Dietary Interventions to Reduce Frailty

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Frailty is a state of accelerated aging that increases susceptibility to adverse health outcomes. Due to its high societal and personal costs, there is great interest in discovering beneficial interventions to attenuate frailty. These interventions can include dietary supplements like vitamins, metalloids, and antioxidants. While many supplements show beneficial results in older pre-clinical models of frailty, these results are often sex specific. Testing these interventions in pre-clinical models can facilitate the understanding of their impact on underlying mechanisms of frailty.

healthspan vitamin supplements frailty index frailty phenotype

1. Introduction

Daily consumption of dietary supplements and other dietary modifications are commonly proposed as a way to improve overall health. In Canada, 65% of women between 51 and 70 years of age use such supplements, but only 42% of men in the same age group do so [1]. While the use of supplements is widespread, there is less information regarding their effectiveness at improving health in older adults. One proposed use for supplements is to reduce frailty or attenuate the negative effects of age on health. While many of these interventions are similar to clinical ones [2], the following sections focus on the evidence found in pre-clinical models.

2. Vitamin Supplements

Many of the interventions designed to attenuate frailty in pre-clinical models use vitamins. Vitamins are biologically active compounds that are important for health and that may or may not be partially synthesised endogenously. While all vitamins are crucial, only a few have been tested as frailty interventions, as discussed below.

2.1. Vitamin D

Many studies have used vitamins as an intervention to attenuate frailty. The most common intervention is vitamin D_3 (25-hydroxyvitamin D). Vitamin D_3 is a prohormone formed in skin by the combination of ultra-violet light and a cholesterol derivative [3]. Interestingly, vitamin D_3 is not readily found in food and must instead be synthesised. This makes vitamin D_3 more like a hormone than a traditional vitamin [3]. Vitamin D_3 has multiple physiological functions, such as maintaining skeletal muscle health [4], increasing bone density [5], and preserving cardiovascular health [6]. This is not surprising, as vitamin D receptors (VDRs) are found throughout the body [7]. The absence of vitamin D_3 has also been linked to multiple pathologies. VDR knockout (KO) mice have higher mortality, lower weight,

increased alopecia, and bone malformations when compared to wildtype controls [8]. VDR KO mice also tend to develop secondary hyperparathyroidism even when fed a high calcium, high phosphorus rescue diet [9]. Another important function of vitamin D_3 is its role in maintaining calcium and phosphate homeostasis [3]. It regulates calcium absorption in the gut and controls serum levels of calcium [10]. Interestingly, similar pathological phenotypes occur in VDR KO mice, even when they are fed a high calcium rescue diet [11]. Another genetic mouse model of low vitamin D_3 involves the hepatic CYP2R1 enzyme, which converts vitamin D_3 into circulating 25-hydroxyvitamin D (25-OHD) [12]. CYP2R1 KO mice have enlarged livers, and very low circulating levels of calcium and phosphorus [12]. Interestingly, levels of the enzyme CYP2R1 decrease with age naturally in mice, leading to low levels of 25-OHD [13]. This suggests that aging mice may greatly benefit from vitamin D_3 supplementation. These multi-system effects of vitamin D_3, along with aging pathologies linked to vitamin D_3 deficiency, make it a prime target as an intervention to mitigate frailty.

The importance of vitamin D_3 in skeletal muscle health suggested to some researchers that it might reduce physical frailty. Studies in mice show that chronic vitamin D_3 deficiency reduces skeletal muscle contractility [14], and more recent work shows that skeletal muscle metabolism is disrupted in VDR KO mice [15]. The use of vitamin D_3 to improve physical health was investigated by Seldeen et al. [16] when young male mice (6 months old) were given diets either deficient in vitamin D_3 (125 IU) or with sufficient levels of vitamin D_3 (1000 IU) for 12 months [16]. Mouse health was assessed by several physical performance measures (grip strength, balance, endurance, and time to exhaustion), but the physical phenotype was not assessed. Mice deficient in vitamin D_3 had lower uphill sprint exhaustion times, reduced stride length and grip endurance but no change in grip strength [16]. These changes in physical performance were associated with an increased expression of genes that code for muscle atrophy pathways in the quadriceps [16]. However, there are no changes in serum markers of inflammation in these vitamin D_3 deficient animals [16]. A follow-up study by the same group used older male mice (24 months old) and measured frailty with the frailty phenotype [17]. Instead of studying vitamin D_3 insufficiency alone, they added another group with a high vitamin D_3 diet (8000 IU). After the 4-month exposure period, mice with both insufficient and normal levels of vitamin D_3 had higher frailty [17]. Importantly, this was not seen in the high vitamin D_3 group [17]. Interestingly, they noted no increase in bone mineral density as might have been expected with high levels of vitamin D_3 supplementation. Similarly, Liu et al. [18] measured frailty using a modified frailty index in middle-aged male rats (13 months) fed a vitamin D_3 supplemented (1.8 IU/kg) diet for 8 months [18]. Rats that took vitamin D_3 had significantly lower frailty index scores than their age-matched controls [18]. Unlike the work by Seldeen et al. [17], they did find a protective effect of vitamin D_3 on bone mineral density in older rats [18]. The difference in results of vitamin D_3 supplementation on bone mineral density may be due to the use of different doses (8000 IU vs. 1.8 IU/kg), varying timeframes (4 vs. 7 months), or differences in species (mouse vs. rat). Taken together, these studies indicate that vitamin D_3 supplementation is a promising intervention to mitigate frailty, even if it is started later in life. This also highlights the importance of having sufficient vitamin D_3 levels, as a lack of this essential nutrient may increase frailty. Importantly, these studies used only male rodents, which limits the applicability of this work. Future work should determine whether vitamin D_3 supplements at similar doses and delivered over similar time frames are effective in older females. As there is still controversy on the precise mechanisms through which vitamin D_3 exerts these beneficial effects, more work in this area is warranted.

2.2. Vitamin C

Vitamin C or ascorbic acid is an essential vitamin that is obtained through the diet. It is absorbed through food and cannot be synthesised by humans. This makes vitamin C, unlike vitamin D, a true vitamin. Physiologically, vitamin C acts in a similar fashion to antioxidants and it is necessary for human health [19]. Vitamin C supplementation has been suggested to augment immune function either via antioxidant protection or by directly enhancing immune cell function [20]. For example, influenza virus A infected male mice show lower expression of proinflammatory cytokines in the lung when they are vitamin C deficient when compared to infected mice with adequate vitamin C levels [21]. By contrast, this result is not found in female mice [21]. There is also evidence that high doses of vitamin C kills cancer cells in mice [22] and that supplementation with this essential nutrient extends lifespan in murine models [23]. Combined, these studies suggest that vitamin C has the potential to affect frailty, especially via beneficial effects on the immune system. A complication related to vitamin C supplementation in mouse models is that, unlike humans, mice synthesise their own vitamin C [19]. Hence, many researchers use a Gulo KO model where the gulo enzyme (L-gulo-y-lactone oxidase), essential for vitamin C synthesis, is knocked out [24]. These mice have lower body weights, a significantly reduced lifespan, and higher serum cholesterol levels [24][25]. These findings suggest that increased levels of vitamin C may improve health by attenuating multiple underlying frailty mechanisms such as those involving inflammation.

Animal studies have not yet explored vitamin C as an intervention for frailty, although some studies show promising effects on both lifespan and overall markers of health. To better investigate vitamin C's antioxidant effects, Selman et al. [26] used female mice exposed to cold stress to increase oxidation. Young wildtype mice were kept in cold conditions (7 °C) and then administered lifelong vitamin C supplementation [26]. They found no improvement in energy expenditure, metabolism, or lifespan in cold-exposed mice fed vitamin C. Interestingly, this study also found that cold exposure alone had no effect on mouse lifespan, unlike previous work that has shown a decrease in lifespan when oxidation levels are increased [27]. Thus, these findings suggest that cold-induced oxidation may not be an ideal oxidation model [26]. Uchio et al. [28] used senescence marker protein 30 knockout (SMP30 KO) male mice to test this intervention. These SMP30 KO mice show increased tissue susceptibility to damage [29] and cannot produce vitamin C [30]. SMP30 KO mice were given either high or regular doses of vitamin C for 2 months before half the mice in each group were given dexamethasone as a glucocorticoid analog to mimic an increase in stress [28]. Mice fed high levels of vitamin C had preserved immune function, normal cytokine levels and preserved T-cell count after dexamethasone treatment [28]. This shows that vitamin C supplementation can maintain immune system function under stress. Thus, these studies show mixed results regarding the beneficial effects of vitamin C supplementation, with preservation of immune function in aging being the best characterised. Interestingly, while the study utilising male mice showed beneficial results [28], the one using females did not [26], suggesting possible sex-specific effects of vitamin C supplementation. Considering the detrimental effects of systemic immune dysfunction with age, future work could focus on vitamin C supplementation and its impact on inflammaging and frailty in both sexes.

2.3. Vitamin E

Vitamin E, or α-tocopherol, is an essential vitamin which is mainly found in animal fats and plant oils. It is generally categorised as an antioxidant. Like other supplements, vitamin E has numerous physiological effects. For example, there is evidence that vitamin E can alter cytokine production in human and animal models [31]. Vitamin E is also implicated in neurological development, as young mice fed a vitamin E deficient diet have reduced cognition and increased brain oxidation [32]. This was further examined using α-tocopherol transfer protein (TTP) knockout mice. TTP plays a role in controlling systemic levels of vitamin E. Adult male and female mice without the TTP protein show inhibition of neurogenesis and increased expression of neurodegeneration genes along with increased signs of anxiety [33]. This suggests the importance of sufficient vitamin E, particularly in maintaining neurological health, which may translate to protection against age-related cognitive decline and potentially also attenuate the degree of frailty.

The impact of vitamin E supplements on frailty have not been fully investigated, but effects on lifespan and physical performance have been explored. Focusing on antioxidant effects, Navarro et al. [34] fed mice a lifelong vitamin E supplementation diet. Interestingly, they found a sex-specific effect on survival, where males fed vitamin E had lower mortality, but this was not seen in females [34]. Using only the male mice, they determined that vitamin E supplementation improved motor coordination and exploratory behavior compared to controls. As in previous work, they found that males given vitamin E had less oxidative damage in their brains compared to controls [34]. This suggests that many of the health benefits of vitamin E may be mediated through protection against oxidation; however, future work is required, especially as these beneficial effects may not occur in females.

2.4. Nicotinamide

Nicotinamide is the amide form of vitamin B_3 and is a key component in the nicotinamide adenine dinucleotide pathway (NAD+). This compound can be both obtained from the diet and endogenously synthesised [35]. Interestingly, NAD+ levels decrease with age and this is linked to cellular senescence [36]. Many other aging processes including DNA damage, cognitive impairment, and mitochondrial changes are linked to lower NAD+ [37]. These are highlighted in an NAD+ deficient mouse model, C57Bl/6RccHsd, which has a nicotinamide nucleotide transhydrogenase gene deletion. Male C57Bl/6RccHsd mice exhibit a reduction in insulin sensitivity and altered metabolism compared to controls [38]. However, there are sex differences in the NAD+ pathway, where female mice are resistant to the metabolic dysfunction resulting from a nicotinamide deficiency unlike males [39]. These beneficial effects are promising as healthspan interventions and suggest that nicotinamide may be a useful intervention to reduce frailty [40].

The effects of nicotinamide supplementation on overall markers of health in pre-clinical models have been investigated, but the effects on frailty directly have not been measured. Mitchell et al. [41] explored the beneficial effects of nicotinamide on metabolism. They fed 12-month-old male mice nicotinamide supplements with or without a high fat diet to induce obesity for their remaining life [41]. Neither of these diets resulted in a change in lifespan, but mice fed a high fat diet had improved locomotor activity when nicotinamide was also consumed [41]. This suggests that nicotinamide can offset some of the negative changes that occur with obesity in older male mice. However, when male mice are injected with nicotinamide supplements for 8 weeks, they develop insulin resistance

and increased lipid accumulation in their skeletal muscle [42]. One reason for these differing results may be the use of different doses of nicotinamide (0.5 g/g and 1.0 g/kg in food vs. 100 mg/kg injected respectively). Beneficial effects were observed with lower doses while detrimental effects occurred at the higher doses, so the concentration-dependence of these effects should be further investigated. In addition, both studies used only male mice so future work should explore the effects of nicotinamide supplementation in females as well.

3. Non-Vitamin Supplements

3.1. Allicin

Allicin is an organosulfur compound commonly found in garlic. While allicin was first investigated as a potential antibacterial agent, more recent studies have shown its potential as an antihypertensive agent [43]. It also has both anti-inflammatory and anti-tumour properties [43]. In addition, it acts as an antioxidant by reacting with thiol-possessing enzymes [43]. Consequently, garlic extracts have been proposed as anti-aging treatments for some time [44]. However, allicin has poor stability, making in vivo studies complex [45]. Like many other organic compounds, allicin impacts multiple biological pathways, and many of these are not well understood. To date only one pre-clinical study has focused on the effects of allicin on frailty. Liu et al. [18] administered low or high doses of allicin to middle-aged male rats (13 months) and followed them for 8 months [18]. They found that allicin attenuated the development of frailty over the intervention time frame, as measured by a frailty index tool [18]. High doses of allicin also increased bone mineral density and bone strength [18]. While the mechanisms underlying these beneficial effects of allicin are still poorly understood, it does show promising results in reducing frailty. Understanding the underlying mechanisms is an important next step. Future research should also incorporate female models, as no studies have yet investigated the effects of allicin on frailty in females.

3.2. Glycine

Glycine is a nonessential amino acid. While it can be synthesised by most mammals and birds, it can also be metabolised from components in many foods. While it is normally considered a nonessential amino acid, chronically low glycine levels can lead to multiple pathologies, so it is generally considered to be a conditionally essential amino acid [46]. Indeed, chronic glycine deficiency can lead to metabolic disorders such as obesity and insulin resistance [47]. Interestingly, glycine levels decline with age, but this occurs only in men [48]. Glycine also declines with age in *Caenorhabditis elegans,* and glycine supplementation increases lifespan in this organism [49]. This work also showed that glycine supplementation can maintain the methionine cycle [49]. Indeed, one small study suggested that glycine supplementation increases lifespan in male rats by reducing methionine toxicity [50]. The methionine pathway is a combination of methionine metabolism and methyltransferases that methylate multiple substrates such as DNA, histones, and telomerase. Disruption in this cycle is a proposed mechanism of aging and frailty [51]. Together, this work shows that glycine can act as an anti-aging compound in *C. elegans* and additional studies in mammalian models would be of interest.

It is not yet clear whether glycine modulates frailty in pre-clinical models. Miller et al. [52] gave both male and female mice lifelong glycine supplementation. They found that glycine extends lifespan and reduces body weight in females but not males [52]. Another study showed that a combination of glycine and the dietary supplement N-acetylcysteine improved lifespan and markers of overall health [53], as discussed in more detail in the next section. This work suggests that glycine supplementation may improve overall health, but potential mechanisms are not well understood and frailty itself has not been measured. It is also not clear whether rodents, like humans, have sex-specific changes in endogenous glycine levels with age. Future work should link changes in the methionine cycle and frailty and should determine if glycine levels change with age in both sexes.

3.3. *N*-Acetylcysteine

N-acetylcysteine (NAC) is a L-cysteine precursor that is used as a dietary supplement, although it is also an approved pharmaceutical agent. NAC was approved by the Food and Drug Administration to treat acetaminophen toxicity. However, NAC is a naturally occurring plant antioxidant that is also sold as a supplement [54]. As a therapeutic agent, NAC acts as a precursor to glutathione and can help treat hepatic toxicity [55]. However, there are other suggested therapeutic uses for NAC. It has been suggested and/or approved for other conditions including bronchopulmonary disorders and cardiovascular disease [54]. NAC has also shown potential in extending lifespan in studies in *C. elegans* and *Drosophila* [56][57]. Thus, NAC has been suggested as an anti-aging therapy and is thought to act on multiple aging mechanisms.

While NAC has not been tested as an intervention to mitigate frailty, it does improve overall markers of health. NAC was initially tested for its anti-aging abilities in the genetically heterogeneous mouse strain known as UM-HET3. This strain was developed by Flurkey et al. [58] to introduce a heterogeneous mouse strain, instead of the more commonly used highly inbred mouse strains, for aging research [58]. With this new mouse model, they tested lifelong NAC supplementation and found that NAC extended lifespan, but only in males. NAC did, however, reduce weight in both sexes compared to controls [58]. As mentioned earlier, NAC has also been combined with glycine (GlyNAC) and given to mice as a supplement [53]. Kumar et al. [53] administered a lifelong GlyNAC diet to adult (65 weeks old) male and female mice. They found a significant increase in longevity (23.7%) in both sexes [53]. This diet also protected them against the mitochondrial damage, nutrient sensing dysfunction, and genomic damage observed in older mice [53]. These findings suggest that the combination of GlyNAC may be even more beneficial at improving overall health compared to either NAC or glycine alone. It would be interesting to know whether these interventions can attenuate frailty.

3.4. Alpha-Ketoglutarate

Alpha-ketoglutarate (AKG) is key compound in the Krebs cycle and therefore is important to overall metabolism and energy production. It helps determine the rate of the citric acid cycle and acts as a precursor for the amino acids glutamate and glutamine. AKG can be produced endogenously but its levels naturally decline with age [59]. This led Chin and colleagues [60] to investigate AKG supplementation in *C. elegans*. They found that AKG extended lifespan by about 50% and prevented the age-related phenotype of rapid, uncoordinated movement characteristic

of older *C. elegans* [60]. They showed that AKG targeted aging mechanisms including the inhibition of ATP synthase and mTOR kinase inactivation [60]. This connection between extended lifespan, mTOR inhibition and AKG was further demonstrated in *Drosophila* where supplemented flies lived longer [61]. The mTOR pathway is a highly conserved pathway that is involved in nutrient sensing, apoptosis, and cell proliferation as well as other functions. This pathway is also involved in regulating innate immunity, and so it functions as an immunosuppressant, and it is the target of the anti-aging drug rapamycin [62]. As rapamycin can reduce frailty [63] through inhibition of mTOR, AKG may also be beneficial to attenuate frailty.

AKG has been directly studied as an intervention to reduce frailty in a recent pre-clinical study. Asadi et al. [64] gave middle-aged (18-month-old) male and female mice an AKG salt supplement diet for their remaining life and measured frailty using a frailty index instrument [64]. They found that while only female mice exhibited an extended lifespan, AKG decreased the amount of time animals spent frail for both sexes [64]. They also noted that AKG played a role in chronic immune regulation by reducing plasma cytokine levels, but this change was more obvious in female mice [64]. While this is only a single study, it does show that AKG is a potential beneficial intervention in the setting of frailty.

3.5. Selenium

Selenium is a trace-essential metalloid that is involved in forming selenoproteins, where the metalloid binds to cysteine residues. These selenoproteins have many physiological functions, including involvement in anti-inflammatory activities, thyroxine synthesis, and antioxidant activities [65]. In humans, selenium levels have been closely correlated with human aging [66], where daily selenium intake is positively correlated with longevity [67]. It seems likely that selenium exerts its beneficial effects through its antioxidant actions [66]. Selenium-deficient mice show higher protein turnover along with increases in glucose, which suggests that selenium plays an important role in metabolism [68]. These multiple physiological functions and anti-aging effects suggest that selenium may be beneficial as an intervention to improve health and reduce frailty.

The impact of selenium on overall health has been investigated in several studies. Yang et al. [69] tested the effects of organic and inorganic selenium supplementation on fertility in middle-aged (12 months old) female mice supplemented for 8 weeks [69]. Selenium supplementation reduces the rate of apoptosis in ovarian tissue and improves antioxidant function [69]. Another study focused on selenium supplementation and physical activity/performance in older mice. Aging mice exposed to selenium showed higher normalised grip strength than control, along with improved skeletal muscle calcium homeostasis [70]. Recently Plummer et al. [71] fed selenium supplements to male mice for 16 weeks and found that selenium supplementation protected mice against diet-induced obesity and reduced insulin-like growth factor 1 levels in both males and females [71]. There is also evidence that selenium affects neurogenesis. Leiter et al. [72] determined that dietary selenium mimics the exercise induced increase in the selenium transporter and that this can reverse age-related cognitive decline [72]. It is possible that other trace-essential metalloids may improve overall health in aging. For example, although the effects of zinc alone have not been investigated in rodent models, a diet low in vitamins, selenium, and zinc reduces muscle force production in aging mice [73]. Taken together, these studies show the potential benefits of

selenium supplementation in the context of aging. Future studies should focus on whether selenium can also improve overall healthspan by attenuating frailty.

3.6. Resveratrol

Resveratrol is a polyphenol stilbenoid produced by many different plants, including grapes, as a natural anti-parasitic. In mammals, it acts as a potent antioxidant [74] and sirtuin activator. It was proposed as an anti-aging supplement after a seminal paper described its anti-cancer properties in 1997 [75]. Since then, resveratrol has been widely studied for its anti-aging and other potential health benefits. In terms of effects on lifespan, a library of the sirtuin family of NAD+-dependent protein deacetylase activators were used in budding yeast *S. cerevisiae* to determine if they could alter lifespan [76]. This study showed that resveratrol extends lifespan by activating sirtuin 1 (SIRT1) [76]. However, these anti-aging effects are species and strain specific, and resveratrol has more effect in worms and yeast than in rodents [77]. It is also possible that instead of being a direct SIRT1 activator, resveratrol activates AMPK which in turn activates SIRT1 [78]. Other than its anti-aging properties, resveratrol may attenuate multiple age-related pathologies. It acts to restore immune system function by activating the nuclear factor kappa beta/N-terminal kinase pathway [79]. Resveratrol also reduces cardiovascular disease [80][81] and cancer [82]. These multiple beneficial effects of resveratrol and its potential as an anti-aging agent suggest that resveratrol may also reduce frailty.

Resveratrol has been used as an intervention to assess its impact on frailty and other markers of health. Kane and colleagues [83] administered resveratrol (100 mg/kg) to older male mice starting at 18 months of age and measured frailty with a frailty index. They showed that mice that received resveratrol had lower frailty scores when compared to age-matched controls [83]. Kan and colleagues [84] assessed exercise performance in resveratrol-treated (25 mg/kg) middle-aged male mice. Although resveratrol did not increase forelimb grip strength or swim times, it did reduce blood lactate levels and increased liver glycogen levels after exercise [84]. A similar study by Rodríguez-Bies and colleagues administered resveratrol (16–17 mg/kg) to old male mice to assess impacts on exercise capacity. Resveratrol reduces lipid biogenesis in skeletal muscles and increases mitochondrial biogenesis but has little effect on exercise capacity [85]. By contrast, others have reported that older male mice supplemented with resveratrol (15 mg/kg) showed increased time to exhaustion with better recovery after exercise [86]. Interestingly, these studies also found synergistic health benefits when combining aerobic exercise and resveratrol supplementation. This suggests that combining dietary supplements with other modifications such as exercise may be a highly effective strategy to mitigate frailty.

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