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Carotenoids as protection against sarcopenia in older adults

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Abstract

Sarcopenia, or loss of muscle mass and strength, plays a major role in the disablement process in older adults and increases the risk of impaired physical performance, falls, physical disability, frailty, and death. Oxidative stress is a major mechanism implicated in the pathogenesis of sarcopenia; aging muscle shows increased oxidative damage to DNA, protein, and lipids. Carotenoids quench free radicals, reduce damage from reactive oxygen species, and appear to modulate redox-sensitive transcription factors such as NF- κ B that are involved in the upregulation of IL-6 and other proinflammatory cytokines. Recent epidemiological studies in community-dwelling older adults show that low serum/plasma carotenoids are independently associated with low skeletal muscle strength and the development of walking disability. These observations are consistent with a growing number of studies showing that a diet with high intake of fruits and vegetables is associated with a reduced risk of inflammation, hypertension, diabetes, cardiovascular disease, and mortality.

Keywords

Aging; Carotene; Carotenoids; Cryptoxanthin; Inflammation; Lutein; Lycopene; Muscle; Sarcopenia; Zeaxanthin

Sarcopenia, a condition characterized by loss of skeletal muscle mass and strength with aging, is considered a key factor in the process of disablement in older adults [1]. Sarcopenia is associated with decreased lower extremity performance [2], functional impairment [3], falls [4,5], physical disability [6–8], and frailty [9]. Low skeletal muscle strength is predictive of disability [10,11] and mortality [12,13]. About one-third of women and two-thirds of men sixty years and older in the US have sarcopenia, and the estimated direct health care cost attributable to sarcopenia in the US in 2000 alone was \$18.5 billion [6].

Humans lose about 20–40% of both skeletal muscle mass and strength from 20 to 80 years of age [14,15]. Age-related changes in skeletal muscle include a decrease in muscle cross-sectional area, a loss of muscle fibers, and fiber atrophy, as well as a decrease in muscle strength. Currently, there is no formal, widely-accepted clinical definition for sarcopenia. Appendicular lean mass as determined by dual-energy X-ray absorptiometry (DEXA)¹ scanning [16] and skeletal muscle index as determined by bioelectrical impedance analysis (BIA) [17] have been used to define sarcopenia. However, the quality and composition of muscle fibers cannot be determined using DEXA or BIA, and alternatively, low skeletal muscle strength has been used as a measure of sarcopenia [18]. Low grip, hip, and knee strength are often used in epidemiological studies of aging to measure sarcopenia. In relation to the risk of

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¹Abbreviations used: DEXA, dual-energy X-ray absorptiometry; BIA, bioelectrical impedance analysis; ROS, reactive oxygen species; JNK, c-Jun N-terminal kinases; CRP, C-reactive protein; WHAS, Women's Health and Aging Studies.

dying, low muscle strength actually appears to be more important than low muscle mass in older adults [13].

The pathogenesis of sarcopenia is multifactorial and is attributed to undernutrition, oxidative stress, inflammation, endocrine changes, inactivity, and early growth. Low birth weight increases the risk of sarcopenia in later life [19,20] and genetic factors also play a role [21]. Anorexia of aging may contribute to sarcopenia, but its role is still uncertain [22]. Testosterone and adrenal androgens decline with age and may contribute to sarcopenia [23,24]. The relationship between physical inactivity and sarcopenia is relatively well established, as resistance training can increase muscle strength [25–27]. Elevations in serum interleukin-6 (IL-6), a major proinflammatory cytokine, have been associated with poor strength, incident mobility disability, decline in walking speed, and poor physical performance [28,29]. Many of the factors that have been implicated in sarcopenia do not act in isolation, and many of their causal pathways intersect or overlap in relation to oxidative stress.

Oxidative stress and sarcopenia

Oxidative stress has been implicated as a central mechanism in the pathogenesis of sarcopenia [30]. Oxidative damage to DNA, proteins, and lipids increases in human skeletal muscle with age. Biopsy studies demonstrate that 8-hydroxy-2-deoxyguanosine, protein carbonyls, and malondialdehyde (markers of oxidative damage to DNA, protein, and lipids, respectively) are elevated in human skeletal muscle in older adults [31–34]. Animal models also show that increased oxidative damage to nuclear DNA, protein, and lipid occurs in skeletal muscle with age [35,36]. Oxidative damage in skeletal muscle has been associated with the atrophy and loss of muscle function and fibers in sarcopenia [30,37].

Mitochondria are a major source of reactive oxygen species (ROS) in skeletal muscle, and mitochondrial DNA may be especially susceptible to oxidative DNA damage [38].

Mitochondrial DNA deletions and mutations increase in human skeletal muscle with increasing age [39–44]. Mitochondrial DNA damage is associated with muscle fiber atrophy and loss [45]. Mitochondrial DNA deletions lead to impaired mitochondrial function and further generation of free radicals, with increased lipid peroxidation and impaired oxidative DNA repair in the nucleus [46]. The accumulation of mitochondrial and nuclear DNA damage is thought to eventually compromise function, leading to the loss of myocytes [14].

Inflammatory cytokines and sarcopenia

Reactive oxygen species can damage muscle tissue directly, but they also provide a trigger for the expression of inflammatory cytokines such as interleukin (IL)-1 β , tumor necrosis factor (TNF)- α , and IL-6. In sepsis, cachexia, and chronic inflammatory conditions, inflammatory cytokines are associated with loss of muscle mass and strength, but whether long-term, low level elevations in proinflammatory cytokines in aging adults cause sarcopenia requires further investigation [1]. Redox-sensitive transcription factors NF- κ B [47,48] and AP-1 [49] are involved in the upregulation of inflammatory cytokines such as IL-6. NF- κ B is a transcriptional regulator that is member of the Rel family proteins [48]. NF- κ B is maintained in the cytoplasm where it is bound to I κ B. ROS enhance the signal transduction pathways for NF- κ B activation in the cytoplasm through serine or tyrosine phosphorylation of I κ B [48], and disruption of the I κ B:NF- κ B interaction is followed by translocation of NF- κ B from the cytoplasm to the nucleus, where NF- κ B regulates the transcription of IL-1 β [50], IL-6 [51], and IL-18 [52]. AP-1 is a nuclear transcription factor that is regulated through synthesis of Jun and Fos proteins and their phosphorylation [49,53]. Two signaling cascades, c-Jun N-terminal kinases (JNK) and p38 mitogen-activated protein kinase (MAPK), lead to the induction of *jun* and *fos* genes [49]. ROS play an important role in the activation of both JNK and MAPK pathways [48,49,

54]. The redox regulation of NF- κ B and AP-1 are involved in the expression of many proinflammatory cytokines, and these cytokines, in turn, are involved in a complex cascade that involves the upregulation of C-reactive protein and feedback loops involving IL-10 and other inflammatory mediators.

In older age, a low-grade inflammatory state characterized by increased concentrations of cytokines and acute phase proteins is common [55,56]. TNF- α , IL-1 β , IL-6, and IL-18, and C-reactive protein (CRP) and fibrinogen are among the cytokines and acute phase proteins that may be elevated in this proinflammatory state [57]. Studies conducted among community-dwelling older adults suggest that the proinflammatory state does have a long-term consequence for sarcopenia. In the Longitudinal Aging Study Amsterdam, elevated IL-6 and CRP were associated with a loss of muscle strength over three years of follow-up [58]. In addition to sarcopenia, the proinflammatory state in older adults may contribute to a wide variety of pathological processes in older adults such as insulin resistance, dyslipidemia, coagulation, lymphocyte activation, atherosclerosis, osteoporosis, cognitive impairment, and mortality [55,56]. Oxidative stress is the pathogenic mechanism that is common to all these processes [59].

Dietary carotenoids in older adults

Given the importance of ROS and redox-sensitive signaling in the upregulation of inflammatory cytokines, what is the relative role of antioxidant nutrients in oxidative stress and inflammation? The six major dietary carotenoids (α -carotene, β -carotene, β -cryptoxanthin, lutein, zeaxanthin, and lycopene) comprise an important component of the antioxidant defense system in humans, and the major dietary sources of carotenoids are fruits and vegetables. Carotenoids are hydrophobic molecules, and thus, carotenoids interact with lipophilic elements of the cell, such as the lipid membrane bilayer. Carotenoids are commonly located within cell membranes, and the location of specific carotenoids within the membrane structure depends upon the chemical structure of the carotenoid. The carotenoids protect against oxidative stress by quenching singlet oxygen, scavenging free radicals, and inhibiting lipid peroxidation [60].

Carotenoids may quench ROS, reduce oxidative stress, and thus modulate redox-sensitive transcription factors that upregulate IL-6. In the Women's Health and Aging Study, a population-based study of older moderate-severely disabled women living in the community in Baltimore, Maryland, low total serum carotenoids were associated with higher levels of serum IL-6, and women with the lowest levels of α -carotene, β -carotene, lutein/zeaxanthin, and total carotenoids were more likely to show subsequent increases in IL-6 over a period of two years [61]. Similarly, among adult 65 years and older in the InCHIANTI study, a low plasma carotenoid level was an independent predictor of an elevated plasma IL-6 level after three years of follow-up (Richard Semba et al., unpublished observations). Thus, two large, population-based studies demonstrate that older adults with low serum/plasma carotenoid levels have a significantly higher risk of developing elevations in IL-6 over time. Could these observations be related to sarcopenia?

Evidence for role of carotenoids in sarcopenia

New investigations are emerging that show that low serum/plasma carotenoids are independently associated with poor skeletal muscle strength and impaired physical performance. Among 669 women aged 70–79 years in the Women's Health and Aging Studies (WHAS) I and II, low serum carotenoid levels were associated with poor muscle strength [62]. In multivariate models adjusting for age, race, smoking, cardiovascular disease, arthritis, and serum IL-6, low total carotenoids were associated with low grip, hip, and knee strength. In the InCHIANTI study, a population-based study of older adults in the Chianti region of Tuscany, Italy, low β -carotene intake was associated with low physical performance [63].

Further investigation in the WHAS I showed that among 554 women without severe walking disability (inability to walk or walking speed <0.4 m/sec) at baseline, women in the lowest quartile of total serum carotenoids at baseline were at significantly higher risk of developing severe walking disability over 36 months of follow-up [64]. In addition, among older men and women in the InCHIANTI study, low plasma carotenoids at enrollment were independently predictive of a higher risk of developing severe walking disability and showing a decline of grip, hip, and knee strength and walking speed over six years of follow-up in multivariate analyses that adjusted for age, sex, education, body mass index, smoking, total energy intake, and chronic diseases (Fulvio Lauretani et al., unpublished observations).

In order to address the argument that older adults only have low serum carotenoid levels because they have difficulty ambulating, shopping, and preparing balanced diets, i.e., reverse causality, we have conducted longitudinal analyses above that exclude those with walking disability or poor muscle strength at baseline. It is important to note in these analyses that low serum or plasma carotenoids at baseline are predictive of the development of severe walking disability or decline in muscle strength. The older adults in these analyses were not disabled and did not have poor muscle strength at baseline, and analyses were adjusted for cognitive status. In the longitudinal design, low serum or plasma carotenoids are temporally placed before the development of severe walking disability or poor muscle strength, and this is the strongest epidemiological evidence that can be gleaned from a natural history study.

In the presence of high levels of oxidative stress, serum or plasma carotenoids can be degraded, since, as mentioned above, carotenoids provide a balance to reactive oxygen species. One might argue that serum or plasma carotenoids are only a marker for oxidative stress and inflammation and may not be in the causal pathway. However, three observations provide further insight against such an argument. Low serum carotenoids have been shown to be independently associated with low muscle strength in multivariate analyses that adjust for serum IL-6 levels [62]. Low serum carotenoid levels are inversely correlated with serum protein carbonyl concentrations, an indicator of oxidative protein damage (Richard Semba et al., unpublished observations). Finally, a recent randomized, double-blind, placebo-controlled clinical trial conducted among post-menopausal women in Boston showed that carotenoid supplementation reduced DNA damage, as assessed by single cell gel electrophoresis [65].

Carotenoids and other related outcomes in adults

The relationships that have been observed between serum/plasma carotenoids and sarcopenia in epidemiological studies should be seen in the broader context of other outcomes such as inflammation and cardiovascular disease. Carotenoids are considered the best biological marker for fruit and vegetable intake [66], and recent large epidemiological studies show that a higher intake of fruits and vegetables is associated with a lower risk of cardiovascular disease [67–69], disability [70], and all-cause mortality [69,71]. A dietary pattern characterized by increased consumption of fruits and vegetables is associated with reduced markers of inflammation and endothelial dysfunction [72]. Diets high in fruits and vegetables lower blood pressure [73–75] and reduce markers of oxidative stress induced by acute hyperlipidemia [76]. Low serum/plasma carotenoids are also associated with an increased risk of insulin resistance [77,78] and the development of diabetes [78]. Adherence to the Mediterranean diet, which is characterized by a high intake of fruits, vegetables, and whole grains, and lower consumption of red meat and saturated fats is associated with lower circulating IL-6 [79], and a recent trial showed the Mediterranean diet reduced IL-6 in adults [80].

Research on the role of oxidative stress in aging-related morbidity has gained considerable momentum since 1956 when Denham Harman proposed the free radical theory of aging [81]. Epidemiological and clinicopathological studies support the idea that a common pathway in

multisystem age-related decline is oxidative stress and inflammation. The carotenoids, as markers of fruit and vegetable intake, are important exogenous antioxidants that are likely involved in redox balance, but there are many other dietary sources of antioxidants that are contained in the same healthy foods, such as plant polyphenols, tocopherol, ascorbate, and selenium. Over the last two decades it has become apparent that megadoses of β -carotene or vitamin E are probably not a good substitute for healthy types of diets that are high in fruits, vegetables, and whole grains and low in saturated fats.

Conclusions

Recent epidemiological studies suggest that carotenoids or carotenoid-rich foods are protective against a decline in muscle strength and walking disability among older community-dwelling adults. This line of investigation also suggests that the possible health benefits of carotenoids or carotenoid-rich foods extend beyond hypertension, atherosclerosis, and cardiovascular disease, the clinical outcomes that are conventionally examined in relationship to dietary factors. Further work is needed to determine whether plasma carotenoids are predictive of changes in balance performance and nerve conduction velocity in older adults, as walking and walking disability represent the integration of skeletal muscle strength, coordination, balance, and proprioception. In addition, the relationship between sarcopenia and other dietary antioxidants, such as α -tocopherol, ascorbate, selenium, and dietary polyphenols should also be examined in cohorts of older adults. If these observations are widely corroborated, this could provide strong rationale and justification for dietary intervention studies aimed at reducing or preventing sarcopenia among older adults.

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