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Influence of iodide ingestion on nitrate metabolism and blood pressure following short-term dietary nitrate supplementation in healthy normotensive adults

Stephen J. Bailey [a, *], Jamie R. Blackwell [a], Lee J. Wylie [a], Annabelle Emery [a], Ellie Taylor [a], Paul G. Winyard [b], Andrew M. Jones [a]

[a] Sport and Health Sciences, College of Life and Environmental Sciences, St. Luke's Campus, University of Exeter, Heavitree Road, Exeter, UK
[b] University of Exeter Medical School, St. Luke's Campus, University of Exeter, Heavitree Road, Exeter, UK

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ABSTRACT

Uptake of inorganic nitrate (NO_3^-) into the salivary circulation is a rate-limiting step for dietary NO_3^- metabolism in mammals. It has been suggested that salivary NO_3^- uptake occurs in competition with inorganic iodide (I^-). Therefore, this study tested the hypothesis that I^- supplementation would interfere with NO_3^- metabolism and blunt blood pressure reductions after dietary NO_3^- supplementation. Nine healthy adults (4 male, mean ± SD, age 20 ± 1 yr) reported to the laboratory for initial baseline assessment (control) and following six day supplementation periods with 140 mL·day^{-1} NO_3^--rich beetroot juice (8.4 mmol NO_3^-·day^{-1}) and 198 mg potassium gluconate·day^{-1} (nitrate), and 140 mL·day^{-1} NO_3^--rich beetroot juice and 450 μg potassium iodide·day^{-1} (nitrate + iodide) in a randomized, cross-over experiment. Salivary [I^-] was higher in the nitrate + iodide compared to the control and NIT trials ($P < 0.05$). Salivary and plasma [NO_3^-] and [NO_2^-] were higher in the nitrate and nitrate + iodide trials compared to the control trial ($P < 0.05$). Plasma [NO_3^-] was higher (474 ± 127 vs. 438 ± 117 μM) and the salivary-plasma [NO_3^-] ratio was lower (14 ± 6 vs. 20 ± 6 μM), indicative of a lower salivary NO_3^- uptake, in the nitrate + iodide trial compared to the nitrate trial ($P < 0.05$). Plasma and salivary [NO_2^-] were not different between the nitrate and nitrate + iodide trials ($P > 0.05$). Systolic blood pressure was lower than control (112 ± 13 mmHg) in the nitrate (106 ± 13 mmHg) and nitrate + iodide (106 ± 11 mmHg) trials ($P < 0.05$), with no differences between the nitrate and nitrate + iodide trials ($P > 0.05$). In conclusion, co-ingesting NO_3^- and I^- perturbed salivary NO_3^- uptake, but the increase in salivary and plasma [NO_2^-] and the lowering of blood pressure were similar compared to NO_3^- ingestion alone. Therefore, increased dietary I^- intake, which is recommended in several countries worldwide as an initiative to offset hypothyroidism, does not appear to compromise the blood pressure reduction afforded by increased dietary NO_3^- intake.

1. Introduction

The gaseous molecule, nitric oxide (NO), regulates an array of physiological processes, but is perhaps best known for its vasodilatory and cardioprotective properties [1,2]. It has been demonstrated that NO can be generated through the O_2-independent reduction of nitrite (NO_2^-) to complement O_2-dependent NO generation through the NO synthases [3–5]. The circulating plasma [NO_2^-] can be increased through dietary supplementation with inorganic nitrate (NO_3^-) and is associated with a reduction in blood pressure and arterial stiffness [6–8], important predictors of future adverse cardiovascular events [9,10]. In addition, NO_3^- supplementation can improve vascular function in healthy older adults [11] and some clinical populations including patients with peripheral artery disease [12] and heart failure [13]. Increasing dietary NO_3^- intake, therefore, appears to confer cardioprotective effects and might hold promise as a nutritional intervention to lower the societal and economic burden of cardiovascular diseases [14].

Approximately 25% of NO_3^- consumed through the diet is actively taken up and concentrated by the salivary glands [15]. NO_3^-

* Corresponding author. School of Sport, Exercise and Health Sciences, Loughborough University, Ashby Road, Loughborough, Leicestershire LE11 3TU, UK.
E-mail address: S.Bailey2@lboro.ac.uk (S.J. Bailey).

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is then transported in saliva to the mouth for second-pass metabolism via the so-called entero-salivary circulation [15–19]. Upon arrival of NO_3^--rich saliva at the oral cavity, microflora on the tongue reduce NO_3^- to NO_2^- [15–19]. After swallowing this NO_2^--rich saliva, NO_2^- is chemically reduced to NO and other reactive nitrogen intermediates in the acidic environment of the stomach [20,21], but it is well documented that the circulating plasma [NO_2^-] is also increased after increased NO_3^- intake [6–8,18]. This circulating plasma NO_2^- can then impact vascular function either through direct NO_2^- action [22,23] or through its subsequent reduction to NO via numerous NO_2^- reductases [24]. While mammalian tissue is capable of reducing NO_3^- to NO_2^- [25], the rate limiting steps for NO_3^- reduction in mammals are NO_3^- transport into the entero-salivary circulation and NO_3^- reduction to NO_2^- by the oral microflora [26]. Importantly, the anions perchlorate (ClO_4^-), thiocyanate (SCN^-), iodide (I^-) and NO_3^- share a common transporter for uptake into the salivary glands, with the order of affinity for salivary uptake being $ClO_4^- > SCN^- > I^- > NO_3^-$ [27]. Although ClO_4^- has the highest affinity for salivary uptake of the aforementioned anions [27], environmental exposure to ClO_4^- is limited [28,29]. Consequently, the competition between SCN^-, I^- and NO_3^- is more likely to be pertinent for NO_3^- transfer into the entero-salivary circulation [28,29] and, subsequently, the stepwise reduction of NO_3^- to NO_2^- and then NO.

It has recently been reported that cigarette smoking [30], which increased salivary and plasma [SCN^-], perturbed aspects of dietary NO_3^- metabolism, and thwarted the lowering of blood pressure, after dietary NO_3^- supplementation. There is evidence to suggest that compared to nitrate iodide is ~8 times more effective at competitively inhibiting the anion transporter in the human thyroid gland [31]. Although I^- has previously been suggested to interfere with salivary NO_3^- uptake [27], the relative potency of I^- to inhibit salivary NO_3^- uptake has yet to be determined. As such, it is unclear whether increasing the circulating [I^-] via I^- supplementation can compromise salivary NO_3^- uptake after NO_3^- ingestion. However, it is also possible that any potential perturbation to salivary NO_3^- uptake after NO_3^- and I^- co-ingestion might be offset by a compensatory increase in NO generation in the stomach. Indeed, nitrous acid (HNO_2), which is formed from the protonation of ingested salivary NO_2^- in the stomach [32], can react with I^- to form NO at an acidic pH [33]. Accordingly, further research is required to assess the extent to which dietary I^- enrichment impacts dietary NO_3^- metabolism and associated vascular health benefits after dietary NO_3^- supplementation. This is important because I^- is present in numerous food sources, with seafood and dairy products, particularly seaweed, white fish, yogurt and milk, being abundant in I^- [34]. Moreover, in excess of 100 countries fortify their salt with I^-, or mandate the use of iodised salt for the production of products such as bread, in an effort to alleviate the prevalence of hypothyroidism [35,36]. These government initiatives have been successful at increasing I^- exposure [36], but it is unclear if this might be to the detriment of dietary NO_3^- metabolism.

The purpose of this study was to examine the effect of co-supplementation with NO_3^- and I^-, compared to NO_3^- supplementation alone, on dietary NO_3^- metabolism and blood pressure. We hypothesised that NO_3^- supplementation would increase salivary and plasma [NO_3^-] and [NO_2^-] and lower blood pressure, but that concurrent NO_3^- and I^- supplementation would attenuate: 1) salivary NO_3^- uptake, 2) the increase in circulating plasma [NO_2^-] and 3) the lowering of blood pressure compared to NO_3^- supplementation alone.

2. Methods

2.1. Subject characteristics

We recruited nine healthy non-smoking adults (4 males, mean ± SD, age 20 ± 1 yr, body mass 71 ± 16 kg, height 1.72 ± 0.11 m) to participate in this study. All procedures employed in this study were approved by the Institutional Research Ethics Committee and subjects gave their written informed consent to participate 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 abolished by antibacterial mouthwash [37,38], subjects were required to refrain from mouthwash use for the duration of the study. Each subject was given a list of NO_3^--rich and SCN^--rich foods and asked to avoid consumption of these foods for the duration of the study, and to abstain from caffeine and alcohol ingestion 6 and 24 h before each test, respectively. Subjects were instructed to maintain their habitual exercise pattern for the duration of the study. 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). Subjects were asked to record their food and beverage consumption on the day of the control test and for the 2 days preceding this test and to replicate this prior to the subsequent trials. After completing the control trial, subjects were randomly assigned to receive six days of supplementation with 2 × 70 mL NO_3^--rich beetroot juice (8.4 mmol NO_3^-) and 2 × 99 mg potassium gluconate placebo capsules (nitrate), or 2 × 70 mL NO_3^--rich beetroot juice and 2 × 225 μg potassium iodide capsules (nitrate + iodide), per day as part of a double-blind, cross-over experimental design (Fig. 1). Subjects consumed 1 × 70 mL NO_3^--rich beetroot juice and 1 × 99 mg potassium gluconate placebo capsule (nitrate) or 1 × 225 μg potassium iodide capsule (nitrate + iodide) in the morning and evening on days 1–5 of supplementation and 2 × 70 mL NO_3^--rich beetroot juice with 2 × 99 mg potassium gluconate placebo capsules (nitrate) or 2 × 225 μg potassium iodide capsules (nitrate + iodide) 2 h prior to arriving at the laboratory on day 6 of supplementation. This was selected to coincide with the peak plasma [NO_2^-] attained following ingestion of 8.4 mmol NO_3^- [8]. A 7–10 day washout separated the supplementation periods. Potassium gluconate and potassium iodide capsules were provided by NOW Sports Nutrition (NOW Foods, Bloomingdale, IL, USA) and were similar in taste, texture and appearance. NO_3^--rich beetroot juice was purchased from James White Drinks (Beet It; James White Drinks, Ipswich, UK).

2.3. Measurements

2.3.1. Blood pressure

After arrival at the laboratory, subjects were required to rest supine for 10 min in an isolated room. Thereafter, blood pressure of the brachial artery was measured whilst the subject was supine using an automated sphygmomanometer (Dinamap Pro, GE Medical Systems, Tampa, USA). Five measurements were taken and the mean of the measurements 2–5 was used for analysis.

2.3.2. Blood and saliva collection

Following blood pressure measurements, venous blood samples