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A stepwise reduction in plasma and salivary nitrite with increasing strengths of mouthwash following a dietary nitrate load

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ABSTRACT

Nitric Oxide (NO) bioavailability is essential for vascular health. Dietary supplementation with inorganic nitrate, which is abundant in vegetables and roots, has been identified as an effective means of increasing vascular NO bioavailability. Recent studies have shown a reduction in resting blood pressures in both normotensive and hypertensive subjects following ingestion of inorganic nitrate. Oral bacteria play a key role in this process and the use of strong antibacterial mouthwash rinses can disable this mechanism. Hence, mouthwash usage, a $1.4 billion market in the US, may potentially be detrimental to cardiovascular health. The purpose of this study was to examine the effects of different strengths of commercially available mouthwash products on salivary and plasma nitrate and nitrite concentrations following 8.4 mmol inorganic nitrate load (beetroot juice). Specifically, we examined the effects of Listerine antiseptic mouthwash, Cepacol antibacterial mouthwash, and Chlorhexidine mouthwash versus control (water).

Twelve apparently healthy normotensive males (36 $\pm$ 11yrs) completed four testing visits in a randomized order, separated by one week. Testing consisted of blood pressure (BP), and saliva and venous blood collection at baseline and each hour for 4 h. Following baseline-testing participants consumed 140 ml of beet juice and then 15 min later gargled with 5 mL of assigned mouthwash. Testing and mouthwash rinse was repeated every hour for 4 h. Linear mixed effects models, followed by pairwise comparisons where appropriate, were used to determine the influence of treatment and time on plasma and saliva nitrate and nitrite, and BP.

Plasma and salivary nitrate increased above baseline (time effect) for all conditions ($p \leq 0.01$). There were time ($p \leq 0.01$), treatment ($p \leq 0.01$), and interaction ($p \leq 0.05$) effects for plasma and salivary nitrite. There was a treatment effect on systolic BP ($p \leq 0.05$). Further examination revealed a differentiation of plasma and salivary nitrite concentration between control/antiseptic and antibacterial/chlorhexidine treatments. When examined in this manner there was a reduction in both SBP ($p \leq 0.01$) and mean arterial BP ($p \leq 0.05$) from the antibacterial/chlorhexidine treatments.

These results suggest a potentially differentiating effect of different commercially available mouthwash solutions on plasma and salivary nitrite concentrations and resting blood pressure responses. This raises potential public health related questions on the appropriate widespread usage of different mouthwash formulations.

1. Introduction

Nitric oxide (NO) is a key regulator of vascular integrity, and a dysfunction of endothelial NO production via nitric oxide synthase (NOS) has been demonstrated in cardiovascular disorders including heart failure [1], type two diabetes [2], peripheral artery disease [3],

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and hypertension [4]. Consequently, an alternative method of increasing bioavailable NO within the vascular system has been an area of recent research interest. One potential therapeutic option involves the conversion of inorganic nitrate (NO_3-) and nitrite (NO_2-) anions into NO (and other biologically active species). This is an attractive approach, as it is biologically distinct from endothelial NOS and inorganic nitrate supplementation can be achieved easily via the diet [5]. In fact, inorganic nitrate is most abundant in green leafy vegetables, beets, celery, lettuce, radishes, and spinach [6].

Research suggests that a diet high in these vegetables and roots may afford protection against the development of atherosclerotic vasculopathy [7,8] and specific studies have shown reductions in blood pressure in normotensive [9,10], pre-hypertensive [7], and hypertensive subjects [11] following supplementation with inorganic nitrate in the form of beetroot juice. It is noteworthy that, the Dietary Approaches to Stop Hypertension (DASH) diet [12], which is recommended to treat hypertension [13,14] and diabetes [15], can have a much higher level of NO_3- than the World Health Organization's Acceptable Daily Intake of 3.7 mg per kg of body weight per day and more than a 2-fold higher than the United States Environmental Protection Agency of 7.0 mg per kg of body weight per day [16]. Lower oral dosages of NO_3-in the range of 0.4–0.8 mmol have also been shown to exert other health [17–19] and exercise performance benefits [7,9,10,20]. Animal models have even documented improvements in revascularization in mice with chronic ischemia following NO_3- administration [21].

The mechanisms responsible for the bioactivation of NO_3- from dietary sources are not completely clear, but involve initial nitrate ingestion into the circulation via the gastrointestinal tract. Although the kidneys clear approximately 75% of the circulating NO_3-, and some maybe distributed and/or stored throughout the body, a significant amount appears to be concentrated in the salivary glands and secreted into the oral cavity [22,23]. Mammals lack specific nitrate reductase enzymes to reduce NO_3- to NO_2-, but the natural oral commensal microbiome appears to readily perform this function [24,25]. This NO_2- is then swallowed and absorbed into the circulation [7,22], with peak plasma NO_2- concentrations occurring approximately three to 4 h post-ingestion [8,24,26], and having an approximate half-life of 6 h [27]. There are numerous pathways in the body for further reduction of NO_2- to NO including the involvement of hemoglobin [28], myoglobin [22], xanthine oxidase [29], ascorbate, polyphenols [30], and protons [22]. These processes appear to be optimized under hypoxic or low oxygen conditions [7,8]. The model of relatively low concentrations of nitrite acting as a storage pool of NO or NO-like species able to exert physiological effects specific to under-perfused tissues is a very attractive one and avoids the hypotensive consequences of non-specific NO-donors.

Although the commensal bacterial-mediated conversion process from oral nitrate intake to plasma nitrite concentration may yield cardiovascular health benefits, there is also a widespread use of commercial mouthwash treatment specifically designed to prevent oral malodor or dental plaque, by inhibiting or eliminating oral bacteria [31–33]. In some circumstances, such as gum disease and acute bacterial infections this may undoubtedly be the best course of action, but in everyday use, mouthwash may be suppressing the beneficial cardiovascular health role of nitrate containing food. A recent study highlighted this dilemma by demonstrating a rise in resting blood pressure in 19 healthy young subjects associated with a reduction in plasma nitrite concentration following antibacterial mouthwash use [33].

Given the wide range of commercially available mouthwash products of various strengths and ingredients, it is difficult to know which ones may affect the conversion process of NO_3- to NO_2-. Therefore, the purpose of this study was to test the effects of a variety of 3 commercially available oral mouthwash solutions (and water as a control) on salivary and plasma NO_3-and NO_2-concentrations. The solutions were selected in order to potentially represent a gradual increase in bacteria-inhibition; (a) Listerine ® antiseptic mouthwash was one of the original oral mouthwash rinse products on the market, with active ingredients including Eucalyptol (0.092%), Menthol (0.042%), methyl salicylate (0.060%), thymol (0.064%) dissolved in up to 25% ethanol [34]. Interestingly 40% ethanol by volume has been shown to toxic to bacterial function. While Listerine ® is established as an effective preventative measure for gingivitis and plaque, it does not appear to cause significant changes within the oral microbiome [35]; (b) Cepacol ® antibacterial mouthwash has been widely used for the last 70 years and contains Cetylpyridinium chloride (0.05%) as the active ingredient. While it provides the same benefits as Listerine in regards to the prevention of gingivitis and plaque, it also acts as an antibacterial agent that alters the oral microbiome [36]; (c) While Chlorhexidine is one of the strongest and most effective mouthwash rinses for combatting plaque and gingivitis, the adverse side effects of teeth staining and metallic taste generally rule it out as a first choice for regular use [37]. It acts as a strong antibacterial agent with the active ingredient chlorhexidine gluconate (0.12%), and is often prescribed for treatment for oral malodor and halitosis, as studies have indicated its effectiveness in significantly reducing organoleptic scores of tongue odor as well as oral pH [38].

We hypothesized there would be an associated graduated effect on NO_3- to NO_2-conversion, as opposed to a threshold effect. Additionally, from previous studies, we anticipated a potential associated effect on resting brachial blood pressures.

2. Methods

2.1. Subjects

Prior to participation, all subjects received an explanation of the study and signed an informed consent document approved by Duke and High Point University Institutional Review Boards. Twelve apparently healthy adult males (six per site) participated in the study. All were non-smokers, with no systemic medication usage, no current use of mouthwash or tongue scrapers, and no recent or current antibiotic use or oral condition (including gingivitis, periodontitis, and halitosis). Subject anthropometric data is shown in Table 1.

2.2. Study design

Subjects completed four testing visits, each separated by at least one week. The individual visits were identical in structure aside from the four mouthwash solution treatments. The order of the visits was randomized, with the sequence determined via a random

Table 1
Subject characteristics and average baseline data.

Subject characteristics (n = 12, mean ± SE)	
Age	36 ± 11
Height (cm)	178.7 ± 5.2
Mass (kg)	79.1 ± 10.2
BMI	24.8 ± 2.2
Average Baseline Data	
Resting SBP	124.2 ± 1.2
Resting DBP	76.1 ± 1.1
Plasma Nitrate (μM)	47.8 ± 4.6
Salivary Nitrate (μM)	684.5 ± 90.4
Plasma Nitrite (nM)	113 ± 16.7
Salivary Nitrite(μM)	280.9 ± 56.1