Potential health effects of dietary nitrate supplementation in aging and chronic degenerative disease

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Abstract

In the United States, latest projections indicate the number of adults 65 years of age and older is expected to double by 2050. Given that increased oxidative stress is a hallmark of aging, it is understandable that waning nitric oxide and chronic degenerative disease arise in tandem. To this end, translational evidence-based strategies are needed to mitigate the impending toll on personal and public health. Dietary nitrate supplementation, particularly in the form of beetroot juice, is an active area of inquiry that has gained considerable attention in recent years. Compelling evidence has revealed beetroot juice can elicit potent physiological responses that may offer associated health benefits for multiple clinical disorders including hypertension, dementia, and sarcopenia. Even in the absence of overt disease, age-related impairments in cardiovascular and skeletal muscle function may uniquely benefit from beetroot juice supplementation as evidence has shown blood pressure lowering effects and improved muscle function/contractility – presumably from increased nitric oxide bioavailability. This, in turn, presents a practical opportunity for susceptible populations to support ease of movement and exercise tolerance, both of which may promote free-living physical activity. A theoretical rationale details the potential health effects of dietary nitrate supplementation, wherein a working framework hypothesizes beetroot juice consumption prior to structured exercise training may offer synergistic benefits to aid healthy aging and independent-living among older adults.

Keywords

arterial stiffness; beetroot juice; endothelial dysfunction; exercise; nitric oxide
Introduction

The increase in life expectancy has given rise to a growing number of older adults worldwide (1). Early deaths attributed to the direct and indirect effects of poor nutrition and infectious disease have been largely curtailed by modern advancements, such that average lifespan often exceeds 80 y in developed countries. The consequence, however, is that individuals are now living long enough to encounter a host of ailments linked to the development and progression of chronic degenerative diseases. Whereas aging itself is a natural consequence of time, it is characterized by marked physiological changes that coincide with an increased vulnerability to disease (2). In fact, cardiovascular disease, cancer, and neurodegenerative disease all share advancing age as a primary risk factor. Waning physiologic function can thus trigger a maladaptive cycle highlighted by physical activity avoidance, systemic deconditioning, and neuromuscular/cognitive impairment (see Figure 1). While habitual exercise training can attenuate many adverse age-related shifts (3), it is of interest to identify other (potentially complementary) non-pharmacologic approaches to combat the complications of advancing age and prevalence of chronic disease. Understandably, quality-of-life concerns abound for individuals and caregivers alike as the metaphorical ‘Silver Tsunami’ fast approaches.

Age-related vascular changes

The endothelium represents the physiologic interface between the flow of blood and surrounding vessel wherein a broad range of active molecules (e.g., prostacyclin, endothelin-1) modulate vascular tone. Adequate blood flow necessitates a coordinated interplay between vessel dilation and constriction to ensure proper tissue perfusion and pressure maintenance. A healthy, phenotypic expression of the endothelial layer favors vasodilation through the release of nitric oxide (NO), which possesses a strong inhibitory effect on coagulation and platelet aggregation thereby improving flow and fluidity. However, physiologic aging is accompanied by functional and structural changes of the endothelium and surrounding vessel layers that make up the vascular wall (4). Prior to a clinical diagnosis, early signs of vascular aging coincide with reduced NO bioavailability – a key feature attributed to diminishing endothelial-dependent vasodilation and arterial stiffening (5).

Mechanisms of reduced NO

It has been over 60 y since the link between aging and oxidative stress was first postulated by Denham Harman (6). Unsurprisingly, considerable work has endeavored to resolve the constraints of human lifespan and attendant comorbidities. Though multi-faceted, the pathophysiological underpinnings that contribute to age-related impairment in vascular function involve the cyclic effect of oxidative stress and inflammation. For instance, excess production of superoxide anions interferes with NO bioactivity, which in turn, weakens the degree of smooth muscle relaxation (i.e., endothelial-dependent vasodilation). Critically, the ultra-rapid reaction between superoxide and NO generates peroxynitrite – a highly reactive oxidant known to impart damaging effects on DNA, lipids, and proteins (7). Additionally, tetrahydrobiopterin, an essential co-factor for NO synthesis is oxidized which
leads to uncoupling of endothelial NO synthase – further reducing NO bioavailability (8). When compared to young adults, and in the context of aging, the functional consequences of these events are best described by the extensive research demonstrating a significant reduction in endothelial-dependent vasodilation (measured via flow-mediated dilation) in older adults (9).

**Nitrate-nitrite-NO pathway**

Given that declining NO is a hallmark feature in aging and many pathophysiological conditions (10, 11), overcoming this problem presents an attractive target to restore optimal ‘downstream’ function (i.e., tissue perfusion). Though a major source of NO occurs via the reaction of L-arginine catalyzed by NO synthases, recent work has confirmed an alternative route wherein dietary nitrate can be reduced to form nitrite. Evidence for the ‘nitrate-nitrite-NO’ pathway (see Figure 2) reveals that under low oxygen and low pH conditions, nitrite can be reduced to form NO (and other nitrosating species) (12). Accordingly, both nitrate and nitrite can be enhanced by several dietary sources particularly the consumption of leafy greens (e.g., spinach) and beetroot. Commensal anaerobic bacteria residing on the dorsal tongue readily convert nitrate to nitrite whereas further reduction to NO is performed by nitrite reductases at the organ-level (13). In the systemic circulation, plasma nitrite levels ‘typically’ peak between 2 to 3 h after consuming dietary nitrate (14). Since nitrite reduces to NO under low oxygen/pH conditions, a characteristic feature of tissue ischemia and several CVD pathologies, supplementation with beetroot juice has attracted considerable attention as a promising adjunct to exercise as well as having potential health implications in its own right (15, 16).

**Dietary nitrate**

Since a ‘lack of time’ is often cited as a barrier to healthy lifestyle practices, increasing dietary nitrate presents a feasible, time-/effort-efficient approach to support NO bioavailability (17). Whereas research involving dietary nitrate has accelerated in the previous 10–12 y, knowledge concerning its health effects to alleviate cardiovascular conditions can be traced to medieval Buddhist manuscripts (18). Green leafy vegetables and beetroot are major sources of nitrate, yet varying environmental and agricultural practices can influence nitrate content (19). Consensus among multiple governing bodies, namely the Joint Food and Agricultural Organization / World Health Organization, have set an acceptable daily intake (ADI) of nitrate ion at 3.7 mg/kg body weight (20). For illustrative purposes, this corresponds to an ADI of 296 mg nitrate for an 80 kg adult. Though fears over nitrate-/nitrite-related toxicities have persisted, scientific inquiry has recently questioned this legitimacy. For example, Hord and colleagues have described the likelihood of significantly exceeding the ADI of nitrate by adhering to the Dietary Approaches to Stop Hypertension (i.e., DASH diet) – known to be rich in vegetables (17). Given that governing entities (e.g., U.S. Department of Health and Human Services) frequently advocate for dietary patterns high in fruits and vegetables, concerns over nitrate-/nitrite-related toxicities may be overstated and should thus be balanced in a specific context (21).
**Hypertension**

The concurrent effects of declining NO bioavailability and arterial stiffening contribute to the link between increased blood pressure and advancing age. While the nitrate-nitrite-NO pathway parallels the L-arginine pathway, it is differentially regulated and becomes more relevant as a source of NO under low oxygen/pH conditions. In this sense, increasing dietary nitrate (and by extension nitrite) is a feasible approach to support vascular health among individuals with occult or overt cardiovascular disease. Consistent with this premise, a recent study involving 68 older (≈56 years) adults with hypertension (n = 34 treated; n = 34 untreated) were randomly assigned to four weeks of daily beetroot juice or placebo (deplete of nitrate). Primary study endpoints were changes in systolic (SBP) and diastolic (DBP) blood pressure (e.g., ambulatory, clinic, and home). Results showed participants supplementing with beetroot juice exhibited significant reductions (≈−8 SBP and ≈−4 DBP mmHg) in all measures of blood pressure whereas the placebo group showed no change. It is worth noting that the blood pressure lowering effects of dietary nitrate have not been universally observed among older adults and/or other at-risk populations. A probable source of this disconnect may involve the conversion of dietary nitrate to nitrite by commensal bacteria (via nitrate reductase) in the mouth. In fact, disruption of the oral microflora from antibacterial mouthwash has been linked to adverse effects on blood pressure regulation. Siervo and colleagues have postulated that differences involving oral microbiota, gastric redox environment, and peripheral blood oxygen/pH may account for some of the individual variance between blood pressure “reducer” and “non-reducer” phenotypes – thus underscoring the need for further investigation. Still, dietary nitrate supplementation has the strong potential to boost NO through the enhancement of its precursor nitrite. This, in turn, represents an opportunity to conduct adequately powered clinical trials to determine biomarkers predictive of individual responsiveness. Resolving this issue would clarify the potential efficacy of dietary nitrate as an adjunct to promote healthy aging as well as determine individual-specific (i.e., age, sex, health status etc.) dosing.

**Dementia**

Diminished brain size, reduced cerebral blood flow, and reduced oxidative metabolism are consequences of advancing age – all of which are linked to poorer cognitive function. While delayed processing speed, visuospatial skills, and executive function generally coincide with age, research has indicated cerebral hypoperfusion may precede the onset of clinical dementia. Hence, habitual consumption of dietary nitrate could offer some utility to modulate the burdens attributed to age-related changes in cerebral blood flow and nutrient (i.e., glucose) delivery. Though several clinical trials are currently poised to examine the effects of beetroot juice on indices of cognition (NCT03617302; NCT03826147), preclinical evidence reveals a diet high in nitrate acutely increases regional perfusion in frontal white matter – known to be involved in working memory, task-switching, and episodic memory retrieval. Following two weeks of daily beetroot juice supplementation, improvement in simple reaction time has also been reported in older (≈67 years) adults with type 2 diabetes. Likewise, a recent investigation tested the effects of exercise training coupled with beetroot juice on motor cortex organization. In this study,
older (≈65 years) adults with hypertension were randomly assigned to consume daily beetroot juice or placebo for 6 week. Supervised exercise training was performed 3×/week wherein participants consumed (depending on randomization) beetroot juice or placebo 1 h before exercise. Interestingly, beetroot juice and exercise training demonstrated positive effects as evidenced by improved functional connections within the motor community. Taken together, these findings have significant practical implications for quality of life outcomes such that further investigations, namely adequately powered randomized controlled trials are warranted.

Sarcopenia

Though some disparity exists concerning the operational definition of ‘sarcopenia,’ the term is generally suggestive of weakened skeletal muscle attributed to advancing age and resultant effects on metabolic health (30). Impairment to the neuromuscular system can thus readily affect overall mobility, as slow ‘preferred’ walking speed is a known predictor of disability among older adults (31). Certainly, age-related changes in muscular strength/power can make even mundane tasks of independent-living troublesome – increasing the likelihood of sedentary/inactive behaviors. Whereas a large number studies have examined dietary nitrate in interest of the cardiovascular system, a concerted effort has also attempted to uncover the benefits of dietary nitrate on muscle contractility. Much of this work has involved younger, healthy adults, however; recent findings indicate improved maximal shortening velocity and power during knee extensor exercise after acute beetroot juice supplementation in middle-aged (≈57 years) individuals with heart failure (32). More recently, Coggan et al. have observed similar results in healthy older (≈71 years) men and women (33). Along the same lines, Justice et al. have described improved rate of torque development during voluntary knee flexion/extension in middle-aged and older adults following 10 weeks of daily nitrite supplementation (34). Despite several hypotheses, the exact mechanism(s) for increased muscle contractility with dietary nitrate supplementation remains incompletely understood. Increased muscle blood flow notwithstanding, greater NO bioavailability may alter calcium (Ca²⁺) signaling (35). From a translational perspective, the implications are notable for clinical populations, as improved strength/power augments exercise tolerance and possibly functional independence (30). Of note, increased leg strength promotes ease of walking (i.e., ↓ heart rate), which in turn, lowers perceptual difficulty for a given task. Because ease of walking is associated with non-exercise activity thermogenesis (critical for weight maintenance) (36), it is reasonable to propose that at-risk (e.g., aging, obesity) individuals may be more inclined to engage in spontaneous forms of free-living physical activities if the consequent activity were perceived to be easier.

Pre-exercise nitrate supplementation

Although exercise training represents a cornerstone to the preservation of functional independence, identifying effective solutions to increase exercise adherence remains elusive. Complications arising from declining physiologic function, including increased fatigue/fatigability, are probable sources contributing to insufficient physical activity in older adults (see Figure 3). Recent work has shown that heighten oxidative stress/inflammation indexed by serum TNF-α, may be linked to arterial elasticity and exercise tolerance among
postmenopausal women (37). Therefore, targeting modifiable lifestyle factors like diet and exercise to attenuate TNF-α are critical for promoting healthy aging and overall well-being. Based on evidence that acute beetroot juice supplementation significantly lowers the energetic cost (i.e., oxygen uptake; VO₂) of moderate-to-vigorous intensity exercise (38), it seems a logical step would be to consume beetroot juice prior to exercise in a structured training paradigm.

**Antioxidant / anti-inflammatory**

Whereas the reduction of nitrate to NO can impart potent physiologic responses, sources of dietary nitrate (e.g., spinach, beetroot) also possess a phytochemical composition rich in carotenoids, flavonoids, and ascorbic acid (39). It is possible these compounds work independently and/or synergistically with nitrate to exert beneficial anti-oxidative / anti-inflammatory effects. Beetroot also contains betalains, a bioactive pigment, characterized by a red-violet color with reportedly strong in vivo anti-inflammatory properties (40). Indeed, Wootton-Beard and colleagues (41) have shown the antioxidant capacity of beetroot juice, as evidenced by the ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays, to be higher than other widely-consumed juices including cranberry, orange, and carrot. Hence, it is reasonable that dietary nitrate could offer beneficial health effects through the expansion of NO bioactivity and reinforcement of host endogenous antioxidant capacity – possibly via activation of the nuclear factor erythroid-2-related factor (Nrf2) (42).

**Hypothesis**

Since peak plasma nitrate/nitrite levels commonly occur within 2–3 hours after consuming beetroot juice, there is a reasonable window of time to start exercise and profit from potential enhancements related to increased muscle blood flow/oxygenation, increased muscle contractility, and reduced energetic cost. Thus, we hypothesis that pre-exercise beetroot juice consumption may be a practical approach to mitigate the physiologic/perceptual difficulty of exercise among older adults and other at-risk populations. It is noteworthy that perceived exercise difficulty is an important factor shown to be independently related to participation in free-living physical activity (43) and predictive of weight gain (44). Under ‘typical’ exercise scenarios, individuals will modulate their level of exertion to a relatively fixed intensity in order to minimize discomfort. Pre-exercise beetroot juice consumption could potentially reduce the strain/effort for a given exercise bout, and thus, result in an individual exercising at a higher intensity (without the commensurate increase in discomfort). Our working framework hypothesizes that repeated exposure to a ‘heightened exercise stimulus’ (via beetroot juice) may elicit more robust cardio-metabolic adaptations compared to exercise training alone (i.e., without beetroot juice). It is also possible that habitual supplementation with beetroot juice coupled with exercise training may shift the redox balance of reactive oxygen species / NO toward ↑NO bioactivity. This, in turn, could promote better end-organ tissue perfusion with advancing age.
Conclusions

Although exercise training represents one of the key strategies to restore and preserve physiologic function independent of age, a number of studies show that dietary nitrate (principally as beetroot juice) may confer favorable health/performance effects in young and older individuals (15, 16). As increased oxidative stress and inflammation are hallmarks of aging and chronic disease – dietary nitrate supplementation may offer health benefits for multiple clinical disorders. In light of the relatively small samples size(s) and noted heterogeneity in the existing evidence – future research should perform adequately powered clinical trials to assess the long-term efficacy of habitual dietary nitrate supplementation on various health parameters including blood pressure, cognitive health, and muscle contractility in older adults. A priority is the determination of individual responsiveness to inform best practices of nitrate dosing. Certainly, the use of dietary nitrate supplementation to offset the burdens of aging is an active area of research with much to be learned. Based on emerging evidence, it is plausible that beetroot juice consumption prior to exercise training may offer synergistic benefits to promote healthy aging and independent-living among older adults.

Acknowledgment

This publication was made possible, in part, with support from the Indiana Clinical and Translational Sciences Institute funded by grant number UL1TR002529 from the National Institutes of Health, National Center for Advancing Translational Sciences, Clinical and Translational Sciences Award, and by grant number AG053606 from the National Institute on Aging. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Conflicts of Interest and Funding: This publication was made possible, in part, with support from the Indiana Clinical and Translational Sciences Institute funded by grant number UL1TR002529 from the National Institutes of Health, National Center for Advancing Translational Sciences, Clinical and Translational Sciences Award, and by grant number AG053606 from the National Institute on Aging. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. The authors declare no conflict of interest.

References


25. Alosco ML, Gunstad J, Jerseky BA, Xu X, Clark US, Hassenstab J, Cote DM, Walsh EG, Labbe DR, Hoge R, Cohen RA, Sweet LH. The adverse effects of reduced cerebral perfusion on...


41. Wootton-Beard PC, Moran A, Ryan L. Stability of the total antioxidant capacity and total polyphenol content of 23 commercially available vegetable juices before and after in vitro...


Fig. 1.
Schematic illustration highlighting a maladaptive cycle attributed to age-related shifts in physiological function. *Note the attendant consequences of systemic deconditioning often correspond with endothelial dysfunction and arterial stiffening, both of which, adversely affect cardiorespiratory fitness, muscle contractility, and metabolic health.
Fig. 2.
Schematic representation of the oxygen-independent nitrate-nitrite-nitric oxide pathway. Note that commensal facultative anaerobic bacteria convert dietary nitrate to nitrite through the obligatory action of nitrate reductase. Further reduction of nitrite to nitric oxide is hastened in the presence of low oxygen tension and low pH. Nitric oxide elicits many important physiologic functions vital to the defense of acute/chronic disease.
Fig. 3. Proposed model describing how age-related changes in aerobic fitness and muscle contractility often occur in tandem such that increased fatigue/fatigability would be expected to reduce the likelihood of spontaneous participation in free-living physical activity.