

Co-Ingestion of Dietary Nitrate and Ascorbic Acid on Nitric Oxide Biomarkers and The Oral Microbiome in Sedentary Hispanic Women.

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ABSTRACT

Nitric oxide bioavailability increases following nitrate supplementation wherein oral microbiota facilitate the metabolism and absorption of nitrate. However, few studies have examined if co-ingestion of nitrate with antioxidants can further elevate nitric oxide bioavailability. Moreover, our understanding on how the oral microbiome responds to nitrate supplementation is limited, especially in women. **PURPOSE:** To examine the effects of ingesting dietary nitrate and ascorbic acid independently and concurrently on markers of nitric oxide bioavailability and oral microbiota species. **METHODS:** Twelve sedentary women of Hispanic descent (mean \pm SD: age 20 ± 1 years; body mass 74 ± 15 kg; height 1.62 ± 0.09 m) consumed nitrate-rich beetroot juice (BR), nitrate-depleted beetroot juice (PL), ascorbic acid (AA), and crystal light (CRY) in four conditions: BR combined with AA (BR+AA); BR only (BR+CRY); AA only (PL+AA); and placebo-control (PL+CRY). Supplements were ingested 2.5 hours prior to a resting blood draw and buccal swab sample. Plasma [nitrate] and [nitrite] were analyzed using gas phase chemiluminescence. Buccal swab samples were used for DNA extraction and isolation. DNA was amplified using polymerase chain reaction (PCR) targeting the V3 - V4 region of the 16S rRNA gene. Following index PCR, amplicons were pooled and sequenced using the iSeq Illumina NGS sequencer. Reads were clustered into amplicon sequence variants and analyzed for alpha and beta diversity and relative abundance. **RESULTS:** BR increased plasma [nitrate] (BR+AA: 641 ± 252 vs. BR+CRY: 528 ± 307 vs. PL+AA: 35 ± 10 vs. PL+CRY: 35 ± 12 μ M, $P < 0.001$) and plasma [nitrite] (BR+AA: 710 ± 336 vs. BR+CRY: 578 ± 428 vs. PL+AA: 209 ± 88 vs. PL+CRY: 198 ± 82 nM, $P < 0.001$) with no differences within BR and PL conditions. Alpha and beta diversity, and the relative abundance of higher and lower taxonomic levels were not significantly different between all conditions ($P > 0.05$) **CONCLUSION:** Concurrent nitrate and AA supplementation did not elicit additional increases to nitric oxide compared to nitrate ingestion alone. Acute beetroot juice and ascorbic acid were ineffective at modulating oral microbial composition. Further research is required to understand the impact of supplementation regimen and population on the physiological effects of dietary nitrate.