) (see Fig. 2). When an action potential is generated and travels down to the pre-synaptic terminal, voltage-gated calcium channels open and the resulting calcium influx triggers translocation of acetylcholine-stored vesicles to the membrane of the axon terminal. Acetylcholine (ACh) is delivered into the synaptic cleft and binds to post-synaptic nicotinic ACh receptors (AChRs) present on the motor endplate to propagate an action potential along the muscle fiber, which results in muscle contraction. The basic functional unit of the neuromuscular system, the motor unit (MU), is comprised of a single lower motor neuron and its innervating muscle fibers that contract simultaneously provided sufficient discharge from the neuron. However, individual motor units are quite different in their contractile response characteristics of muscle fiber. Muscle fibers are generally classified as either type I (slow-twitch) or type II (fast-twitch), with the latter displaying greater contractile speed, force generation, and susceptibility to fatigue, as well as less mitochondria and myoglobin content. An individual MU innervates muscle fibers that only belong to a single fiber type, and muscle fibers require innervation for survival.

Although the following subsections highlight the predominant age-related neuromuscular changes seen in humans, further neuromuscular changes that occur in animal models may translate to aging humans (see [20, 21]). For example, a reduced capacity of successful motor neuron reinnervation to muscle (reviewed in [22]), a degradation of muscle contractile protein

machinery [23], and a loss of regenerative capacity and stem cell function [24] may also occur in humans with aging. Altogether, these changes may lead to the age-related excitation-contraction uncoupling (reviewed in [12]). Furthermore, rodent models of human aging have corroborated that neural changes precede, and may cause, the age-related myofiber atrophy [25, 26]. Recent insights on signaling pathways, like dysregulated autophagy, sympathetic activity, and agrin-MuSK-Lrp4 and Wnt signaling, are involved in the aging neuromuscular junction, but these are not discussed (see [27]).

2.2.1. Motor unit loss

In aging, both neural and muscular changes can affect the MU, as it undergoes several age-related structural and physiological changes that are involved in the concomitant decrease in motor performance. Most fundamentally, the number of motor neurons in the spinal cord progressively decline in old age [28, 29], with one study showing instances of aged subjects demonstrating only half of the motor neuron counts found in middle-aged subjects [30]. This age-related motor neuron loss results in fewer MUs and supports the loss of spinal gray matter seen with aging [31, 32]. However, McNeil et al. [33] have shown that, despite a decrease in the number of MUs in older adults compared to young, maximal isometric strength in the tibialis anterior did not differ. Conversely though, very old adults with a more pronounced decline in the number of MUs had weaker maximal isometric strength in the tibialis anterior compared to young adults. These findings suggest that the progressive loss of motor neurons needs to reach a critical threshold before presenting functional impairments in maximal isometric strength of the tibialis anterior. Presumptively, the preservation of this functional parameter early in adult life

may result from the maintenance of muscle fibers through collateral reinnervation by surviving motor neurons, which describes the MU remodeling that is observed with electromyography in aging (i.e., larger and less MUs) [34]. Furthermore, this MU remodeling in aging is implicated in not only strength deficits but also the decrease in peak muscle power [35].

2.2.2. Dysfunctional motor unit remodeling

In another study investigating the maximal isometric strength of the index finger (i.e., abduction of the second digit), Kamen et al. [36] detected weaker force production in old compared to young adults and that the MU discharge rate in old adults was 64% of that in young adults. The investigators proposed that reductions in maximal force capacity of older adults are partially a result from an impaired ability to fully drive the surviving MUs. The more variable discharge from single MUs has also been suggested to reduce the ability of older subjects to perform steady muscle contractions [37]. Collectively, these studies demonstrate that, although no differences may occur in maximal strength of specific muscles when full compensatory MU remodeling exists, the resulting larger MUs have different physiological properties. These different physiological properties may be in response to a motor neuron having to maintain more muscle fibers, which may consequently lead to the age-related impairments in fine motor control (i.e., reduced force steadiness and accuracy) [38, 39].

Remodeling of the MU in aging is also accompanied by a change in the motor innervation pattern at the endplate. In particular, an age-related increase in the number of axonal branches entering the endplate has been reported [40]. Age-related increases in axonal arborization may

explain the observed intrusion of Schwann cell processes into the synaptic cleft [41]. Since Schwann cells produce the myelin sheath insulating axons, the processes may need to be as close as possible to previously denervated end plates to facilitate successful reinnervation via myelination of newly sprouted axonal branches. Neural changes at the NMJ are also accompanied by changes at the muscular membrane. Specifically, degeneration of junctional folds and an expansion of the postsynaptic area appear during aging, with the latter resulting from increasing length and branching of the motor endplate [41]. Along with this postsynaptic expansion in aging, an increase in the number of aggregated AChRs on the postsynaptic endplate follows [40]. Together, these age-related morphological and physiological changes of the NMJ may be involved in the general decrease in excitability of spinal reflexes seen in aging [42-44].

Motor neuron loss can also be accompanied by extensive muscle fiber loss when reinnervation reaches its capacity and, presumably, fat or fibrous tissue partially replace this muscle loss [45]. Consequently, this motor neuron loss may explain the minor degree of muscle fiber type grouping in old age [41], the observed age-related increase in intermuscular fat [9], and the decrease in muscle size with age. However, the decreasing size of aging muscle occurs because of not only muscle fiber loss but also muscle fiber atrophy, which appears to affect type II muscle fibers to a greater extent [46]. These type II fibers are essential for fast reactions to loss of balance and, thus, preventing falls.

2.3. Features of neuromuscular dysfunction

2.3.1. Mitochondrial dysfunction

Oxidative stress has been implicated in neuromuscular dysfunction [47] and is mediated by reactive oxygen and nitrogen species, like free radicals, which are largely a byproduct of mitochondrial oxidative phosphorylation. In aging, production of these reactive species increases due to mitochondrial dysfunction [48] caused in part by age-related aberrations in mitochondrial DNA [49]. If these reactive species are not neutralized by endogenous or exogenous antioxidants, they can induce oxidative damage to cellular infrastructure and subsequently impair function (reviewed in [50]). For example, aging rats display dramatic structural changes in mitochondria of distal motor axon terminals, but not of motor neurons within the ventral horn of the spinal cord [51]. The regional specificity of structural changes in mitochondria is particularly interesting given the presence of apoptotic markers and their colocalization with retrograde transport proteins in the soma indicating an early degenerative stage initiated distally at the NMJ. Even though mitochondrial dysfunction is quite ubiquitous in the aging body, presumably some anatomical and cellular specificity contribute to its etiology. With the contribution of the age-related decline in adaptive responses that help to neutralize reactive species, an increase in oxidative-induced damage has been demonstrated in aging human muscle [52] and aging peripheral nerves of rodent models [53].

2.3.2. Inflammation

Aging is also accompanied by chronic, mild inflammation [54], which is marked by an elevated amount of circulating proinflammatory cytokines [55] and has been demonstrated to be a risk

factor for accelerated decline in muscle mass and strength [56]. Exactly how inflammation is involved in the decline in muscle function is unclear, but many potential pathways may mediate this relationship. For example, many proinflammatory cytokines are known to negatively interact with the bioactivity and production of the anabolic hormone insulin-like growth factor 1 (IGF-1) [57], which is consistent with the aging endocrine and paracrine decline of IGF-1 discussed in the following subsection.

Another example drawn from rodent studies is that Schwann cell senescence is correlated with inflammatory cytokine (interleukin 6) overexpression, which implicates inflammation in age-related changes in myelination [58]. A recent study suggests that the detected inflammation in aging rats may perturb cholesterol homeostasis and contribute to impaired function of the spinal cord [59]. Given that cholesterol is a major constituent of cell membranes and myelin, WM integrity may be compromised due to this perturbation and would certainly exhibit functional consequences. Furthermore, the observed changes mostly occurred by middle-age, which suggests that disrupted cholesterol homeostasis may be an early event in the age-related motor deficits. Moreover, a recent study found that in aging rats the downregulation of cholesterol biosynthesis induces neuromuscular dysfunction by disrupting myelination [83].

2.3.3. Endocrine factors

Normal, healthy aging is accompanied by changes in circulating endocrine factors that are implicated in neuromuscular dysfunction [21]. Given that muscle atrophy partly contributes to the age-related functional deficits, the well-documented decline in circulating anabolic hormones

[60-62], like testosterone, growth hormone (GH), and IGF-1, is of particular interest. Accordingly, hormonal supplementation studies in aging have consistently shown increases in lean body mass while occasionally demonstrating only marginal improvements to muscular strength or function [63-65]. Although greater efficacy exists for combination therapies (reviewed in [66]), anabolic hormones may be favorably acting through mechanisms other than the obvious muscular growth. For example, even though IGF-1 has compelling anabolic effects on muscle, it also has potent neurotrophic effects that promote dendritic arborization and synaptogenesis, as well as facilitate in the myelination of axons, prevention of motor neuron apoptosis, stimulation of axonal sprouting, and restoration of damaged axons (reviewed in [67]). Therefore, the age-related decline in IGF-1 may be contributing to the neural changes that occur with the aging motor unit.

3. Targeted nutritional intervention

The aim of this section is to evaluate nutritional interventions that show promise in preventing or attenuating the age-related decline in motor abilities by impacting the underlying mechanisms of neuromuscular dysfunction. Deficient states of one or more nutrients certainly can confound findings in regards to dietary supplementation and, when possible, are mentioned. Furthermore, a limitation is that many studies reviewed herein have been performed in conjunction with an exercise protocol, which makes it difficult to assess whether dietary supplementation would be beneficial when minimal physical activity also exists.

Although several dietary supplements have shown benefits in preventing or attenuating the age-related decline in muscle and physical function, the focus of this review is on nutritional supplements that specifically demonstrate the capability to influence the aging neuromuscular junction or its functional output. Currently, limited aging human evidence has been reported with only some evidence corroborated through aging rodent studies. Nonetheless, this section begins with some relevant essential nutrient considerations, since malnutrition is not uncommon in the elderly and dietary supplementation should be considered as an adjunct to usual dietary intake patterns.

3.1. Essential nutrient considerations

3.1.1. Protein

In regards to macronutrient and micronutrient requirements, a clear distinction exists between minimal requirements and a more optimal level of intake. For example, protein, a macronutrient of interest in regards to skeletal muscle health, has a recommended dietary allowance (RDA) of 0.8g/kg body weight per day and, at this amount, is not adequate to maintain muscle in aging [68]. However, higher protein intake, even in the absence of exercise intervention, has been associated with smaller losses of lean mass in both middle-aged [69] and aged adults who lose weight and with greater gains of lean mass in aged adults who gain weight [70]. In fact, many experts recommend a protein intake of 1.2-2 g/kg bodyweight per day [71]. Additionally, the combined effects of a high protein diet and exercise are additive for improving lean body mass during weight loss [72]. The metabolic basis for these changes in lean mass are determined by

the net muscle protein balance (NBAL), which is the difference between muscle protein synthesis (MPS) and breakdown (MPB). Although no difference exists in basal NBAL between young and old adults [73], the main cause for the negative NBAL in aged adults is due to anabolic resistance, which is indicated by the reduced MPS in response to anabolic stimuli, such as feeding [74], exercise [75], and insulin [76]. To a lesser extent, the age-related decline in insulin's suppressive action on MPB is involved in the negative NBAL in aging as well [77].

MPS is modulated by several dietary factors, with the essential amino acids (EAAs) from protein being the most efficient activator. For that reason, ingestion of EAA in elderly individuals stimulates MPS to a greater extent than an isocaloric ingestion of whey protein [78]. Among the EAAs, branched-chain amino acids (BCAAs) appear to be the most responsible for directly stimulating MPS. Leucine, one particular BCAA, has been acknowledged to be a potent stimulator of MPS by mechanisms that involve mammalian target of rapamycin (mTOR) signaling [79]. As a result, recent systematic reviews and meta-analyses suggest that leucine is effective in addressing sarcopenia, since it does indeed increase MPS and improve lean body mass [80, 81]. However, considering that an age-related deficit in the muscle anabolic response to nutritional stimuli exists, a higher proportion of leucine is required for optimal stimulation of MPS by EAAs in the elderly [82].

In regards to the impaired response to anabolic stimuli in aged muscle, it has been suggested that a defect in activating an mTOR signaling protein (S6K1) that targets a ribosomal component to stimulate MPS is likely responsible [76]. Another contribution is that insulin sensitivity is known to decrease with age. Thus, higher protein diets may be favorable, since a hypocaloric

high-protein, as opposed to high-carbohydrate, diet can improve insulin sensitivity and spare lean body mass [83]. In fact, EAA supplementation in aged adults with sarcopenia has been shown to improve not only lean body mass [84] but also insulin sensitivity and IGF-1 serum concentrations, as well as decrease serum concentrations of tumor necrosis factor-alpha, a systemic inflammatory marker [85]. It should be mentioned that IGF-1 also activates mTOR signaling.

3.1.2. Vitamin D

A recent study found that exercise and supplementation with protein, EAAs, and vitamin D in sarcopenic elderly people increased fat-free mass, strength, IGF-1, well-being, and daily life function relative to not only controls but also exercise alone [86]. Therefore, in addition to the supplementation of protein in aging, the benefit of supplementing micronutrients, like vitamin D, may be crucial [87]. This is to some extent due to the high prevalence of vitamin D insufficiency in middle-aged and aged adults: nearly half are either at risk for deficiency or inadequacy based on serum 25-hydroxyvitamin D (25-OHD) levels of less than 30 nmol/L or 30-49 nmol/L, respectively [88]. Both vitamin D_2 (ergocalciferol) and D_3 (cholecalciferol) can be ingested from the diet and supplements, but D_3 is also synthesized in the skin from cholesterol and its synthesis is dependent on sun exposure (i.e., by UVB radiation). Both vitamin D_2 and D_3 are hydroxylated in the liver to two respective 25-OHD metabolites, which collectively are measured in serum to determine vitamin D status of an individual. However, these metabolites are further hydroxylated by 1α-hydroxylase (1α-OHase) principally within the kidneys to form the biologically active hormonal forms of vitamin D, ercalcitriol (1,25-dihydroxyergocalciferol) and

calcitriol (1,25-dihydroxycholecalciferol), which are collectively referred to as 1,25-dihydroxyvitamin D or 1,25$(OH)_2$D.

Vitamin D status based on serum 25-OHD concentrations in older adults is positively correlated with healthful muscular fat infiltration [89], improved functional performance, psychomotor function, and strength [90], as well as suppressed rates of decline in performance [91], which suggests a role of vitamin D in neuromuscular function [92]. In support of this, systematic reviews and meta-analyses have shown vitamin D supplementation, in deficient elderly men and women, enhances strength [93] and balance [94], as well as reduces insulin resistance [95] and the risk of falls [96]. Additionally, based on a more recent meta-analysis, the increase in muscle strength found with vitamin D supplementation in the elderly is most evident in the lower limbs (i.e. most commonly assessed my knee extension), which includes muscles generally more susceptible to sarcopenia (i.e., proximal leg muscles), and is not accompanied by an increase in muscle mass [97], suggesting an influence on the neuromuscular system.

Vitamin D deficiency, which results in motor decline and myopathy that predominantly affects the number of type II fibers, can be ameliorated with dietary supplementation of vitamin D (reviewed in [98]). Currently, the daily RDA for vitamin D is 600 International Units (IU) (15 μg) for those 1-70 years of age and 800 IU (20 μg) for those older. Although the beneficial effects of supplemental vitamin D in aging individuals that are already at a sufficient status remains inconclusive, some evidence exists that supplementation may still be beneficial in these individuals. For example, a recent meta-analysis showed that daily dosages of ≥4000 IU vitamin D in healthy young adults significantly increased lower and upper limb muscle strength [99].

Whether this can extrapolate to aging individuals at already sufficient levels of vitamin D requires further corroboration. However, a meta-analysis that focused on fall prevention in the elderly, determined that <700 IU of daily supplemental vitamin D or attained serum 25-OHD levels <60 nmol/L yielded no reduction in falls, whereas ≥700 IU or ≥60 nmol/L significantly reduced the risk of falling [100]. These findings are certainly influenced by baseline vitamin D levels prior to intervention, supplementation strategy, and supplemental form of vitamin D. For instance, marginally greater prevention may be achieved with supplemental vitamin D_3 in lieu of vitamin D_2. Thus, additional studies are needed to define proper timing and duration of intervention, doses, and risks of each individual vitamin D form.

Though vitamin D appears to be the most promising and extensively studied micronutrient in the context of age-related neuromuscular dysfunction, little is known about its mechanism in tissues beyond the gut, kidney, and bone. Vitamin D likely plays a beneficial role through both the direct activation of vitamin D receptor (VDR) and indirect action of regulating calcium and phosphate. Interestingly, aging in humans is associated with decreased VDR expression in muscle, regardless of muscle location or serum 25-OHD levels [101]. From both animal and *in vitro* studies, we know that VDR activation regulates gene expression that is involved in muscle cell development, differentiation, and growth. Moreover, a nonnuclear or membrane-associated VDR is presumably responsible for rapid, non-transcriptional signaling that mediates the actions of calcium influx and contraction, as well as involves pathways downstream of IGF-1 that regulate growth (reviewed in [102]). IGF-1 signaling is further implicated as vitamin D activates a specific tyrosine kinase, Src [103], which can then activate the IGF-1 receptor [104]. Lastly, myoblasts and myotubes have been shown to have functionally active 1α-OHase [105],

indicating that muscle may be a target tissue for 25-OHD as it can be converted to biologically active vitamin D.

Aside from the effects of vitamin D in muscle, some evidence of neurotrophic and anti-inflammatory effects exists as well (reviewed in [106]). First and foremost, vitamin D deficient diets in rodents have some deleterious effects on performance and, interestingly, result in alterations in the genomic and proteomic profile of muscle, specifically in NMJ-related genes and proteins [107]. Additionally, 25-OHD and $1,25(OH)_2D$ have been detected in human cerebrospinal fluid [108], and the well-characterized and widespread distribution of VDR and 1α-OHase in neurons and glial cells within the human brain suggests autocrine/paracrine properties of vitamin D in the brain [109]. On a similar note, an *in vitro* study has demonstrated that activated macrophage cells (microglia) in the brain can convert 25-OHD into $1,25(OH)_2D$ [110], suggesting that the brain may react to inflammation by increasing $1,25(OH)_2D$ concentrations. *In vitro* studies have also revealed that $1,25(OH)_2D$ can regulate the expression of several neurotrophic factors [111], stimulate VDR expression in oligodendrocytes [112], trigger anti-inflammatory responses in human brain pericytes [113], and inhibit proinflammatory cytokine production in microglia [114]. Furthermore, vitamin D has been shown in rats to enhance cholinergic activity [115], induce nerve growth factor production [116, 117], improve nerve recovery and myelination after injury [118], and protect against neural aging [119]. A recent systematic review by Minshull et al. [120] indicated that vitamin D may expedite neuromuscular remodeling and repair in animal models of injury, specifically with a 24 to 140% enhancement of recovery compared to controls. However, this same systematic review, acknowledged that the effects in humans are inconclusive and actually do no show an effect of

vitamin D supplementation on neuromuscular strength adaptations following exercise. Still, meta-analysis of the data was limited due to considerable heterogeneity of methodology and outcomes across studies, which echoes the need for further research. Overall, although not directly tied to the aging NMJ, the cumulative clinical and preclinical evidence points to a benefit of vitamin D on the neuromuscular system.

3.1.3. Omega-3 PUFA

The only essential omega-3 fatty acid alpha-linolenic acid, which is commonly found in plant oils, can be converted into eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The latter two omega-3 PUFA are known to be associated with many healthy effects and are found in fish, phytoplankton, marine algae, and animal products (e.g. egg yolks). In fact, fish oil supplements are generally taken for these beneficial health effects and as an inexpensive source of the polyunsaturated fatty acids (PUFA).

In regards to its beneficial effects on the neuromuscular system, just 21 days of supplementation with 5 ml of seal oil (0.38 EPA and 0.51 g DHA) daily in young athletic adults has been shown to improve peripheral neuromuscular function, energy, and overall performance [121]. These beneficial effects appear to extrapolate to the aging population, too. For instance, higher plasma PUFA levels in older adults are associated with greater muscle size and strength [122], whereas lower levels are predictive of a greater age-related decline in peripheral nerve function [123]. Furthermore, twice a day ingestion of omega-3 PUFA (totaling 1.86 g EPA and 1.50 g DHA a day) for six months in older adults increased strength and thigh muscle volume, but only

marginally improved power output, even in the absence of a structured exercise program [124]. Likewise, in the presence of a training program, the improvement in both neuromuscular capacity and functional performance observed in old aged individuals was greater in the supplemental groups, i.e., with fish oil at each meal providing a total of ~0.4 g EPA and 0.3 g DHA per day for at least 90 days [125]. In contrast, in another study, 12 weeks of 1.3 g of PUFA supplementation twice a day, totaling 0.66 g EPA and 0.44 g DHA per day, did not affect the evaluated parameters on body composition, strength, and physical performance in older adults [126]. The reason for this discrepancy is not known, but it could be due to population differences (baseline PUFA levels, genetic differences) or differences in study design. Additional studies are needed to assess whether individuals with diets adequate in PUFA still confer benefits from supplementation.

The beneficial effects of omega-3 PUFA supplementation to neuromuscular function in aging is corroborated by a considerable amount of evidence at the molecular level. Dietary fish oil supplementation has been shown to regulate the muscle transcriptome in older adults. In particular, pathways involved in mitochondrial function and extracellular matrix organization were increased, whereas pathways involved in proteolysis and inhibition of the main anabolic regulator, mTOR, were decreased [127]. The impact on mitochondrial function can lead to a decrease in reactive species production and thus indirectly impact NMJ health (as described in section 3.10). These beneficial effects of dietary omega-3 PUFA on muscle composition, quality, and protein metabolism in older adults are further reviewed in Smith, 2016 [128].

3.2. Other nutritional ingredients

3.2.1. Beta-hydroxy-beta-methylbutyrate

A minor leucine metabolite, known as beta-hydroxy-beta-methylbutyrate (HMB), is an ingredient commonly used to maintain muscle in elderly populations. A recent systematic review and meta-analysis has substantiated that HMB supplementation preserves muscle mass in older adults [129]. Aside from preserving muscle mass, HMB supplementation has been shown to improve strength and muscle quality without training in older adults [130], as well as increase both fat-free mass gain and percent body fat loss in old aged individuals engaged in a strength training program [131]. Furthermore, HMB can improve endurance performance (i.e., physical working capacity) in untrained men and women, as it appears to delay the onset of neuromuscular fatigue [132]. Although the optimal dosage of HMB for neuromuscular benefits remains inconclusive, most studies show beneficial effects with the use of 2 to 3 g/day. Additionally, oral doses of 6 g of HMB per day for 1 month have been shown to be well-tolerated in humans with no side-effects, and doses up to 100 g/day have been used in animal models [129].

While it is unclear whether these neuromuscular effects are mediated by peripheral neurotrophic effects, a growing literature is exploring the effects of HMB in the brain as it crosses the blood-brain barrier in rats [133]. For example, HMB is known to promote neurite outgrowth *in vitro* [134], and long-term supplementation in aging rats preserves the dendritic tree of pyramidal neurons in the medial prefrontal cortex [135], which may account for the beneficial cognitive

effects observed in aging [136, 137]. The beneficial effects of HMB have been proposed to be mediated through inhibiting proteolysis and upregulating the GH/IGF-1 axis, mTOR signaling, and presumably cholesterol biosynthesis (reviewed in [138]). mTOR signaling is known to regulate autophagy, which is a dysregulated pathway recently implicated in age-related NMJ dysfunction [27]. Overall, HMB appears to be promising for the aging neuromuscular system; however, further research is needed to confirm these findings and understand the exact mechanism of action on the neuromuscular system

3.2.2. Creatine

Creatine is a non-essential nutrient naturally synthesized in the human body from glycine, arginine, and methionine that helps to supply cellular energy. By being taken up and stored as phosphocreatine in tissues, the high-energy phosphate group can be used to resynthesize ATP from ADP. Considering the high-energy needs of muscle and nervous tissue, creatine plays a vital role, especially in aging when there is mitochondrial dysfunction. Creatine is also found in meat and additively contributes to circulating creatine and its storage. Furthermore, creatine supplementation, generally in the form of creatine monohydrate, appears to be beneficial for cognition [139] and muscle performance [140]. Lastly, the optimal dosage of creatine appears to be 3 to 5 g/day. At this dosage, creatine is well-tolerated, whereas at higher single doses of 10 g or more are occasionally associated with mild gastrointestinal discomfort (reviewed in [141]).

In regards to its effects on the neuromuscular system, creatine supplementation has been shown in young healthy individuals to improve functional parameters assessed by electromyography

[142]. This beneficial effect of creatine on neuromuscular function is also seen in the elderly, as it has been shown to improve physical work capacity by delaying neuromuscular fatigue [143]. Although limited studies exist on neuromuscular function per se, a recent meta-analysis supports a beneficial role for creatine supplementation during resistance training in aging individuals by improving muscle mass gain, strength, and functional performance over resistance training alone [144]. Moreover, a review of the current literature suggests that creatine supplementation, even without resistance training, in the elderly can potentially delay muscle atrophy and improve muscular endurance, muscular strength, and bone strength [145]. Furthermore, a natural precursor to creatine, guanidinoacetic acid, is currently being investigated as a performance-enhancing supplement; however, much of this research is preliminary and whether its beneficial effects can be applied in the context of aging has yet to be determined (reviewed in [146]).

3.2.3. Dietary phospholipids

Dietary milk fat globule membrane (MFGM), composed of macronutrients as well as a substantial amount of phospholipids (e.g., phosphatidylcholine, phosphatidylserine, sphingomyelin), may be beneficial for the neuromuscular system in aging adults. Given that the dietary phospholipids found in MFGM support myelination in the developing nervous system of rodents [147] and upregulate factors that aid in NMJ formation and myotrophy, it is unsurprising that MFMG supplementation with exercise in mice has been shown to improve age-related deficits in muscle function [148].

Likewise in middle-aged adults, 1g of MFGM supplemented daily for 4 weeks combined with exercise significantly improved strength and neuromuscular output relative to those in the exercise alone group [149], whereas 10 weeks of supplementation further benefited neuromuscular output, physical performance, and muscle size [150]. A recent study has shown that at the same dosage for 16 weeks in the elderly, MFGM with exercise could improve frailty status; however, MFGM alone had minimal effects on frailty status [151]. These differences could be related to baseline population differences (sarcopenia/frailty status) since it is known that sarcopenia severity may impact response to nutritional intervention [152]. Nonetheless, MFGM appears promising and further evaluation is warranted in populations with neuromuscular dysfunction. Lastly, no side-effects from dietary supplementation with MFGM have been reported, but an optimal dose remains unknown.

4. Future Research

More research is needed to elucidate the integration and temporal relationships of contributing factors to neuromuscular dysfunction in aging. Additionally, investigating the underlying cause of the age-related neuromuscular dysfunction will help guide the development of targeted interventions for aging individuals and may even lead to insights on neuromuscular diseases. In regards to dietary interventions, more research is certainly needed to elucidate the mechanisms by which dietary supplementation can impact the neuromuscular system, especially in the context of aging. Furthermore, further research is essential to define the optimal initiation, dosage, and duration of dietary supplementation. Indeed, other promising dietary supplements

especially those with strong antioxidant properties may be useful, if future studies can demonstrate a clinical benefit on neuromuscular health.

5. Conclusions

There are many aging features, especially within the neuromuscular system, that have been described as contributing factors to the age-related decline in physical function. These include the structural, physiological, and functional diminution of neural and muscular tissue, as well as systemic changes, like mitochondrial dysfunction, augmented oxidative stress and inflammation, and diminished levels of anabolic hormones.

In view of these well-characterized features of aging, the consequences of many lifestyle factors have been explored. Although physical activity is important for healthy neuromuscular aging [153], less is known about the role of nutrition. Aside from getting the required minimal intake of micronutrients and optimal intake of macronutrients (as defined for older adults), a potential role for specific nutrients or nutritional ingredients may exist by providing targeted benefit to the neuromuscular system. To date, vitamin D, omega-3 PUFA, HMB, creatine, and MFGM provide the most substantial evidence in promoting healthy neuromuscular aging. In particular, nutritional supplementation with these dietary supplements may be beneficial for promoting healthy neuromuscular aging as they target precise mechanisms that are affected: (1) vitamin D can promote myotrophic, neurotrophic, and anti-inflammatory effects, (2) omega-3 fatty acids can positively affect muscle transcriptome, specifically with pathways involved in mitochondrial function, muscle integrity, and anabolism, (3) HMB can be neurotrophic, anti-catabolic, and

indirectly anabolic, (4) creatine can improve cellular bioenergetics, (5) and MFGM may have both neurotrophic and myotrophic effects.

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Figure Legends

Figure 1. A summary of the contributing factors to age-related neuromuscular dysfunction. Many systemic changes, like mitochondrial dysfunction, augmented oxidative stress and inflammation, and reduced levels of anabolic hormones, are implicated in the age-related degeneration of the neuromuscular system. Altogether, these age-related changes result in neuromuscular dysfunction. Key: ↑: increased; ↓: decreased; ROS: reactive oxygen species; AchRs: Acetylcholine receptors

Figure 2. A depiction of the neuromuscular system and junction. Motor neurons from the spinal cord project to muscle appending at the neuromuscular junction, which consists of a pre-synaptic motor nerve terminal, the synaptic cleft, and the post-synaptic motor endplate (i.e., the muscle membrane). The basic functional unit of the neuromuscular system, the motor unit, is comprised of a single motor neuron and its innervating muscle fibers that contract simultaneously provided sufficient discharge from the neuron. Motor units can have different contractile response characteristics dependent on muscle fiber type innervation. Type I (slow-twitch) muscle fibers display lower contractile speed, force generation, and susceptibility to fatigue, as well as greater mitochondria and myoglobin content.

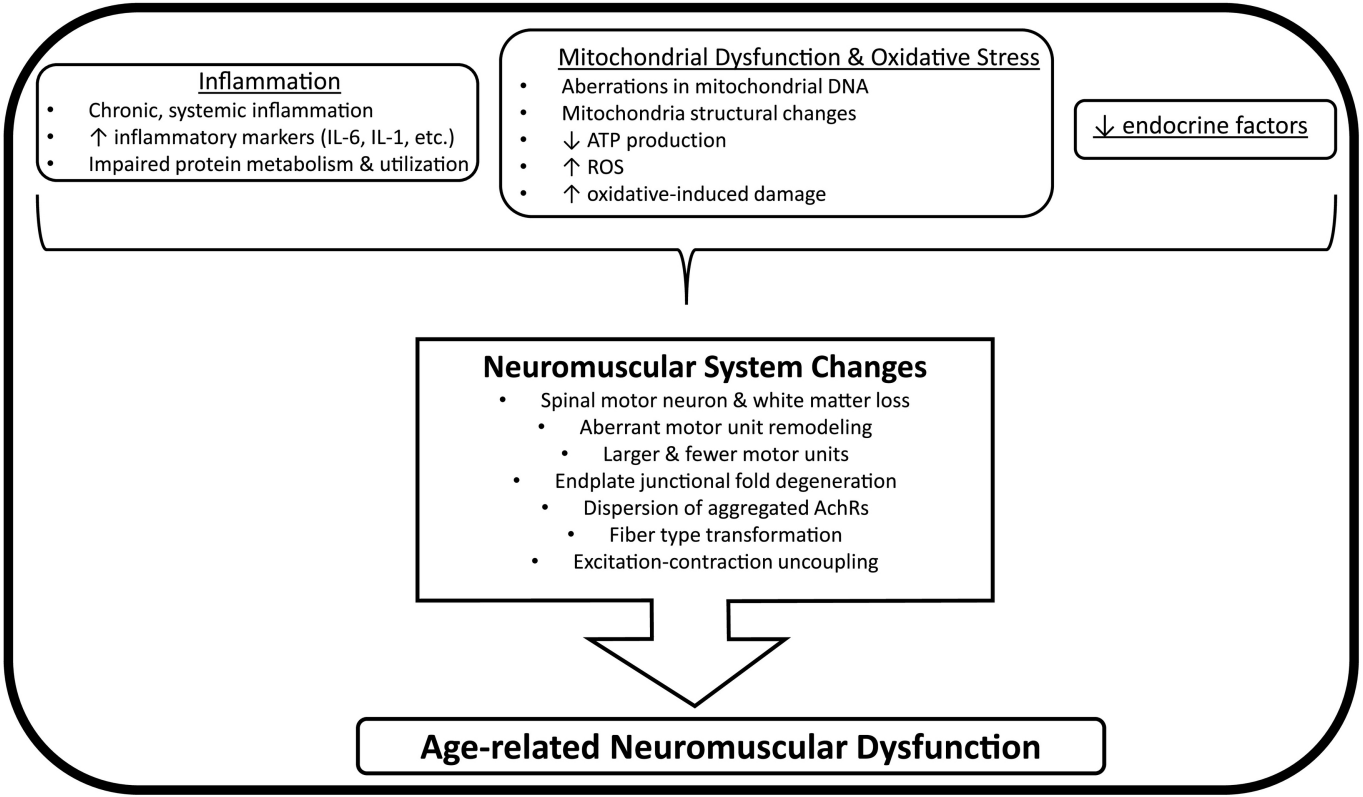
Inflammation
• Chronic, systemic inflammation
• ↑ inflammatory markers (IL-6, IL-1, etc.)
• Impaired protein metabolism & utilization
Mitochondrial Dysfunction & Oxidative Stress
• Aberrations in mitochondrial DNA
• Mitochondria structural changes
• ↓ ATP production
• ↑ ROS
• ↑ oxidative-induced damage
↓ endocrine factors
Neuromuscular System Changes
• Spinal motor neuron & white matter loss
• Aberrant motor unit remodeling
• Larger & fewer motor units
• Endplate junctional fold degeneration
• Dispersion of aggregated AchRs
• Fiber type transformation
• Excitation-contraction uncoupling
Age-related Neuromuscular Dysfunction

Figure 1

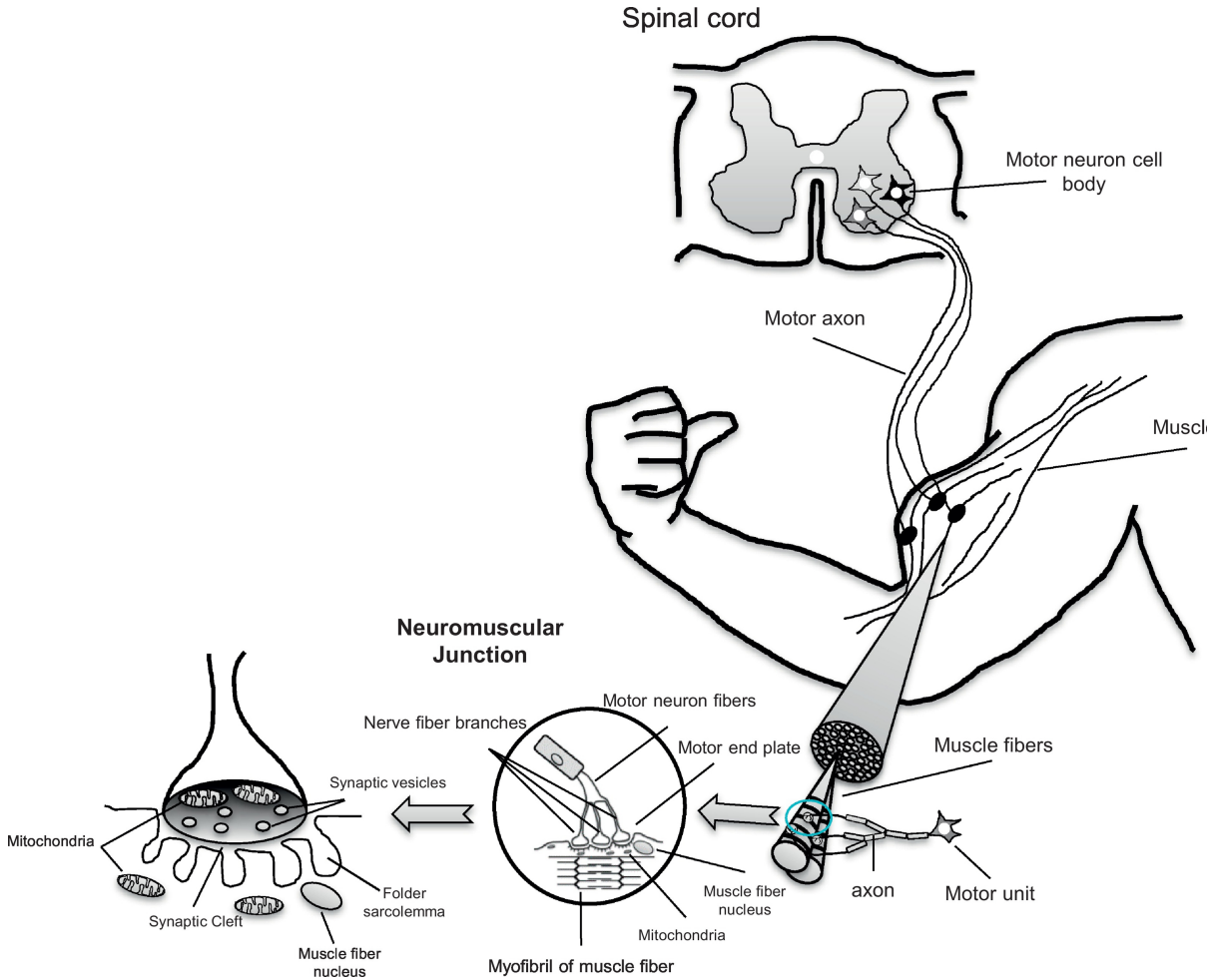
Spinal cord
Motor neuron cell body
Motor axon
Muscle
Neuromuscular Junction
Motor neuron fibers
Nerve fiber branches
Motor end plate
Muscle fibers
Synaptic vesicles
Mitochondria
Folder sarcolemma
Synaptic Cleft
Muscle fiber nucleus
Muscle fiber nucleus
axon
Motor unit
Mitochondria
Myofibril of muscle fiber

Figure 2