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## Dietary Nitrate Reverses Vascular Dysfunction in Older Adults With Moderately Increased Cardiovascular Risk



**To the Editor:** The average human life span increases continuously and with it the percentage of sexagenarians and older people. Aging deteriorates vascular integrity, characterized by endothelial dysfunction and increasing vascular stiffness. The latter jointly precede and entail incident systolic blood pressure (SBP), with an increase in cardiovascular morbidity and mortality (1). Strategies to attenuate the aging process are sparse. Studies such as the DASH (Dietary Approaches to Stop Hypertension) trial have demonstrated that certain dietary patterns may influence blood pressure (2). The micronutrient inorganic nitrate, abundant in vegetables, acutely lowered diastolic blood pressure (DBP) in healthy young volunteers and improved vascular remodeling in animals (3,4). We here sought to investigate the effects of daily dietary supplementation with inorganic nitrate on vascular function in older adults.

Elderly volunteers without history, signs, or symptoms of cardiovascular disease, although with moderate cardiovascular risk (mean [SD]; HeartScore 4.7 [2.7]; age 63 [5] years), were included in a randomized, placebo-controlled double-blind trial. Study procedures were in accordance with the Declaration of Helsinki and the Heinrich-Heine University Düsseldorf Institutional Ethics Committee. Volunteers gave informed consent and were treated daily with dietary nitrate supplementation (sodium nitrate 150  $\mu\text{mol/kg}$  body weight, dissolved in drinking water; dose equivalent to a portion (300 g) of spinach). The study comprised 11 volunteers (7 men; mean age 63 [6] years; body mass index 24 [4]  $\text{kg/m}^2$ ; cholesterol 238 [37]  $\text{mg/dl}$ ; HeartScore 4.7 [2.5]) receiving sodium nitrate for 4 weeks, compared with controls (sodium chloride 150  $\mu\text{mol/kg}$  body weight; 10 volunteers: 6 men, mean age 63 [4] years; body mass index 26 [3]  $\text{kg/m}^2$ ; cholesterol 219 [40]  $\text{mg/dl}$ ; HeartScore 4.7 [3.1]); no difference was seen between groups. Measurements were taken at baseline and 1 day after the last intake of nitrate or placebo. Paired 2-tailed Student *t* test was used for statistical analyses within the groups; unpaired Student *t* test was used for comparing the groups on change from baseline.

To evaluate effects on endothelial function, we assessed flow-mediated dilation of the brachial artery and carotid intima-media thickness (IMT) (Vivid i ultrasound, GE Healthcare, Munich, Germany) (5). Dietary nitrate supplementation improved endothelial function modestly, with no effect in controls (nitrate group: flow-mediated dilation 6.0% [0.8%] to 6.5% [0.8%],  $p = 0.004$ ; controls: 6.0% [0.8%],  $p = 0.97$ ; unpaired  $p = 0.0058$ ) (Fig. 1). The impact of dietary nitrate on vascular stiffness was evaluated by measures of aortic stiffness and pressure pulsatility, namely pulse wave velocity and augmentation index (at 75 beats/min) derived from arterial applanation tonometry (SphygmoCor, AtCor Medical, Meinerzhagen, Germany). Vascular stiffness only improved in nitrate-supplemented volunteers (pulse wave velocity for

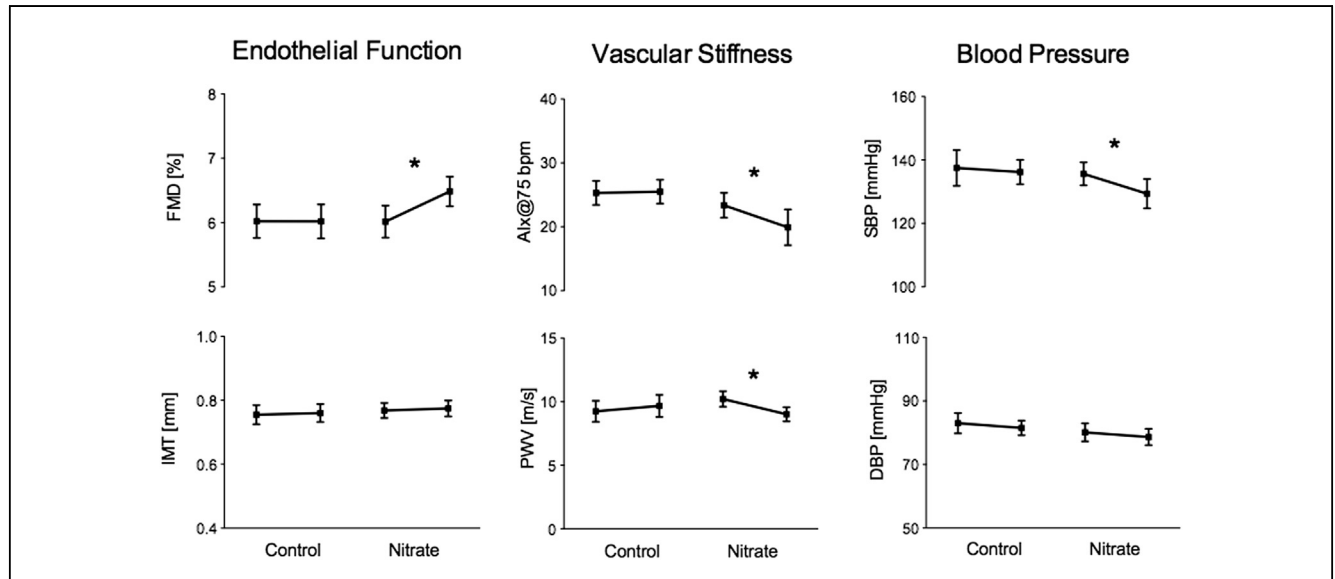
nitrate group 10.2 [2.0] to 9.0 [1.8]  $\text{m/s}$ ,  $p = 0.006$ ; controls: 9.2 [2.6] to 9.6 [2.7]  $\text{m/s}$ ,  $p = 0.148$ ; unpaired:  $p = 0.002$ ); augmentation index at 75 beats/min for nitrate group: 23.4 [6.5] to 19.9 [9.3],  $p = 0.012$ ; controls: 25.3 [6.0] to 25.5 [5.9],  $p = 0.85$ ; unpaired  $p = 0.029$ ) (Fig. 1).

These vascular changes suggest an impact on blood pressure. SBP was measured twice noninvasively using a standard sphygmomanometer. Remarkably, 1 day after the last intake of nitrate, a reduction in systolic pressure from 137 [13] to 129 [15]  $\text{mm Hg}$  ( $p = 0.008$ ) was observed in older adults with mild hypertension, with no effect in controls (138 [18] to 136 [12]  $\text{mm Hg}$ ;  $p = 0.71$ ; unpaired  $p = 0.037$ ) (Fig. 1). Of note, DBP, heart rate, and IMT remained unchanged (DBP in nitrate group 80 [9] to 79 [9]  $\text{mm Hg}$ ,  $p = 0.39$ ; controls 83 [10] to 81 [7]  $\text{mm Hg}$ ,  $p = 0.51$ ; unpaired  $p = 0.89$ ; HR for nitrate group 65 [8] to 66 [9]  $\text{beats/min}$ ,  $p = 0.59$ ; controls: 62 [8] to 62 [8]  $\text{beats/min}$ ,  $p = 0.73$ ; unpaired  $p = 0.33$ ; IMT in nitrate group 0.77 [0.08] to 0.77 [0.08]  $\text{mm}$ ,  $p = 0.3$ ; controls 0.76 [0.1] to 0.76 [0.09]  $\text{mm}$ ,  $p = 0.5$ ; unpaired  $p = 0.88$ ).

Dietary nitrate supplementation increased plasma nitrite and nitrate levels as measured by high-performance liquid chromatography (5), with no differences in controls (nitrite in nitrate group 85 [25] to 330 [226]  $\text{nM}$ ,  $p = 0.005$ ; controls 99 [41] to 111 [44]  $\text{nM}$ ,  $p = 0.4$ ; unpaired  $p = 0.005$ ; nitrate in nitrate group 32 [13] to 263 [180]  $\mu\text{M}$ ,  $p = 0.002$ ; controls 31 [19] to 40 [18]  $\mu\text{M}$ ,  $p = 0.06$ ; unpaired  $p = 0.001$ ).

Mechanistically, nitrate and nitrite can be viewed as stable storage pools for nitric oxide (NO)-like bioactivity. Nitrate is initially bioactivated via reduction to nitrite by symbiotic bacteria in the oral cavity. Nitrite then undergoes conversion to NO by numerous enzymatic as well as nonenzymatic processes in blood and tissue (3–5). A role for nitrite and especially NO has been suggested for the regulation and modulation of blood flow, endothelial function, and blood pressure. The nutritional aspects of the vasculoprotective effects of nitrite and nitrate are intriguing because nitrate and nitrite are abundant in our everyday diet. Major dietary sources of nitrite and nitrate are green leafy vegetables. Long-term effects are unknown but likely include adaptive and regenerative processes.

Dietary supplementation with inorganic nitrate improves endothelial dysfunction and vascular stiffness and reduces SBP in the elderly with moderately increased cardiovascular risk. These prognostic relevant outcome measures have been shown to predict cardiovascular events in older adults. Our findings provide evidence that dietary modulation with micronutrients improves vascular dysfunction. This might prevent or delay development of essential hypertension and have an impact on the deleterious sequelae and clinical outcome in an aging population. (Effects of Dietary Nitrate on Age-Related Vascular Function; NCT01729234).



**Figure 1** Effects of Dietary Nitrate Supplementation on Endothelial Function, Vascular Stiffness, and Blood Pressure

Dietary nitrate supplementation improved endothelial function as determined by flow-mediated dilation (FMD), reduced vascular stiffness as assessed by augmentation index (Aix@75bpm) and aortic pulse wave velocity (PWV), and lowered systolic blood pressure (SBP). Carotid intima-media thickness (IMT) and diastolic blood pressure (DBP) remained unaffected. Values are presented as mean ± SEM. \*p < 0.05 (n = 10 and n = 11, respectively).

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#### REFERENCES

1. Kaess BM, Rong J, Larson MG, et al. Aortic stiffness, blood pressure progression, and incident hypertension. *JAMA* 2012;308:875-81.
2. Appel LJ, Moore TJ, Obarzanek E, et al. A clinical trial of the effects of dietary patterns on blood pressure. DASH Collaborative Research Group. *N Engl J Med* 1997;336:1117-24.

3. Larsen FJ, Ekblom B, Sahlin K, Lundberg JO, Weitzberg E. Effects of dietary nitrate on blood pressure in healthy volunteers. *N Engl J Med* 2006;355:2792-3.
4. Hendgen-Cotta UB, Luedike P, Totzeck M, et al. Dietary nitrate supplementation improves revascularization in chronic ischemia. *Circulation* 2012;126:1983-92.
5. Rassaf T, Heiss C, Mangold S, et al. Vascular formation of nitrite after exercise is abolished in patients with cardiovascular risk factors and coronary artery disease. *J Am Coll Cardiol* 2010;55:1502-3.

#### Letters to the Editor

### Prognostic Stratification of Patients With Vasospastic Angina



I read with interest the report by Takagi et al (1). I was very impressed by the amount of information they were able to gather about a topic that is not frequently seen by any individual practitioner. I congratulate the authors for trying to assess the risk of vasospastic angina by developing a risk score.

I have 1 concern regarding the risk score: the history of out-of-hospital cardiac arrest. Although I admit this is a risk for a future event, it is also an endpoint for a major adverse cardiac event, and I am not sure it belongs in the same category as smoking, angina at rest, organic coronary stenosis, multivessel spasm, ST-segment elevation during angina, and beta-blocker use.