AMERICAN JOURNAL OF PHYSIOLOGY
HEART AND CIRCULATORY PHYSIOLOGY

*Am J Physiol Heart Circ Physiol* 328: H937–H944, 2025.
*First published March 11, 2025; doi:10.1152/ajpheart.00065.2025*

**SHORT REPORT**

*Women's Health Research and Cardiovascular Disease*

# Effect of 12-wk dietary nitrate supplementation on carotid arterial stiffness in postmenopausal females

**Vivian dos Santos Pinheiro,[1] David N. Proctor,[2] Rogerio Nogueira Soares,[3] and Thiago Silveira Alvares[1]**

[1]*Food and Nutrition Institute, Multidisciplinary Center UFRJ-Macaé, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil;* [2]*Integrative Vascular Physiology Lab, Department of Kinesiology, College of Health and Human Development, The Pennsylvania State University, University Park, Pennsylvania, United States; and* [3]*Division of Kinesiology, Health, and Sports Studies, Wayne State University, Detroit, Michigan, United States*

## Abstract

Menopause is associated with reduced nitric oxide (NO) bioavailability, a key contributor to increased arterial stiffness and, consequently, greater risk of cardiovascular disease-related mortality in postmenopausal females. Even though dietary nitrate has been shown to increase NO bioavailability in postmenopausal females acutely, previous studies showed no impact of dietary nitrate supplementation on arterial stiffness in postmenopausal females. Their findings were likely limited by the acute and/or short-term design. Thus, this study aimed to determine whether 12 wk of dietary nitrate supplementation via beetroot extract improves carotid artery stiffness in postmenopausal females. A randomized, double-blind, placebo-controlled, and parallel-design trial was conducted with 20 postmenopausal females (60–85 yr). Participants received nitrate-rich (NR-BEETx, 8.8 mmol/day) or nitrate-depleted (ND-BEETx) beetroot extract. Carotid stiffness parameters—pulse wave velocity (PWVβ), β stiffness, pressure-strain elastic modulus, augmentation index (AIx), and arterial compliance—were measured at baseline and *weeks 4*, *8*, and *12*. Serum nitrate and nitrite concentrations and blood pressure were also assessed. Compared with ND-BEETx, NR-BEETx supplementation significantly reduced PWVβ, β stiffness, elastic modulus, and AIx at *weeks 4*, *8*, and *12*, whereas arterial compliance increased by *week 12*. Serum nitrate and nitrite concentrations were elevated five- to sixfold and 1.5- to 2-fold, respectively, in the NR-BEETx group, with peak concentrations occurring at *week 8* and showing a plateau or slight decrease at *week 12*. Blood pressure remained unchanged in both groups. Twelve weeks of nitrate-rich beetroot extract supplementation improved carotid artery stiffness and increased NO bioavailability without altering blood pressure. These findings suggest that beetroot extract supplementation can be recommended as an alternative nutritional strategy to mitigate carotid artery stiffening in postmenopausal females.

**NEW & NOTEWORTHY** Postmenopausal females experience reduced nitric oxide (NO) bioavailability and elevated carotid artery stiffness, a well-established independent risk factor for end-organ damage and all-cause mortality. In this study, we demonstrate that 12 wk of dietary nitrate supplementation through beetroot extract significantly increased NO bioavailability and improved carotid artery stiffness in postmenopausal females.

*artery stiffness; beetroot extract; cardiovascular risk; postmenopausal females; vascular aging*

## INTRODUCTION

A primary mechanism underlying the increased cardiovascular risk in older postmenopausal females is the loss of estrogen (1, 2) and, consequently, a reduction in nitric oxide (NO) bioavailability (3–5). Impairments in endothelium- and NO-dependent pathways induce changes in vascular properties (i.e., endothelial cell stiffness, increased collagen deposition, and elastin degradation) associated with augmented arterial stiffness and increased risk for cardiovascular disease (CVD)-related death (6, 7). Postmenopausal females have been shown to have increased central and carotid arterial stiffness compared with young premenopausal females (8). In addition to the association between central stiffness and risk for CVD (9, 10), a recent large-scale study reported that carotid artery stiffness is associated with end-organ damage and all-cause mortality (11). Thus, establishing alternative interventions aimed at increasing nitric oxide bioavailability and potentially reducing carotid artery stiffness in postmenopausal females is critical.

Correspondence: T. S. Alvares (alvares@macae.ufrj.br); R. N. Soares (rogerio.nogueira@wayne.edu).
Submitted 27 January 2025 / Revised 6 February 2025 / Accepted 5 March 2025

In this sense, acute dietary nitrate supplementation has been shown to improve cardiovascular health and increase circulating nitrite (precursor of NO) via the endothelium-independent NO production pathway (nitrate → nitrite → NO pathway) in older populations (12). In a recent study (13), derived aortic waveforms were measured during supine rest in 13 healthy postmenopausal females before and 100 min after consumption of 140 mL of either nitrate-rich juice containing 9.7 mmol (~600 mg) nitrate or nitrate-depleted beetroot juice. Compared with placebo, acute nitrate supplementation did not alter the pulse wave velocity (PWV) or wave reflection characteristics. Similarly, Delgado Spicuzza et al. (14) did not observe changes in brachial-ankle PWV in postmenopausal females after 7 days of consuming 70 mL of nitrate-rich beetroot juice daily containing 6.4 mmol (~400 mg) of nitrate. The lack of change in the aortic pulse wave characteristics in these studies was likely due to the inability of a single or short-term dose of nitrate to reverse age- and menopause-associated aortic remodeling. In this regard, Bock et al. (15) demonstrated that 8 wk of beetroot extract supplementation containing ~4.03 mmol (250 mg) nitrate reduced peripheral and central systolic blood pressure and aortic augmentation index (AIx) in males and females with type 2 diabetes. Altogether, these findings suggest that a longer duration of dietary nitrate supplementation may be needed to alter arterial wave reflection indices in individuals with or at high risk of CVD.

Accordingly, the purpose of the present study was to test the hypothesis that a longer dietary nitrate intake intervention (i.e., 12 wk) via beetroot extract supplementation would improve common carotid artery stiffness in postmenopausal females.

## MATERIAL AND METHODS

### Participants

To first investigate whether our cohort of older postmenopausal females ($n = 20$) has increased carotid artery stiffness and lower circulating markers of nitric oxide bioavailability, we compared baseline data (*week 0*) from the older postmenopausal females' cohort with a group of young premenopausal females ($n = 10$). Next, we examined the impact of dietary nitrate supplementation on carotid artery stiffness in older postmenopausal females, as described in *Experimental Design*. All participants were recruited through announcements on websites and advertisements during community events. The participants' characteristics are shown in Table 1. The inclusion criteria included participants aged between 60 and 85 yr. The exclusion criteria included heart failure and/or a history of myocardial infarction, uncontrolled hypertension, neurological disease, or cancer. The additional exclusion criteria for older females were the use of estrogen replacement therapy and not being able to confirm a minimum of 2-year periods between the onset of menopause and the study visits. Participants were not excluded for being hypertensive (having a blood pressure of ≥130/85 mmHg), having controlled diabetes (HbA1c ≤ 8%), or for taking antihypertensive, statin, or hypoglycemic medications. All eligible participants provided written informed consent to participate. Participants were excluded from the study if changes in lifestyle (i.e., physical activity) or medication were reported within the 12-wk intervention period. The study was approved by the Institutional Review Board for Research Involving Human Subjects at the Federal University of Rio de Janeiro (CAAE: 55245622.2.0000.5699), in accordance with the principles outlined in the Declaration of Helsinki.

**Table 1.** *Participants' characteristics at the beginning of the study*

| | | Postmenopausal Females | | |
|---|---|---|---|---|
| | Young | NR-BEETx | ND-BEETx | One-Way ANOVA (*P* Value) |
| *N* | 10 | 10 | 10 | |
| Age, yr | 23 ± 3* | 66 ± 5 | 67 ± 5 | **<0.001** |
| Menopause time, yr | | 22 ± 8 | 22 ± 7 | 0.765‡ |
| Height, cm | 160.5 ± 5.5 | 156.9 ± 4.5 | 158.8 ± 4.6 | 0.286 |
| Weight, kg | 58.3 ± 10.7 | 64.8 ± 9.7 | 66.9 ± 12.3 | 0.202 |
| BMI, m/kg$^2$ | 22.5 ± 3.1 | 26.5 ± 4.8 | 26.4 ± 4.0 | 0.058 |
| Total cholesterol, mg/dL | 178.4 ± 30.1 | 218.0 ± 47.7# | 170.7 ± 28.7 | **0.017** |
| HDL-c, mg/dL | 67.1 ± 10.5 | 54.6 ± 9.6 | 60.9 ± 14.2 | 0.097 |
| LDL-c, mg/dL | 97.0 ± 15.9 | 142.9 ± 48.8† | 90.9 ± 30.1 | **0.004** |
| Fasting glucose, mg/dL | 87.4 ± 9.1# | 88.3 ± 8.3 | 100.9 ± 19.1 | **0.041** |
| SBP, mmHg | 107 ± 8* | 123 ± 9 | 121 ± 9 | **<0.001** |
| DBP, mmHg | 71 ± 8 | 79 ± 11 | 78 ± 9 | 0.130 |
| MAP, mmHg | 83 ± 8* | 94 ± 9 | 93 ± 8 | **0.008** |
| PAL, min/wk | 128 ± 59 | 188 ± 76 | 153 ± 100 | 0.268 |
| Risk factor for CVD, *n* | | | | |
| Hypertension | | 4 | 6 | |
| T2D | | 0 | 1 | |
| Dyslipidemia | | 3 | 2 | |
| Medications use, *n* | | | | |
| Antihypertensive | | 4 | 6 | |
| Hypoglycemiant | | | 3 | |
| Lipid lowering | | 3 | 2 | |

Values are means ± SD. Data for the three group comparisons (Young vs NR-BEETx vs ND-BEETx) were analyzed using an One-way ANOVA. An unparied t-test was used to compare menopause time comparison between NR-BEETx X ND-BEETx groups. Statistically significant data are in bold. BMI, body mass index; DBP, diastolic blood pressure; HDL-c, high-density lipoprotein, LDL-c, low-density lipoprotein; MAP, mean arterial blood pressure; ND-BEETx, nitrate-depleted beetroot extract; NR-BEETx, nirate-rich beetroot extract; PAL, physical activity level; SBP, systolic blood pressure; T2D, type 2 diabetes mellitus. *$P < 0.05$ vs. postmenopausal females (Bonferroni post hoc). #$P < 0.05$ vs. ND-BEETx (Bonferroni post hoc). †$P < 0.05$ vs. Young and ND-BEETx (Bonferroni post hoc). ‡Unpaired *t* test.

All participants provided written informed consent, and the study was registered in the Brazilian Registry of Clinical Trials (RBR-87qh649). Baseline data from four young premenopausal and five older postmenopausal females have been published in a previous study examining different research questions (16).

### Experimental Design

This was a cross-sectional (young premenopausal × older postmenopausal) followed by a randomized, double-blind, placebo-controlled parallel study in which only the older postmenopausal female participants were randomly assigned to one of two groups: nitrate-rich beetroot extract (NR-BEETx, $n = 10$) or nitrate-depleted beetroot extract (ND-BEETx, $n = 10$). Older postmenopausal females visited the laboratory on four occasions over a 12-wk study period. On the first visit (*week 0*), participants arrived at the laboratory and underwent seated blood pressure and anthropometric measurements. A venous blood sample was collected to assess baseline serum nitrate and nitrite concentrations. Afterward, participants rested quietly in the supine position for 10 min to measure carotid artery stiffness. At the end of the first visit, participants were given the first 4 wk' supply of NR-BEETx or ND-BEETx. Participants returned to the laboratory every 4 wk to receive the next 4 wk' supply and for blood samples, blood pressure, and carotid artery stiffness measurements. Participants were asked to consume two sachet bags, each containing 10 g of NR-BEETx daily (totaling ~8.8 mmol nitrate/day; SABEET, Sabinsa Corporation) or the same amount of ND-BEETx (~0.7 mmol of nitrate; Sabinsa Corporation). The sachets were diluted in water and consumed separately, one in the morning and the other in the afternoon.

The ND-BEETx intervention had exactly the same nutritional composition, appearance, color, and taste as the NR-BEETx but without nitrate. Participants were asked to avoid nitrate-rich foods such as spinach, arugula, or beetroot and were instructed to avoid using mouthwash during the study period. To monitor adherence to the supplementation protocol, weekly reminders were delivered individually to each participant, either in person or by phone, and a weekly questionnaire was administered. Participants were asked to report whether they had taken the supplement daily or skipped any days. All testing visits were performed in the morning, between 8:00 AM and 10:00 AM, following a 12-h overnight fast. On the day before each study visit, participants were instructed to abstain from alcohol and caffeine and to avoid engaging in exercise. Notably, randomization was performed using a free web-based randomization tool (www.randomization.com). Both the participants and the investigators were blinded to the group allocation.

### Carotid Artery Stiffness Analysis

The elastic characteristics of the common carotid artery were assessed using a high-definition echo-tracking ultrasound system (Prosound Alpha 6; Aloka, Tokyo, Japan) (16, 17). A high-resolution linear array transducer (7.5 MHz) was paired with computer-assisted analysis software (e-TRACKING system; Aloka Co., Tokyo, Japan) featuring an automated edge detection system for artery diameter measurement synchronized to the R-wave of the QRS complex using a 3-lead electrocardiogram. Pressure waveforms were obtained using a diameter change of the right carotid artery calibrated to blood pressure. The following arterial stiffness parameters were assessed as the mean of at least five consecutive beats: β index (stiffness parameter), calculated as $\beta \text{ index} = \ln(P_s/P_d)/[D_s - D_d)/D_d]$, where $P_s$ and $P_d$ are systolic and diastolic brachial pressures (surrogates for carotid pressures), and $D_s$ and $D_d$ are carotid systolic and diastolic diameters; pressure-strain elastic modulus ($Ep$), calculated as $Ep = (P_s - P_d)/[(D_s - D_d)/D_d]$, indicating vessel stiffness; pulse wave velocity (PWVβ), derived from $\text{PWV}\beta = \sqrt{(\beta P_d/2\rho)}$, where ρ represents blood density (1,050 kg/m$^3$); arterial compliance (AC), calculated as $\text{AC} = \pi(D_s \times D_s - D_d \times D_d)/[4 \times (P_s - P_d)]$, reflecting vessel compliance; and augmentation index (AIx), calculated as $\text{AIx} = [\Delta P/(P_s - P_d)] \times 100$, where $\Delta P$ is the difference between maximal pressure and the pressure at the first peak of the carotid pressure waveform. All parameters were derived from calibrated waveforms and synchronized measurements.

### Serum Nitrate and Nitrite Analysis

Blood samples were collected from the antecubital vein into tubes with a gel clot activator and immediately centrifuged at 3,000 *g* for 10 min at 4°C. The resulting serum was aliquoted and stored at −80°C for subsequent analysis. Serum nitrate and nitrite concentrations were quantified using a high-performance liquid chromatography system with photodiode array detection for nitrate and fluorescence detection for nitrite, following previously established methods (18, 19).

### Statistical Analysis

One-way ANOVA followed by Bonferroni correction was used for baseline data comparisons between older postmenopausal and young premenopausal females (Table 1 and Fig. 1). Differences in artery stiffness parameters, blood pressure, and serum nitrate and nitrite over the 12-wk supplementation period were determined using a two-way repeated measures ANOVA. Bonferroni correction was used as a post hoc test when a significant group × time interaction $F$ test was found (Fig. 2). All analyses were performed using SPSS v. 27 (IBM, Chicago, IL). A $P$ value ≤0.05 was considered to be statistically significant. Data are expressed as means ± standard deviation (SD) unless otherwise informed.

## RESULTS

### Participant Characteristics

Baseline characteristics of the participants are reported in Table 1 and Fig. 1. There was no statistical significance in the participant's characteristics except for total cholesterol and LDL-c, which were higher in the NR-BEETx group compared with the ND-BEETx group. Older postmenopausal females assigned to ND-BEETx or NR-BEETx supplementation had increased PWVβ, β stiffness, AIx, and elastic modulus compared with young premenopausal females (Fig. 1, *A–D*). Arterial compliance of both older postmenopausal groups was smaller and reduced compared with the young

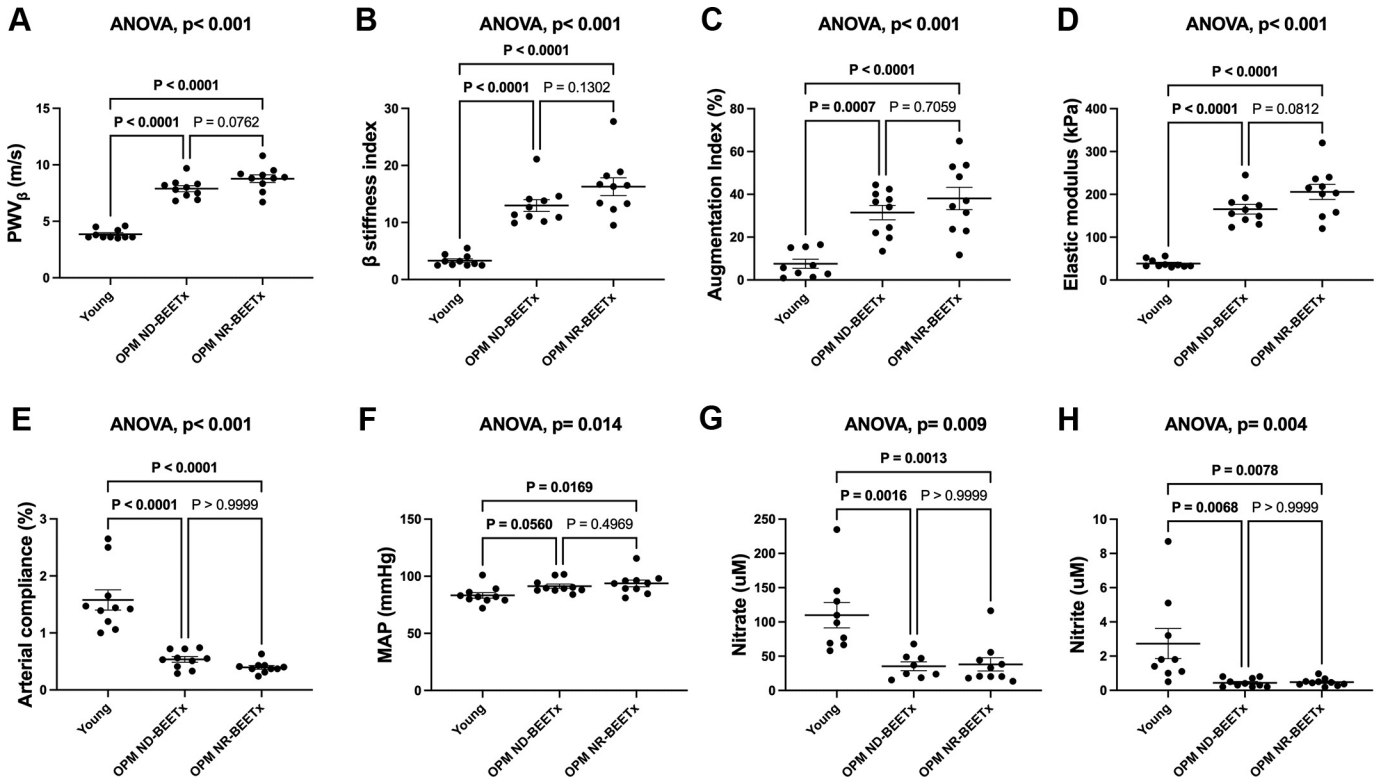


**Figure 1.** Baseline carotid artery stiffness and circulating nitrate and nitrite in young and older postmenopausal females at baseline. Older postmenopausal females from both nitrate-depleted (OPM ND-BEETx, $n = 10$) and nitrate-rich beetroot extract (OPM NR-BEETx, $n = 10$) had increased pulse-wave velocity ($PWV_{\beta}$) (*A*), β-stiffness index (*B*), augmentation index (*C*), and elastic modulus (*D*) compared with young females (young, $n = 10$). *E*: arterial compliance of both OPM ND-BEETx and OPM NR-BEETx groups was reduced compared with young. *F*: the mean arterial pressure (MAP) of older postmenopausal females was greater than the MAP of the young group. *G* and *H*: serum nitrate and nitrite were higher in young females compared with older postmenopausal females. Data were analyzed by one-way ANOVA followed by Bonferroni post hoc when appropriate. Significant *P* values are in boldface.

premenopausal group (Fig. 1*E*). Older postmenopausal females had greater mean arterial pressure compared with young premenopausal females (Fig. 1*F*). Circulating nitrate and nitrite were reduced in older postmenopausal females compared with young premenopausal females (Fig. 1, *G* and *H*). No significant differences were found between older postmenopausal females in the ND-BEETx compared with the NR-BEETx group for all variables depicted in Fig. 1.

### Arterial Stiffness Parameters, Blood Pressure, and Circulating Nitrate and Nitrite Responses to 12-Wk Placebo or Nitrate-Rich Beetroot Extract Intake

One-point carotid stiffness and hemodynamic parameters at baseline and after 4, 8, and 12 wk of nitrate-rich beetroot extract and placebo interventions are summarized in Fig. 2. Significant time × intervention interaction effects were observed for PWVβ, β-stiffness parameter, AIx, elastic modulus, and arterial compliance. Following the nitrate-rich beetroot extract intervention, PWVβ, β-stiffness parameter, and Ep significantly decreased at *weeks 4*, *8*, and *12* (Fig. 2, *A*, *B*, and *D*), the augmentation index significantly decreased at *weeks 8* and *12* (Fig. 2*C*), and arterial compliance significantly increased at *week 12* (Fig. 2*E*). In contrast, no significant changes were observed in these parameters in the ND-BEETx group. In addition, systolic blood pressure, diastolic blood pressure, and mean arterial pressure remained unchanged ($P > 0.05$) over the 12-wk study period in both groups. Figure 2, *G* and *H*, illustrates changes in serum nitrate and nitrite concentrations in the NR-BEETx and ND-BEETx groups over the 12-wk study period. A two-way repeated measures ANOVA revealed significant interaction effects for both serum nitrate and nitrite concentrations (all $P < 0.05$). Follow-up pairwise comparisons indicated that in the NR-BEETx group, serum nitrate levels were significantly elevated at *weeks 4*, *8*, and *12*, whereas serum nitrite levels were significantly elevated at *weeks 8* and *12*, compared with the ND-BEETx group (Fig. 2, *G* and *H*).

## DISCUSSION

To our knowledge, this study is the first to examine the effect of 12-wk dietary nitrate supplementation (via beetroot extract) on carotid artery stiffness parameters in postmenopausal females. The main findings were that the carotid pulse wave velocity (PWV), β stiffness, pressure-strain elastic modulus, and augmentation index (AIx) decreased, whereas arterial compliance increased after 12 wk of supplementation. In addition, nitrate-rich beetroot extract supplementation increased fasting serum nitrate (five- to sixfold) and nitrite concentrations (1.5- to 2-fold), with peak concentrations occurring at *week 8* and showing a plateau or slight decrease at *week 12*. No significant changes in mean arterial blood pressure were observed in either group.

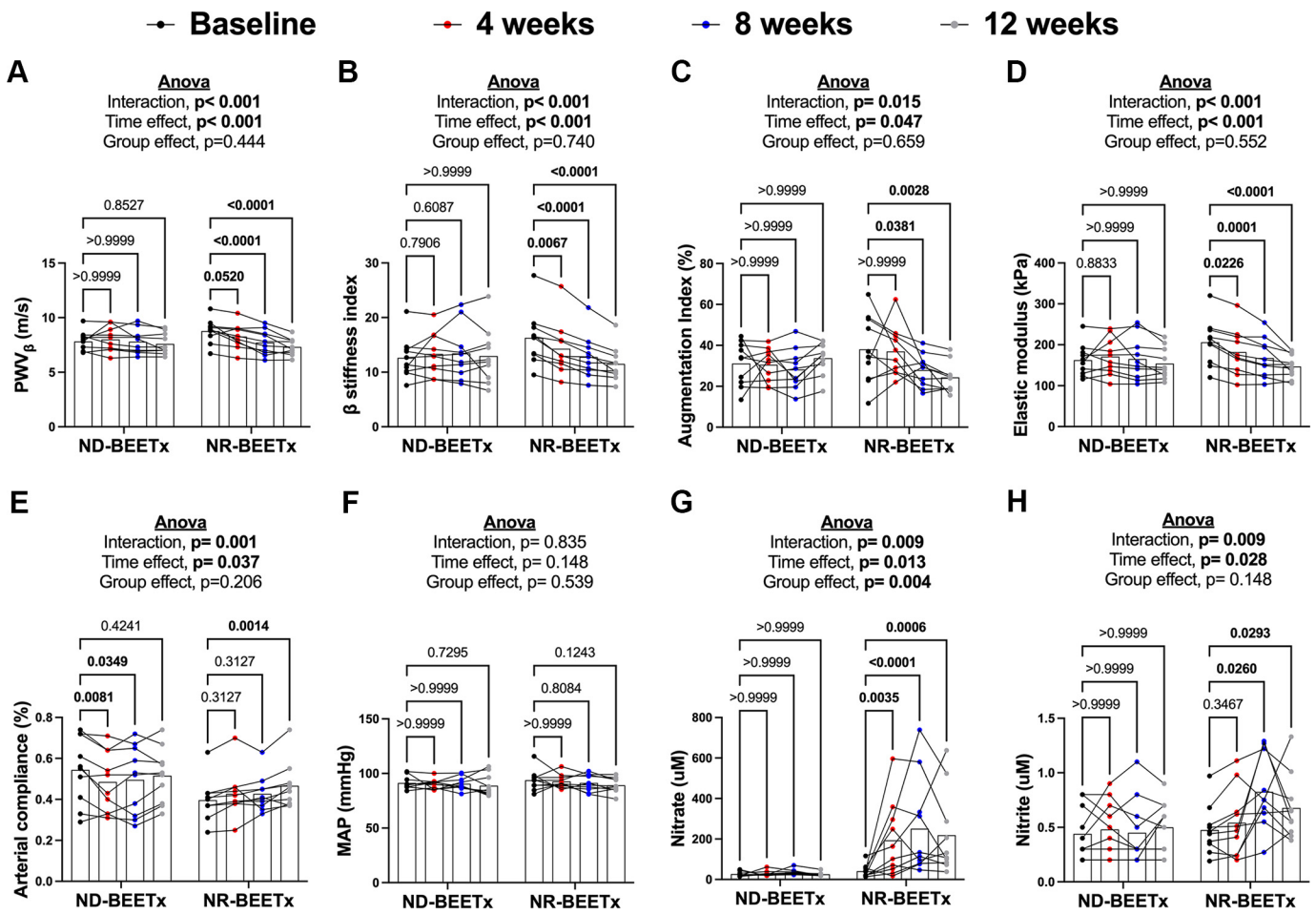


**Figure 2.** Changes in carotid stiffness after 4, 8, and 12 wk of nitrate-rich beetroot extract and placebo interventions. Pulse-wave velocity ($PWV_{\beta}$) (*A*), β stiffness (*B*), augmentation index (*C*), elastic modulus (*D*), arterial compliance (*E*), mean arterial pressure (MAP) (*F*), serum nitrate (*G*) and nitrite (*H*) at baseline and after 4, 8, and 12 wk of nitrate-depleted (ND-BEETx) and nitrate-rich (NR-BEETx) beetroot extract supplementation in older postmenopausal females. Data were analyzed by two-way repeated measures ANOVA followed by Bonferroni post hoc when appropriate. Significant *P* values are in boldface.

Previous studies of acute (single dose) or short-term (<7 days) nitrate supplementation in older populations (12, 20), including studies exclusively involving postmenopausal females (13, 14), have failed to show improvements in measures of arterial stiffness. For instance, Liu et al. (20) found that carotid-femoral PWV and AIx remained unchanged in older adults following a nitrate-rich meal containing 3.54 mmol (220 mg) of nitrate. Similarly, Hughes et al. (12) reported no significant changes in AIx after older participants consumed a single dose of 500 mL of nitrate-rich beetroot juice containing 9.4 mmol (583 mg) of nitrate. Kim et al. (13) also observed no significant changes in AIx or aortic PWV in postmenopausal females after consumption of a single dose of 140 mL of nitrate-rich beetroot juice containing 9.7 mmol (600 mg) of nitrate. Nitrate supplementation over 7 days (400 mg of nitrate/day via beetroot juice) also failed to improve PWV in early and late postmenopausal females (14). The present findings of significant carotid artery remodeling (de-stiffening) after 4 wk and continuing for up to 12 wk of daily nitrate-rich beetroot extract supplementation in postmenopausal females are consistent with the premise that longer durations of inorganic nitrate supplementation (>7 days) are needed to significantly improve age- and menopause-associated structural aspects of the arterial wall.

Notably, approximately half of the postmenopausal females in the present study (4 in the NR-BEETx group and 6 in the ND-BEETx group) were taking anti-hypertensive medications at the time of this study. Given the small subgroup sample sizes, we cannot conclusively determine whether the de-stiffening effects of nitrate supplementation are more pronounced in postmenopausal females taking hypertensive medication. To our knowledge, only one prior study involving long-duration nitrate supplementation examined arterial stiffness outcomes in hypertensive subjects, finding reductions in both PWV and AIx after 4 wk of daily nitrate-rich beetroot juice consumption (21). That study included more than 50% females, many of whom would have been postmenopausal. Sex differences were not examined, but medication use did influence the change in PWV, with significant reductions in treated hypertensives (significant reduction) versus drug-naïve patients (no change). Future research will be needed to determine whether dietary nitrate treatment effects on carotid stiffness are stronger in postmenopausal females with versus without cardiovascular disease.

Although the exact mechanism underlying the reductions in carotid stiffness parameters observed in the present study remains uncertain, it can be speculated that a sustained increase in NO bioavailability may have indirectly influenced conduit artery structural properties. NO has been shown to inhibit advanced glycation end-products (22), reduce collagen deposition (23), and stimulate elastin expression (24), thereby lowering arterial stiffness, especially in aging populations. In addition, previous studies suggest that changes in actin filaments in endothelial and smooth muscle cells, such as actin filament polymerization, may also play a major role in increased arterial stiffness in conditions such as type 2 diabetes and aging (25, 26). For instance, Soares et al. (25) showed that 6 wk of treatment with empagliflozin reduced arterial stiffness and vascular actin polymerization in a rodent model of aging. Interestingly, no changes in arterial collagen and elastin content were observed. Indeed, more studies are needed to elucidate the mechanisms linking the increase in NO bioavailability and vascular remodeling in older postmenopausal females.

Our findings revealed that nitrate-rich beetroot extract resulted in substantial increases in fasting serum nitrate and nitrite concentrations, with peak levels observed at *week 8*, followed by a plateau or slight decrease by *week 12*. This suggests that enhanced reduction of nitrate to nitrite and the subsequent increase in resting NO bioavailability become physiologically significant after ~8 wk of supplementation. Moreover, these findings highlight the possibility that circulating nitrate and nitrite levels during long-term dietary nitrate supplementation are regulated by a homeostatic mechanism (27, 28). This mechanism may involve sialin, a nitrate transport channel protein that can be upregulated with prolonged dietary nitrate supplementation, facilitating nitrate transport into cells (27, 29–31). Such regulation could help maintain a balance between extracellular and intracellular nitrate concentrations, promoting systemic nitrate-nitrite-NO homeostasis or a long-term steady state (28). Absorbed nitrite can be further reduced to NO via several pathways (32). It is believed that the NO originating from the reduction of diet-derived nitrate to nitrite in the mouth provides a substantial contribution to the total NO pool in the body (32).

In the present study, no significant changes in systolic, diastolic, or mean arterial blood pressure were observed. This contrasts with several previous studies reporting significant reductions in both systolic and diastolic blood pressure following dietary nitrate intake (33–35). However, Broxterman et al. (36) reported that the beneficial effects of nitrate supplementation on blood pressure were only observed in patients with hypertension who were not taking antihypertensive medications, suggesting that the efficacy of dietary nitrate to improve blood pressure may be dependent upon one's initial degree of blood pressure elevation. The participants in the present study had relatively normal baseline blood pressure, likely influenced by the use of antihypertensive medications in 50% of the participants, therefore leaving less room for improvement. Furthermore, it has been suggested that peripheral measurements of brachial artery blood pressure may not be sensitive to aging-related changes in large-conduit artery walls and arterial stiffening (37). Yaginuma et al. (38) showed that nitroglycerin administration reduced the ascending aorta reflected wave and systolic pressure by ~20 mmHg, whereas the brachial artery blood pressure remained unchanged. Whether or not long-term nitrate supplementation may have a greater impact on central stiffness and blood pressure that cannot be detected in the brachial artery of postmenopausal females remains to be determined.

The present study has some limitations. First, the use of antihypertensive medications may have masked any potential effects of the intervention on blood pressure. Second, we did not measure aortic and leg PWV to determine the specific arterial segments affected by dietary nitrate supplementation. However, local carotid PWV has been shown to provide similar information on arterial stiffness as aortic PWV (39). Third, the findings of the present study may not be generalizable to other populations, such as men or younger females, thereby limiting their broader applicability. Fourth, lifestyle factors, such as physical activity, diet, or medication use, might also have contributed to variations in arterial stiffness, adding another layer of complexity to the interpretation of these results. In addition, the majority of the postmenopausal females in the present study were in the late phase, leaving the efficacy of prolonged dietary nitrate supplementation in reducing arterial stiffness among early-phase postmenopausal females unclear.

Taken together, the results observed in the present study provide novel evidence of the potential of dietary nitrate as a complementary lifestyle behavior intervention for age-related carotid artery stiffening, particularly in postmenopausal females who are at increased risk of cardiovascular diseases.

## DATA AVAILABILITY

The data underlying this article will be shared upon reasonable request to the corresponding authors.

## ACKNOWLEDGMENTS

Dr. Thiago S. Alvares acknowledges Fundação Carlos Chagas de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) and the National Council for Scientific and Technological Development (CNPq) for the research productivity scholarship.

## GRANTS

This research project was supported by Fundação Carlos Chagas de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ), Grant No. E-26/200.546/2023 and by the National Council for Scientific and Technological Development (CNPq), Grant No. 309262/2023-2 and 402948/2024-6.

## DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

## AUTHOR CONTRIBUTIONS

V.d.S.P., R.N.S., and T.S.A. conceived and designed research; V.d.S.P. performed experiments; V.d.S.P., R.N.S., and T.S.A. analyzed data; V.d.S.P., D.N.P., R.N.S., and T.S.A. interpreted results of experiments; V.d.S.P., R.N.S., and T.S.A. prepared figures;

V.d.S.P., D.N.P., R.N.S., and T.S.A. drafted manuscript; D.N.P., R.N.S., and T.S.A. edited and revised manuscript; V.d.S.P., D.N.P., R.N.S., and T.S.A. approved final version of manuscript.

## REFERENCES


1. **Mitchell GF**, **Parise H**, **Benjamin EJ**, **Larson MG**, **Keyes MJ**, **Vita JA**, **Vasan RS**, **Levy D.** Changes in arterial stiffness and wave reflection with advancing age in healthy men and women: the Framingham Heart Study. *Hypertension* 43: 1239–1245, 2004. doi:10.1161/01.HYP.0000128420.01881.aa.
2. **Mercuro G**, **Zoncu S**, **Piano D**, **Pilia I**, **Lao A**, **Melis GB**, **Cherchi A.** Estradiol-17beta reduces blood pressure and restores the normal amplitude of the circadian blood pressure rhythm in postmenopausal hypertension. *Am J Hypertens* 11: 909–913, 1998. doi:10.1016/s0895-7061(98)00096-x.
3. **Prorock AJ**, **Hafezi-Moghadam A**, **Laubach VE**, **Liao JK**, **Ley K.** Vascular protection by estrogen in ischemia-reperfusion injury requires endothelial nitric oxide synthase. *Am J Physiol Heart Circ Physiol* 284: H133–H140, 2003. doi:10.1152/ajpheart.00957.2001.
4. **Xing D**, **Nozell S**, **Chen YF**, **Hage F**, **Oparil S.** Estrogen and mechanisms of vascular protection. *Arterioscler Thromb Vasc Biol* 29: 289–295, 2009. doi:10.1161/ATVBAHA.108.182279.
5. **Simoncini T**, **Hafezi-Moghadam A**, **Brazil DP**, **Ley K**, **Chin WW**, **Liao JK.** Interaction of oestrogen receptor with the regulatory subunit of phosphatidylinositol-3-OH kinase. *Nature* 407: 538–541, 2000. doi:10.1038/35035131.
6. **Vasan RS**, **Pan S**, **Xanthakis V**, **Beiser A**, **Larson MG**, **Seshadri S**, **Mitchell GF.** Arterial stiffness and long-term risk of health outcomes: the Framingham Heart Study. *Hypertension* 79: 1045–1056, 2022. doi:10.1161/HYPERTENSIONAHA.121.18776.
7. **Marti CN**, **Gheorghiade M**, **Kalogeropoulos AP**, **Georgiopoulou VV**, **Quyyumi AA**, **Butler J.** Endothelial dysfunction, arterial stiffness, and heart failure. *J Am Coll Cardiol* 60: 1455–1469, 2012. doi:10.1016/j.jacc.2011.11.082.
8. **Zaydun G**, **Tomiyama H**, **Hashimoto H**, **Arai T**, **Koji Y**, **Yambe M**, **Motobe K**, **Hori S**, **Yamashina A.** Menopause is an independent factor augmenting the age-related increase in arterial stiffness in the early postmenopausal phase. *Atherosclerosis* 184: 137–142, 2006. doi:10.1016/j.atherosclerosis.2005.03.043.
9. **Willum-Hansen T**, **Staessen JA**, **Torp-Pedersen C**, **Rasmussen S**, **Thijs L**, **Ibsen H**, **Jeppesen J.** Prognostic value of aortic pulse wave velocity as index of arterial stiffness in the general population. *Circulation* 113: 664–670, 2006. doi:10.1161/CIRCULATIONAHA.105.579342.
10. **Mitchell GF**, **Hwang SJ**, **Vasan RS**, **Larson MG**, **Pencina MJ**, **Hamburg NM**, **Vita JA**, **Levy D**, **Benjamin EJ.** Arterial stiffness and cardiovascular events: the Framingham Heart Study. *Circulation* 121: 505–511, 2010. doi:10.1161/CIRCULATIONAHA.109.886655.
11. **Pewowaruk R**, **Korcarz C**, **De Boer I**, **Kestenbaum B**, **Heckbert SR**, **Tedla YG**, **Gepner AD.** Carotid artery stiffness mechanisms are associated with end organ damage and all-cause mortality: MESA (Multi-Ethnic Study of Atherosclerosis). *J Am Heart Assoc* 12: e027517, 2023. doi:10.1161/JAHA.122.027517.
12. **Hughes WE**, **Ueda K**, **Treichler DP**, **Casey DP.** Effects of acute dietary nitrate supplementation on aortic blood pressure and aortic augmentation index in young and older adults. *Nitric Oxide* 59: 21–27, 2016. doi:10.1016/j.niox.2016.06.007.
13. **Kim DJ**, **Roe CA**, **Somani YB**, **Moore DJ**, **Barrett MA**, **Flanagan M**, **Kim-Shapiro DB**, **Basu S**, **Muller MD**, **Proctor DN.** Effects of acute dietary nitrate supplementation on aortic blood pressures and pulse wave characteristics in post-menopausal women. *Nitric Oxide* 85: 10–16, 2019. doi:10.1016/j.niox.2019.01.008.
14. **Delgado Spicuzza JM**, **Gosalia J**, **Zhong L**, **Bondonno C**, **Petersen KS**, **De Souza MJ**, **Alipour E**, **Kim-Shapiro DB**, **Somani YB**, **Proctor DN.** Seven-day dietary nitrate supplementation clinically significantly improves basal macrovascular function in postmenopausal women: a randomized, placebo-controlled, double-blind, crossover clinical trial. *Front Nutr* 11: 1359671, 2024. doi:10.3389/fnut.2024.1359671.
15. **Bock JM**, **Hughes WE**, **Ueda K**, **Feider AJ**, **Hanada S**, **Casey DP.** Dietary inorganic nitrate/nitrite supplementation reduces central and peripheral blood pressure in patients with type 2 diabetes mellitus. *Am J Hypertens* 35: 803–809, 2022. doi:10.1093/ajh/hpac068.
16. **Alvares TS**, **Pinheiro V**, **Gomes T**, **Murias JM**, **Nogueira Soares R.** Vasculature of older females shows heterogeneity in the association between cardiovascular risk and vascular function. *Am J Physiol Heart Circ Physiol* 328: H93–H100, 2025. doi:10.1152/ajpheart.00731.2024.
17. **Vriz O**, **Aboyans V**, **Minisini R**, **Magne J**, **Bertin N**, **Pirisi M**, **Bossone E.** Reference values of one-point carotid stiffness parameters determined by carotid echo-tracking and brachial pulse pressure in a large population of healthy subjects. *Hypertens Res* 40: 685–695, 2017. doi:10.1038/hr.2017.24.
18. **Croitoru MD.** Nitrite and nitrate can be accurately measured in samples of vegetal and animal origin using an HPLC-UV/VIS technique. *J Chromatogr B Analyt Technol Biomed Life Sci* 911: 154–161, 2012. doi:10.1016/j.jchromb.2012.11.006.
19. **Alvares TS**, **Conte-Junior CA**, **Silva JT**, **Paschoalin VM.** Acute L-arginine supplementation does not increase nitric oxide production in healthy subjects. *Nutr Metab (Lond)* 9: 54, 2012. doi:10.1186/1743-7075-9-54.
20. **Liu AH**, **Bondonno CP**, **Croft KD**, **Puddey IB**, **Woodman RJ**, **Rich L**, **Ward NC**, **Vita JA**, **Hodgson JM.** Effects of a nitrate-rich meal on arterial stiffness and blood pressure in healthy volunteers. *Nitric Oxide* 35: 123–130, 2013. doi:10.1016/j.niox.2013.10.001.
21. **Kapil V**, **Khambata RS**, **Robertson A**, **Caulfield MJ**, **Ahluwalia A.** Dietary nitrate provides sustained blood pressure lowering in hypertensive patients: a randomized, phase 2, double-blind, placebo-controlled study. *Hypertension* 65: 320–327, 2015. doi:10.1161/HYPERTENSIONAHA.114.04675.
22. **Asahi K**, **Ichimori K**, **Nakazawa H**, **Izuhara Y**, **Inagi R**, **Watanabe T**, **Miyata T**, **Kurokawa K.** Nitric oxide inhibits the formation of advanced glycation end products. *Kidney Int* 58: 1780–1787, 2000. doi:10.1046/j.1523-1755.2000.00340.x.
23. **Han G**, **Nguyen LN**, **Macherla C**, **Chi Y**, **Friedman JM**, **Nosanchuk JD**, **Martinez LR.** Nitric oxide-releasing nanoparticles accelerate wound healing by promoting fibroblast migration and collagen deposition. *Am J Pathol* 180: 1465–1473, 2012. doi:10.1016/j.ajpath.2011.12.013.
24. **Sugitani H**, **Wachi H**, **Tajima S**, **Seyama Y.** Nitric oxide stimulates elastin expression in chick aortic smooth muscle cells. *Biol Pharm Bull* 24: 461–464, 2001. doi:10.1248/bpb.24.461.
25. **Soares RN**, **Ramirez-Perez FI**, **Cabral-Amador FJ**, **Morales-Quinones M**, **Foote CA**, **Ghiarone T**, **Sharma N**, **Power G**, **Smith JA**, **Rector RS**, **Martinez-Lemus LA**, **Padilla J**, **Manrique-Acevedo C.** SGLT2 inhibition attenuates arterial dysfunction and decreases vascular F-actin content and expression of proteins associated with oxidative stress in aged mice. *Geroscience* 44: 1657–1675, 2022. doi:10.1007/s11357-022-00563-x.
26. **Power G**, **Lateef OM**, **Ramirez-Perez FI**, **Lazo-Fernandez Y**, **Augenreich MA**, **Ferreira-Santos L**, **Soares RN**, **Gonzalez-Vallejo JD**, **Morales-Quinones M**, **Norton CE**, **Manrique-Acevedo C**, **Martinez-Lemus LA**, **Padilla J.** Reduced cofilin activity as a mechanism contributing to endothelial cell stiffening in type 2 diabetes. *Am J Physiol Heart Circ Physiol* 328: H84–H92, 2025. doi:10.1152/ajpheart.00667.2024.
27. **Zhou J**, **Liu H**, **Kagami H**, **Wang S.** Nitrate and body homeostasis. *Medicine Plus* 1: 100003, 2024. doi:10.1016/j.medp.2023.100003.
28. **Qin L**, **Liu X**, **Sun Q**, **Fan Z**, **Xia D**, **Ding G**, **Ong HL**, **Adams D**, **Gahl WA**, **Zheng C**, **Qi S**, **Jin L**, **Zhang C**, **Gu L**, **He J**, **Deng D**, **Ambudkar IS**, **Wang S.** Sialin (SLC17A5) functions as a nitrate transporter in the plasma membrane. *Proc Natl Acad Sci USA* 109: 13434–13439, 2012. doi:10.1073/pnas.1116633109.
29. **Wylie LJ**, **Park JW**, **Vanhatalo A**, **Kadach S**, **Black MI**, **Stoyanov Z**, **Schechter AN**, **Jones AM**, **Piknova B.** Human skeletal muscle nitrate store: influence of dietary nitrate supplementation and exercise. *J Physiol* 597: 5565–5576, 2019. doi:10.1113/JP278076.
30. **Srihirun S**, **Park JW**, **Teng R**, **Sawaengdee W**, **Piknova B**, **Schechter AN.** Nitrate uptake and metabolism in human skeletal muscle cell cultures. *Nitric Oxide* 94: 1–8, 2020. doi:10.1016/j.niox.2019.10.005.
31. **Park JW**, **Piknova B**, **Walter PJ**, **Cai H**, **Upanan S**, **Thomas SM**, **Tunau-Spencer KJ**, **Schechter AN.** Distribution of dietary nitrate and its metabolites in rat tissues after. *Sci Rep* 13: 3499, 2023. doi:10.1038/s41598-023-28190-2.
32. **Lundberg JO**, **Gladwin MT**, **Ahluwalia A**, **Benjamin N**, **Bryan NS**, **Butler A**, **Cabrales P**, **Fago A**, **Feelisch M**, **Ford PC**, **Freeman BA**, **Frenneaux M**, **Friedman J**, **Kelm M**, **Kevil CG**, **Kim-Shapiro DB**,

**Kozlov AV**, **Lancaster JR Jr**, **Lefer DJ**, **McColl K**, **McCurry K**, **Patel RP**, **Petersson J**, **Rassaf T**, **Reutov VP**, **Richter-Addo GB**, **Schechter A**, **Shiva S**, **Tsuchiya K**, **van Faassen EE**, **Webb AJ**, **Zuckerbraun BS**, **Zweier JL**, **Weitzberg E.** Nitrate and nitrite in biology, nutrition and therapeutics. *Nat Chem Biol* 5: 865–869, 2009. doi:10.1038/nchembio.260.

33. **Siervo M**, **Lara J**, **Ogbonmwan I**, **Mathers JC.** Inorganic nitrate and beetroot juice supplementation reduces blood pressure in adults: a systematic review and meta-analysis. *J Nutr* 143: 818–826, 2013. doi:10.3945/jn.112.170233.
34. **Jackson JK**, **Patterson AJ**, **MacDonald-Wicks LK**, **Oldmeadow C**, **McEvoy MA.** The role of inorganic nitrate and nitrite in cardiovascular disease risk factors: a systematic review and meta-analysis of human evidence. *Nutr Rev* 76: 348–371, 2018. doi:10.1093/nutrit/nuy005.
35. **Bahrami LS**, **Arabi SM**, **Feizy Z**, **Rezvani R.** The effect of beetroot inorganic nitrate supplementation on cardiovascular risk factors: a systematic review and meta-regression of randomized controlled trials. *Nitric Oxide* 115: 8–22, 2021. doi:10.1016/j.niox.2021.06.002.
36. **Broxterman RM**, **La Salle DT**, **Zhao J**, **Reese VR**, **Richardson RS**, **Trinity JD.** Influence of dietary inorganic nitrate on blood pressure and vascular function in hypertension: prospective implications for adjunctive treatment. *J Appl Physiol (1985)* 127: 1085–1094, 2019. doi:10.1152/japplphysiol.00371.2019.
37. **O'Rourke M.** Arterial stiffness, systolic blood pressure, and logical treatment of arterial hypertension. *Hypertension* 15: 339–347, 1990. doi:10.1161/01.hyp.15.4.339.
38. **Yaginuma T**, **Avolio A**, **O'Rourke M**, **Nichols W**, **Morgan JJ**, **Roy P**, **Baron D**, **Branson J**, **Feneley M.** Effect of glyceryl trinitrate on peripheral arteries alters left ventricular hydraulic load in man. *Cardiovasc Res* 20: 153–160, 1986. doi:10.1093/cvr/20.2.153.
39. **Gaszner B**, **Lenkey Z**, **Illyés M**, **Sárszegi Z**, **Horváth IG**, **Magyari B**, **Molnár F**, **Kónyi A**, **Cziráki A.** Comparison of aortic and carotid arterial stiffness parameters in patients with verified coronary artery disease. *Clin Cardiol* 35: 26–31, 2012. doi:10.1002/clc.20999.