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Lowering of blood pressure after nitrate-rich vegetable consumption is abolished with the co-ingestion of thiocyanate-rich vegetables in healthy normotensive males

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ABSTRACT

A diet rich in vegetables is known to provide cardioprotection. However, it is unclear how the consumption of different vegetables might interact to influence vascular health. This study tested the hypothesis that nitrate-rich vegetable consumption would lower systolic blood pressure but that this effect would be abolished when nitrate-rich and thiocyanate-rich vegetables are co-ingested. On four separate occasions, and in a randomized cross-over design, eleven healthy males reported to the laboratory and consumed a 750 mL vegetable smoothie that was either: low in nitrate (~0.3 mmol) and thiocyanate (~5 μmol), low in nitrate and high in thiocyanate (~72 μmol), high in nitrate (~4 mmol) and low in thiocyanate and high in nitrate and thiocyanate. Blood pressure as well as plasma and salivary [thiocyanate], [nitrate] and [nitrite] were assessed before and 3 h after smoothie consumption. Plasma [nitrate] and [nitrite] and salivary [nitrate] were not different after consuming the two high-nitrate smoothies, but salivary [nitrite] was higher after consuming the high-nitrate low-thiocyanate smoothie (1183 ± 625 μM) compared to the high-nitrate high-thiocyanate smoothie (941 ± 532 μM; $P < .001$). Systolic blood pressure was only lowered after consuming the high-nitrate low-thiocyanate smoothie (−3 ± 5 mmHg; $P < .05$). The acute consumption of vegetables high in nitrate and low in thiocyanate lowered systolic blood pressure. However, when the same dose of nitrate-rich vegetables was co-ingested with thiocyanate-rich vegetables the increase in salivary [nitrite] was smaller and systolic blood pressure was not lowered. These findings might have implications for optimising dietary guidelines aimed at improving cardiovascular health.

1. Introduction

Cardiovascular disease (CVD) is the leading cause of mortality globally and places a significant burden on healthcare services [1]. In an effort to attenuate CVD morbidity cost-effectively, many governments actively promote the consumption of a diet rich in fruit and vegetables [2–4] based on evidence that adhering to such diets can mitigate the risks associated with CVD morbidity [5,6]. However, emerging evidence indicates that the cardioprotective effect afforded by vegetable consumption outweighs that of fruit consumption [7], and that leafy green vegetables rich in inorganic nitrate (NO_3^-) might be particularly effective at improving the health of the cardiovascular system [8–11].

The cardiovascular benefits associated with NO_3^--rich vegetable consumption has been ascribed to the stepwise reduction of NO_3^- to nitrite (NO_2^-) and then nitric oxide (NO) [12]. Since humans have a limited capacity to directly reduce NO_3^- to NO_2^-, with this being critically dependent on the NO_3^- reducing bacteria on the tongue [13,14], a rate limiting step for the chemical reduction of NO_3^- is the delivery of NO_3^- to the oral cavity through the enterosalivary circulation [15]. The uptake of NO_3^- into the salivary glands occurs in competition with other anions, including thiocyanate (SCN^-), with evidence that SCN^- has a higher affinity for transport into the salivary glands compared to NO_3^- [16]. It has recently been reported that

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cigarette smokers, who manifest higher plasma and salivary [SCN^-], exhibited smaller increases in salivary [NO_3^-] and plasma [NO_2^-] compared to non-smokers after ingesting the same NO_3^- dose, and that blood pressure was only lowered post NO_3^- supplementation in the non-smokers [17]. Therefore, increased exposure to SCN^- has the potential to perturb dietary NO_3^- metabolism and its beneficial effect on vascular health.

Although some green vegetables, including spinach and rocket, are a rich source of NO_3^- [18,19], other green vegetable varieties, including members of the *Brassica* family such as cabbage, cauliflower and broccoli, have been reported to increase serum [SCN^-] [20]. *Brassica* vegetables are rich in glucosinolates [21,22]. During processes that damage plant cell membranes, such as mastication, glucosinolates are exposed to myrosinase which catalyses the hydrolysis of glucosinolates to SCN^- [23,24]. Therefore, co-ingestion of glucosinolate/SCN^--rich vegetables with NO_3^--rich vegetables has the potential to perturb dietary NO_3^- metabolism and the beneficial effect of NO_3^- on vascular health markers.

The purpose of this study was therefore to investigate the independent and combined effect of ingesting NO_3^--rich and SCN^--rich vegetables on dietary NO_3^- metabolism and blood pressure at doses within the recommended daily fruit and vegetable intake of 400 g·day^{-1} [3,25]. We hypothesised that, compared to the ingestion of vegetables high in NO_3^- and low in SCN^-, the increases in salivary [NO_3^-] and [NO_2^-] and plasma [NO_2^-] would be blunted, and the lowering of blood pressure would be attenuated when the same dose of NO_3^--rich vegetables was co-ingested with SCN^--rich vegetables. We also hypothesised that the ingestion of vegetables low in NO_3^- would not alter blood pressure, irrespective of their SCN^- content, due to a lack of change in plasma [NO_2^-].

2. Subjects and methods

2.1. Subjects characteristics

Eleven healthy, non-smoking males (mean ± SD, age 21 ± 1 yr, height 1.82 ± 0.03 m, body mass 80 ± 9 kg) were recruited from the University student community to participate in this trial. The participant number was determined *a priori* via a statistical power calculation. Specifically, to detect an effect size of 1, which was based on the peak reduction in systolic blood pressure following the ingestion of NO_3^--rich beetroot juice providing 4 mmol NO_3^- from the study by Wylie et al. [26], with a high statistical power (0.80) and an α error probability of 0.05, 10 subjects were required. We recruited 11 participants to account for potential participant drop out. This calculation was conducted using G*Power (Version 3.1.9.2). All procedures employed in this study were approved by the Institutional Research Ethics Committee. Subjects gave their written informed consent to participate in this trial prior to the commencement of the study and 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 state after an overnight fast. Since the chemical reduction of NO_3^- to NO_2^- in the oral cavity is thwarted by antibacterial mouthwash [13], subjects were required to refrain from mouthwash use for the duration of the study. Each subject was also asked to avoid consumption of NO_3^--rich and glucosinolate/SCN^--rich foods for 48 h, and from caffeine and alcohol ingestion 12 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. All tests were performed at the same time of day (± 1 h).

2.2. Experimental design

Subjects were required to report to the laboratory on five occasions over a 3–5 week period to complete the experimental testing. Since environmental (e.g. humidity, temperature, water content and exposure to sunlight) and agricultural factors can influence vegetable NO_3^- and SCN^- concentrations [19,22,27,28], each vegetable was obtained from the same supplier (Tesco Stores Ltd, UK) and the trial was completed within a 4-month period to limit the potential for variability in these factors.

On the first visit to the laboratory, subjects were familiarized with the blood pressure measures and venous blood and saliva collection methods described below. On the subsequent four laboratory visits, seated blood pressure was assessed, and saliva and venous blood samples were obtained, after arrival at the laboratory. Subjects then consumed a vegetable smoothie beverage prepared using vegetables either low in NO_3^- and SCN^- (LoN-LoT); low in NO_3^-, high in SCN^- (LoN-HiT); high in NO_3^-, low in SCN^- (HiN-LoT); or high in NO_3^- and SCN^- (HiN-HiT) along with a standardised breakfast of 54 g of porridge oats ("Oats So Simple", Quaker Oats) prepared with 180 mL of tap water and one 20 g sachet of golden syrup (Lyle's golden syrup). The smoothies were administered in a randomized, doubled-blind, counterbalanced, cross-over experimental design. Blood pressure was measured and venous blood and saliva were collected three hours post vegetable smoothie consumption to align with the peak plasma [NO_2^-] and blood pressure reduction following dietary supplementation with 4.2 mmol NO_3^- [26]. Plasma and salivary [NO_3^-], [NO_2^-] and [SCN^-] were determined using the procedures described below. Subjects were naive to the experimental hypotheses and were informed that the aim of this study was to investigate the effects of consuming different vegetables on vascular health.

2.3. Supplementation procedures

To inform the composition of the vegetable smoothie beverages administered in this study, the NO_3^- and SCN^- content of a variety of vegetables was assessed prior to experimental testing. These preliminary analyses revealed that garden peas were low in both NO_3^- (~0.8 mg/100 g) and SCN^- (< 0.1 mg/100 g), spinach and rocket were high in NO_3^- (~143.0 and 360.5 mg/100 g, respectively) and low in SCN^- (both < 0.1 mg/100 g), and the inner leaves of a cabbage were high in SCN^- (~5.0 mg/100 g) and low in NO_3^- (~7.3 mg/100 g). Therefore, these vegetables were selected to prepare the four vegetable smoothie beverages administered in this study. Further pilot work to assess drink palatability and the potential for side effects, including gastrointestinal (GI) discomfort, revealed that subjects were able to ingest 750 mL of each of the four vegetable smoothie beverages prepared in this study with minimal GI discomfort.

The four vegetable smoothies administered in this study were all 750 mL and comprised 250 mL tap water, 150 mL of Ribena blackcurrant cordial (Ribena, Lucozade Ribena Suntory Limited, United Kingdom) and 340 g of blended green vegetables. The 340 g of green vegetables comprised 340 g garden peas in the LoN-LoT beverage; 122 g garden peas and 218 g inner cabbage leaves in the LoN-HiT beverage; 218 g garden peas, 87 g spinach and 35 g rocket in the HiN-LoT beverage; and 218 g inner cabbage leaves, 87 g spinach and 35 g rocket in the HiN-HiT beverage. Vegetables were weighed using digital scales sensitive to 100 mg (Ohaus Valor 3000 Xtreme, Ohaus Corporation, USA) and blended on the morning of testing. Each smoothie was then refrigerated at 4 °C for > 15 min prior to consumption. Vegetables were blended raw to preserve nutritional content, since conventional cooking methods reduce the intake of glucosinolates/SCN^- [22] and NO_3^- [29]. A 30 mL aliquot was obtained from each of the four vegetable smoothies from which subsequent 1.5 mL aliquots were collected and analyzed to determine the NO_3^- and SCN^- content of the vegetable smoothie beverages (see below for further details). These analyses were completed at the start and end of the study to gauge any changes in the vegetable NO_3^- and SCN^- content across the study. The NO_3^- and SCN^- content of the four vegetable smoothie beverages at the start and end of the study are presented in Table 1, with the mean of these two