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Title: Dietary Nitrate Supplementation and Thermoregulation During Exercise

NCT#: NCT03506646

Protocol

Background

The vast majority of the research involving dietary nitrate supplementation, namely beetroot juice, has been performed mostly on samples of healthy populations. A few clinical populations have been studied, including heart failure with preserved ejection fraction, chronic obstructive pulmonary disease (COPD), and peripheral artery disease (PAD). In various populations, beetroot juice (BRJ) has been shown to decrease overall blood pressure, increase blood flow, increase muscle tissue oxygenation, improve body core temperature regulation, and increase exercise tolerance while decreasing the oxygen cost of exercise.

BRJ supplementation has been shown to have an immediate effect on reducing blood pressure, with the most substantial decreases recorded within approximately 2.5-3 hours following supplement consumption. Systolic, diastolic, and mean arterial blood pressures showed decreases of 10.4±3 mmHg, 8.1±2.1 mmHg, and 8.0±2.1 mmHg, respectively, in healthy volunteers. During exercise in healthy individuals, systolic blood pressure remained lower throughout varying exercise intensities. Blood pressure was also reduced in heart failure patients with preserved ejection fraction. After an acute dose of BRJ, resting systolic blood pressure significantly decreased when compared to placebo conditions (BRJ 127 ± 14 mmHg; placebo134 ± 14 mmHg). A slight reduction in systolic blood pressure during exercise may be beneficial to PAD patients. Hypertension, or high blood pressure, is a major risk factor for the development of PAD. Decreasing overall blood pressure and slightly decreasing systolic blood pressure in response to light activity would result in a decrease in unnecessary stress placed on the cardiovascular system at rest and during exercise.

In a study involving PAD patients where 7 of the 8 participants were classified as either hypertensive or prehypertensive, BRJ consumption caused a significant reduction in diastolic blood pressure during rest that was maintained during exercise testing. The study concludes that their data suggest that BRJ supplementation reduces blood pressure in PAD patients and this effect is maintained throughout exercise.

Oxygen demand of working muscle increases as activity level increases the efficiency of oxygen delivery and oxygen utilization is crucial to muscle function, as well as a necessary increase in blood flow. This is especially important in PAD patients; increasing oxygenation to areas of skeletal muscle ischemia may increase physical function. By using near-infrared spectroscopy, muscle tissue oxygenation can be examined. During cycling in healthy males supplementing with BRJ, the right vastus lateralis muscle oxyhemoglobin concentrations were greater than that of the placebo condition6. While cycling at a moderate intensity, the male cyclists had a 13% reduction in deoxyhemoglobin concentration amplitude post-supplementation, which indicated a reduction in fractional oxygen extraction in the right vastus lateralis muscle. These results suggest that BRJ supplementation may promote a better balance between localized oxygen delivery and utilization as an index of muscle fractional oxygen extraction. Overall, BRJ supplementation has been shown to increase working muscle tissue oxygenation during exercise.

In PAD patients, the oxygenation of the gastrocnemius with the worst PAD symptoms was monitored during a walking cardiopulmonary exercise test. Subjects showed a 48% reduction in deoxyhemoglobin concentration amplitude following BRJ supplementation, which indicated that fractional oxygen extraction was reduced. During the exercise protocol, deoxyhemoglobin amplitude measures for the BRJ supplementation group at 100 and 200 seconds into exercise were reduced by 44% and 53%, respectively. This response implies that BRJ supplementation in PAD patients improves a balance between local oxygen delivery and utilization as an index of muscle fractional oxygenation extraction by the working muscle.

Increasing oxygenation to areas of skeletal muscle ischemia in PAD patients may increase physical function, but in order for this to be as efficient as possible, a dose-response relationship must be determined.

Increases in activity and oxygen demand of working skeletal muscle necessitate increases in blood flow. In PAD patients, increasing blood flow to working muscle becomes difficult due to atherosclerotic occlusions in the lower extremity arteries. Research in healthy populations showed significant increases in forearm blood flow during hand grip exercise in hypoxic conditions following an acute dosage of BRJ in comparison to placebo (BRJ 373 ± 38 mL/min; placebo 343 ± 32 mL/min). Nitric oxide signals smooth muscle within the blood vessels (endothelium) to relax, which in turn increases blood flow to the localized area of vasodilation. In healthy populations, dietary nitrate supplementation increases vasodilation near the surface of the skin. This increase in vasodilatory capacity and blood flow would create a stronger temperature gradient at the level of the skin, which would facilitate more efficient heat exchange as blood is cooled at the level of the skin (sweat evaporative, conductive, and convective cooling), causing decreased strain on the body. In PAD patients, nitrite-related nitric oxide signaling showed to increase peripheral blood flow to hypoxic tissue, which is supported by a decrease in gastrocnemius deoxygenation and a decrease in blood pressure. However, during brachial artery flow mediated dilation, peak dilation did not significantly change (BRJ 42.6 ± 10.6 seconds; placebo 41.0 ± 10.39 seconds), which suggested that endothelial production of nitric oxide did not change. The unchanging vasodilatory response is likely due to the study not examining a BRJ dose response relationship and its effects on vasodilation.

BRJ supplementation has been shown to decrease oxygen cost during low-intensity and moderate-intensity exercise. In trained populations supplementing with BRJ, significant decreases in oxygen cost during the beginning stages of exercise have been detected. Oxygen cost during walking was shown to decrease by approximately 12% following BRJ supplementation. BRJ supplementation showed a 20% decrease in oxygen cost during moderate intensity cycling in recreationally trained men. This reduction in oxygen cost implies an increase in exercise efficiency in light-to-moderate level exercise, which in turn increases exercise tolerance. In high-intensity exercise, time-to-exercise-failure increased by 15% following BRJ supplementation, which is also suggestive of an increase in exercise tolerance.

BRJ supplementation in clinical populations has also shown to improve exercise tolerance. In heart failure with preserved ejection fraction patients, submaximal endurance exercise improved by 24% following BRJ supplementation in comparison to placebo conditions (BRJ 449 ± 180 seconds; placebo 363 ± 125 seconds). In patients with COPD, walking distance increased by 11% and time to exercise fatigue increased by 6%. In PAD patients, BRJ supplementation showed an 18% (32 second) increase to onset claudication pain and a 17% (65 seconds) increase peak walking time in comparison to placebo conditions. These results in PAD patients show a substantial acute response to BRJ supplementation (~2 hours of consumption) during exercise testing.

In various populations, BRJ supplementation has shown to decrease blood pressure, improve blood flow, increase muscle tissue oxygenation, safely maintain core temperature, and increase exercise tolerance. The effects of BRJ specifically in PAD patients showed decreases in blood pressure, increases in time to onset claudication and exercise tolerance, but did not show a significant effect on endothelial function. Examining a dose-response relationship is necessary to determine the responses to BRJ supplementation (endothelial function, leg function, and blood vessel oxygen carrying capacity) in PAD patients. In this study, a higher dose of nitrate (280 mL, 16.8 mmol nitrates) will be examined. BRJ supplementation has shown reductions in blood pressure, increased muscle tissue oxygenation, blood flow, and thermoregulatory response. These mechanisms all contribute to improving overall cardiovascular function.

If these results are observed during this study, PAD patients may experience less claudication pain as well as better tolerance to daily physical activities and exercise.

Methods

Upon receiving informed consent, baseline measurements will be obtained: height/weight (standard scale and stadiometer), resting heart rate (12-lead EKG), blood pressure (automated sphygmomanometer), hand grip strength (hand grip dynamometer), body composition (handheld bioelectrical impedance analysis), blood nitrate/nitrite levels (nitrate/nitrite assay kit will quantify the amount of nitrate/nitrite in 10mL of blood), endothelial function (using flow mediated dilation), arterial stiffness (peripheral using brachial-to-ankle pulse-wave velocity analysis, central using carotid-to-femoral pulse-wave velocity analysis), autonomic function (heart rate variability measured with a heart rate monitor), leg blood flow (femoral and popliteal blood flow will be measured in both legs using a Terason uSmart 3300 ultrasound imaging system), leg muscle oxygenation (measured in the ischemic gastrocnemius using near-infrared spectroscopy), walking test (time to onset claudication using Gardner treadmill protocol), and core body temperature (rectal thermometer). The same testing procedures will occur for post-supplementation and post-placebo assessments. The initial testing visit will take 2 hours and the successive two testing visits will take 3 hours each.

After completion of baseline measurements, participants will be assigned either the beetroot juice supplement or the placebo in accordance with the prospective randomized (1:1 ratio), double-blinded, crossover study design. Supplementation dosage will be determined by mmol nitrate/kg body weight and normalized to the body mass of our participants.

Statistical Analysis

The statistical power analysis is reflective of a crossover experimental design. Independent t-tests, dependent t-tests, and repeated measures ANOVA will be used. Based on preliminary data of previous studies of this type, testing at the significance $p = 0.05$, two-tailed, will have a power of 80% to detect a difference of 0.90 standard deviations in the difference between the beetroot juice supplement group versus the placebo group. The power and sample size determination package, PASS (PASS, Number Cruncher Statistical Systems Statistical Software) was used for this analysis.