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Improvement in blood pressure after short-term inorganic nitrate supplementation is attenuated in cigarette smokers compared to non-smoking controls

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ABSTRACT

Dietary supplementation with inorganic nitrate (NO_3^-) has been reported to improve cardiovascular health indices in healthy adults. Cigarette smoking increases circulating thiocyanate (SCN^-), which has been suggested to competitively inhibit salivary nitrate (NO_3^-) uptake, a rate-limiting step in dietary NO_3^- metabolism. Therefore, this study tested the hypothesis that dietary NO_3^- supplementation would be less effective at increasing the circulating plasma nitrite concentration ([NO_2^-]) and lowering blood pressure in smokers (S) compared to non-smokers (NS). Nine healthy smokers and eight healthy non-smoking controls reported to the laboratory at baseline (CON) and following six day supplementation periods with 140 mL day^{-1} NO_3^--rich (8.4 mmol NO_3^- day^{-1}; NIT) and NO_3^--depleted (0.08 mmol NO_3^- day^{-1}; PLA) beetroot juice in a cross-over experiment. Plasma and salivary [SCN^-] were elevated in smokers compared to non-smokers in all experimental conditions ($P < 0.05$). Plasma and salivary [NO_3^-] and [NO_2^-] were elevated in the NIT condition compared to CON and PLA conditions in smokers and non-smokers ($P < 0.05$). However, the change in salivary [NO_3^-] (S: 3.5 ± 2.1 vs. NS: 7.5 ± 4.4 mM), plasma [NO_3^-] (S: 484 ± 198 vs. NS: 802 ± 199 μM) and plasma [NO_2^-] (S: 218 ± 128 vs. NS: 559 ± 419 nM) between the CON and NIT conditions was lower in the smokers compared to the non-smokers ($P < 0.05$). Salivary [NO_2^-] increased above CON to a similar extent with NIT in smokers and non-smokers ($P > 0.05$). Systolic blood pressure was lowered compared to PLA with NIT in non-smokers ($P < 0.05$), but not smokers ($P > 0.05$). These findings suggest that dietary NO_3^- metabolism is compromised in smokers leading to an attenuated blood pressure reduction compared to non-smokers after NO_3^- supplementation. These observations may provide novel insights into the cardiovascular risks associated with cigarette smoking and suggest that this population may be less likely to benefit from improved cardiovascular health if they increase dietary NO_3^- intake.

1. Introduction

Cardiovascular disease is the leading cause of mortality in developed countries and costs the global economy approximately US$3.7 trillion per annum [1]. As such, interventions that can lower cardiovascular disease morbidity will be of epidemiological and economic importance. It has long been appreciated that a diet rich in fruit and vegetables lowers the risk of developing cardiovascular diseases and the incidence of adverse cardiovascular events such as stroke, heart failure and coronary heart disease [2,3]. Consequently, there are numerous government-driven initiatives to increase fruit and vegetable consumption including the Dietary Approaches to Stop Hypertension (DASH) diet in the United States of America [4], the 5-A-Day diet in the United Kingdom [5] and variations of this latter diet in countries within the European Union [6].

It has been suggested that the cardio-protective effects of diets rich in fruit and vegetables might be linked to their high inorganic nitrate (NO_3^-) content [7–9]. Vegetable consumption accounts for 60–80% of dietary NO_3^- intake [10] with leafy-green vegetables (e.g., spinach and lettuce varieties) and beetroot being particularly

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rich in NO_3^- [7]. It has been reported that consuming 5 portions of NO_3^--rich vegetables for 7 days, which provided a daily NO_3^- intake of ~317 mg (5.1 mmol), lowered systolic blood pressure, whereas a control diet where participants avoided NO_3^--rich vegetables, resulting in a daily NO_3^- intake of ~8 mg (0.1 mmol), did not [9]. Increased dietary NO_3^- intake in the form of NO_3^--rich beetroot [11–13] or spinach [14,15] supplementation alone has also been shown to lower resting blood pressure. These findings are consistent with the emerging body of evidence to support improved vascular health following dietary NO_3^- supplementation (4–16 mmol·day^{-1}) in younger [11–13] and older [16,17] normotensive adults, and in individuals with hypertension [18], peripheral artery disease [19] and heart failure [20]. Therefore, enriching the diet with NO_3^-, at a dose that can be readily achieved by a diet high in vegetables [4,7,9], might represent a practical and cost-effective intervention to lower cardiovascular disease morbidity and mortality.

After oral ingestion, approximately 25% of NO_3^- passes into the entero-salivary circulation [21]. Subsequently, NO_3^- is concentrated and delivered within saliva to the oral cavity where facultative microflora reduce NO_3^- to nitrite (NO_2^-) [21–25]. NO_2^--rich saliva is then ingested and NO_2^- is further reduced to nitric oxide (NO) and other reactive nitrogen intermediates in the acidic environment of the stomach [26,27]. It is also clear that a portion (in the nM range) of the ingested NO_2^- passes into the systemic circulation [24] where it can impact vascular function directly [28,29] or through its subsequent reduction to NO via a number of NO_2^- reductases [30]. Although mammalian tissues have the capacity to directly metabolise NO_3^- [31], the entero-salivary delivery of NO_3^- to the oral cavity and its subsequent reduction to NO_2^- by lingual anaerobes, are key rate limiting steps of NO_3^- metabolism in mammals [32]. There is evidence to suggest that the uptake of NO_3^- into the salivary circulation occurs in competition with perchlorate, thiocyanate (SCN^-) and iodide [33]. Therefore, increased exposure to perchlorate, SCN^- or iodide may interfere with dietary NO_3^- metabolism and might subsequently blunt the improvements in vascular health that have typically been observed after increased dietary NO_3^- intake.

Cigarette smoking is a major risk factor for cardiovascular disease morbidity and mortality [34–36], and a leading cause of preventable death worldwide [35,37]. In spite of global government initiatives to facilitate smoking cessation, there are still an estimated 1 billion smokers worldwide [38]. Cigarette smoke contains over 7000 noxious chemicals, including cyanide [35]. Following consumption, cyanide is rapidly detoxified to thiocyanate (SCN^-) via transsulfuration reactions catalysed by the enzymes, thiosulfate sulfotransferase (rhodanase) and 3-mercaptopyruvate sulfurtransferase [39]. Consequently, cigarette smokers have elevated plasma and salivary [SCN^-] compared to non-smoking controls [e.g., [40]. Importantly, and consistent with a competitive inhibition of salivary NO_3^- uptake by SCN^- [33], it has been reported that salivary [NO_3^-] is lower in cigarette smokers after NO_3^- ingestion, compared to non-smoking controls [41,42]. However, in spite of a lower salivary [NO_3^-] after NO_3^- ingestion in smokers, the increase in salivary [NO_2^-] was not different between the smokers and non-smokers [41]. It is therefore unclear whether cigarette smoking interferes with the increases in plasma [NO_2^-] and the associated reduction of blood pressure that has been observed following dietary NO_3^- ingestion in non-smokers [12,13,31]. Further research is required to elucidate the effects of cigarette smoking on dietary NO_3^- metabolism and its implications for vascular health. If dietary NO_3^- metabolism is indeed perturbed by cigarette smoking, this may provide new insights into the mechanisms by which cigarette smoking increases cardiovascular disease morbidity.

The purpose of this study was to assess the effects of six days dietary NO_3^- supplementation on plasma and salivary [NO_3^-], [NO_2^-] and [SCN^-] and resting blood pressure in smokers and non-smoking controls. It was hypothesized that the increases in salivary [NO_3^-], plasma [NO_3^-] and plasma [NO_2^-], but not salivary [NO_2^-], after dietary NO_3^- supplementation would be attenuated in cigarette smokers compared to non-smoking controls. It was also hypothesized that dietary NO_3^- supplementation would lower blood pressure in non-smokers, but not in smokers.

2. Materials and methods

2.1. Subjects

We recruited nine cigarette smokers (5 males, mean ± SD, age 24 ± 7 yr, body mass index 23 ± 2 kg m^2; smoking history 7 ± 6 pack years) and eight age- and BMI-matched non-smoking controls (4 males, mean ± SD, age 24 ± 5 yr, body mass index 23 ± 4 kg m^2) from the University staff and student communities to participate in this study. Both the smokers [forced vital capacity (FVC) 4.68 ± 1.02 L; forced expiratory volume in 1-s (FEV_1) 4.26 ± 0.99 L; FEV_1/FVC 91 ± 6%] and non-smokers (FVC, 4.17 ± 0.76 L; FEV_1, 3.64 ± 0.57 L; FEV_1/FVC 88 ± 5%] exhibited normal resting pulmonary function and had a similar level of habitual physical activity, as assessed by the Baecke et al. [43] questionnaire (smokers, 7.2 ± 1.7; non-smokers 7.2 ± 1.7), upon recruitment to the study. All procedures employed in this study were approved by the Institutional Research Ethics Committee and subjects gave their written informed consent to participate prior to the commencement of the study, after the experimental procedures, associated risks, and potential benefits of participation had been explained. Subjects were instructed to arrive at each laboratory testing session in a rested and fully hydrated state, at least 3 h postprandial. Since the reduction of NO_3^- to NO_2^- in the oral cavity is compromised by antibacterial mouthwash [44], subjects were required to refrain from mouthwash use for the duration of the study. Each subject was also asked to avoid consumption of nitrate-rich foods for the duration of the study, and from caffeine and alcohol ingestion 6 and 24 h before each test, respectively. All subjects were instructed to maintain their habitual physical activity pattern for the duration of the study, and to avoid strenuous exercise in the 24 h preceding the testing sessions. Smokers were asked to maintain their habitual smoking patterns for the duration of the study, but were required to abstain from smoking for 3 h before each testing session. All tests were performed at the same time of day (±2 h).

2.2. Supplementation procedures

All subjects were required to report to the laboratory on three occasions over a 3–4 week period. Subjects did not undergo dietary supplementation prior to their first visit to the laboratory (the control condition; CON). Subjects were asked to record their food and beverage consumption on the day of the CON test and for the 5 days preceding this test and to replicate this prior to the subsequent trials. After completing the CON trial, subjects were randomly assigned to receive six days of supplementation with either NO_3^--rich (NIT) or NO_3^--depleted beetroot juice as a placebo (PLA) as part of a double-blind, cross-over experimental design. In the NIT and PLA conditions, subjects ingested 70 mL of concentrated beetroot juice containing 4.2 and 0.04 mmol NO_3^-, respectively, in the morning and evening over the first five days of supplementation. Subjects ingested 140 mL of beetroot juice 2 h before reporting to the laboratory on day six of NIT and PLA supplementation. This was selected to coincide with the peak plasma [NO_2^-] attained following ingestion of 8.4 mmol NO_3^- [13]. A 7–10 day washout separated the supplementation periods.