



Potential Influence of Red Beetroot Juice Consumption on Endothelial Function

Functional Foods
& Human Health Laboratory

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Background

- Cardiovascular disease (CVD) is the leading cause of death in the United States.
- Aging is the primary risk factor for CVD.
- Overweight/obesity and menopause can exacerbate CVD risk.
- Atherosclerosis is the main pathophysiological process underlying the development of CVD. (Figure 1)
- Vascular endothelial dysfunction is a major initiating step in atherosclerosis.
- The vascular endothelium lines the inner wall of arteries and functions to produce vasoactive molecules that regulate the function and health of arteries, including vasodilation.
- Vascular endothelial dysfunction occurs when the endothelium is no longer able to maintain this normal functional state.
- Functional foods rich in bioactive compounds have been shown to improve endothelial function and may represent a therapeutic strategy for preventing or slowing the development of atherosclerosis.
- Red beetroot juice (RBJ) contains a variety of bioactive compounds beneficial to cardiovascular health including phenolic acids, flavonoids and betalains, as well as inorganic nitrates. (Figure 2)

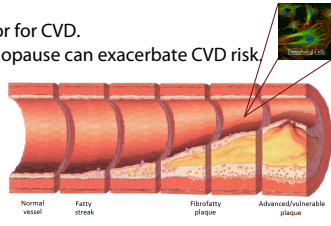


Figure 1: Development of atherosclerosis. The inner most layer facing the lumen of the blood vessel is the endothelium.

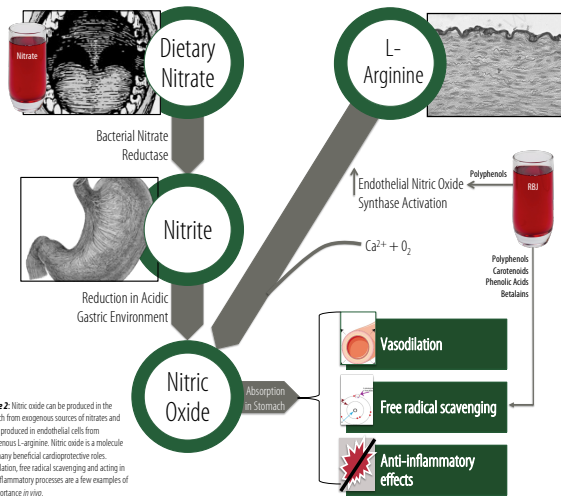


Figure 2: Nitric oxide can be produced in the stomach from endogenous sources of nitrates and can be produced in endothelial cells from endogenous L-arginine. Nitric oxide is a molecule with many beneficial cardiovascular roles. Vasodilation, free radical scavenging and acting in anti-inflammatory processes are a few examples of its importance *in vivo*.

Objective

- To evaluate the effects of RBJ vs. its isolated bioactive components on endothelial function in healthy, overweight/obese men and postmenopausal women aged 40-65 years.

Study Design & Methods

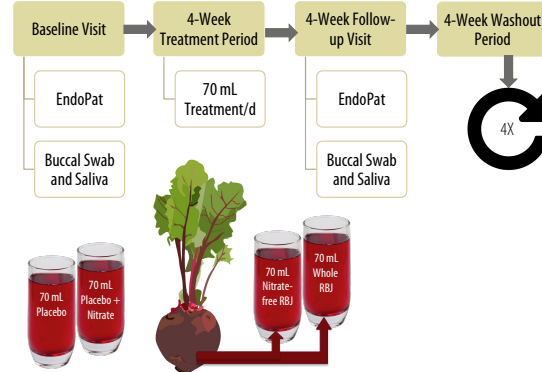


Figure 3: Study design and treatments. Abbreviations: Red beetroot juice (RBJ).

Baseline/Follow-Up Tests

- Anthropometrics, blood pressure, and arterial stiffness are assessed at baseline.
- EndoPat is used to quantify endothelial function. (Figure 4)
- Buccal swabs and saliva are collected to quantify nitrate and nitrite and profile oral microbiome.

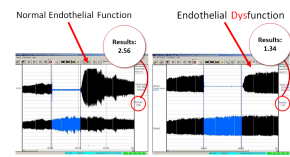


Figure 4: Example EndoPat test results. Endothelial function is assessed as reactive hyperemia index (RHI) determined by EndoPAT at baseline and after 4 weeks of consumption of each treatment. RHI is determined through measurement of arterial pulsatile blood volume changes in response to a forearm reactive hyperemic stimulus. A RHI score below 1.67 is indicative of endothelial dysfunction.

Laboratory Assays

- DNA will be extracted from buccal swabs. (Figure 5)
- Oral nitrate-reducing bacterial genes will be quantified by qPCR.
- Oral microbial communities will be characterized using 16S sequencing.
- Saliva nitrate and nitrite levels will be quantified using a commercially available colorimetric assay.

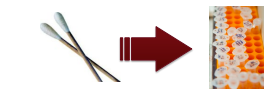


Figure 5: Through a series of steps, DNA is extracted from the swab used to collect bacteria from the inside of the participants' cheeks and gums. The DNA will then be used for downstream steps such as PCR and DNA sequencing.

Preliminary Results

| Characteristic | Mean ± SE | Range (Min-Max) |
|----------------|-------------|-----------------|
| Sex | 7M:7F | N/A |
| Age | 52.6 ± 1.9 | 42-61 |
| BMI | 29.6 ± 0.91 | 26.19-36.55 |
| SBP (mmHg) | 120.6 ± 3.0 | 97-136.3 |
| DBP (mmHg) | 78.9 ± 2.7 | 62-96 |
| PWV | 7.20 ± 0.4 | 4.57-10 |
| RHI Score | 1.74 ± 0.1 | 1.31-2.38 |

Figure 6: Baseline characteristics of currently enrolled/completed study participants. Abbreviations: SE=Standard Error; M=Male; F=Female; SBP=systolic blood pressure; DBP=diastolic blood pressure; PWV=pulse wave velocity.

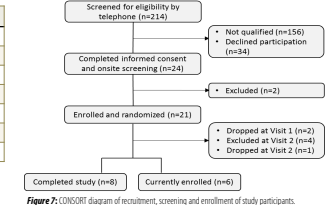


Figure 7: CONSORT diagram of recruitment, screening and enrollment of study participants.

Expected Results

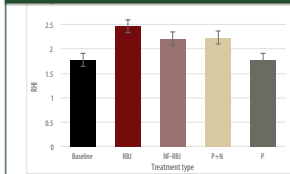


Figure 8: Hypothesized treatment effects on endothelial function measured by EndoPat. Abbreviations: RBJ=Red beetroot juice; NF-RBJ=Nitrate-Free RBJ; P+N=Placebo+Nitrate; P=Placebo.

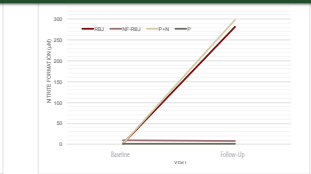


Figure 9: Expected nitrite levels (µM) at baseline and follow-up visits. Abbreviations: RBJ=Red beetroot juice; NF-RBJ=Nitrate-Free RBJ; P+N=Placebo+Nitrate; P=Placebo.

| Nitrate-Reducing Bacteria | RBJ | | | |
|--|--------|-----|---|----|
| | NF-RBJ | P+N | P | NC |
| <i>Streptococcus, Haemophilus, Neisseria, Veillonella, Granulicatella, Prevotella, Leptotrichia, Actinomyces</i> | ↑ | NC | ↑ | NC |

Figure 10: Expected increase in nitrate-reducing bacteria after consumption of whole red beetroot juice and placebo + added nitrate groups. No change (NC) in the concentration in nitrate-reducing bacteria will likely be seen in placebo and nitrate-free red beetroot juice groups.

Implications

- We hypothesize consumption of RBJ for 4 weeks will lead to the greatest improvement in endothelial function.
- We also expect that these improvements will be associated with alterations in the oral microbiome.
- These results would suggest that the bioactive compounds found in RBJ work synergistically to improve endothelial function.
- RBJ consumption could be a potential therapeutic strategy for preventing or slowing the development of atherosclerosis and reducing CVD risk. Future research could explore potential underlying mechanisms, including the role of the gut microbiota.

References

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