Manuscript Details

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.

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Figure 1.tif [Figure]

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Figure 3 - Salivary Nitrate and Nitrite .tif [Figure]

Figure 4 - Plasma Nitrate and Nitrite.tif [Figure]

Figure 5 - Salivary-Plasma Nitrate and Nitrite Ratio.tif [Figure]

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To the Editor,

We thank you and the reviewers for considering our initial manuscript, Ms. No.: NOX-2016-120 entitled "Influence of iodide ingestion on nitrate metabolism and blood pressure following short-term dietary nitrate supplementation in healthy normotensive adults", for publication in Nitric Oxide. Please find enclosed our revised manuscript entitled "Influence of iodide ingestion on nitrate metabolism and blood pressure following short-term dietary nitrate supplementation in healthy normotensive adults" for consideration by Nitric Oxide. We thank the editor for allowing us to submit a revised manuscript to be considered for publication in Nitric Oxide. We are grateful to the reviewers for their detailed reports and have responded to each of their comments in the "response to reviewers" document. The manuscript has been adjusted as required. Through the process of addressing the reviewers' comments, we feel the manuscript is much improved and hope that you deem it of a standard acceptable for publication in Nitric Oxide.

Yours sincerely,

Stephen J. Bailey PhD

Ms. No.: NOX-2016-120 entitled "Influence of iodide ingestion on nitrate metabolism and blood pressure following short-term dietary nitrate supplementation in healthy normotensive adults"

Comment to the Editor: We thank the editor for allowing us to submit a revised manuscript to be considered for publication in Nitric Oxide. We are grateful to the reviewers for their detailed reports and have responded to each of their comments below. The manuscript has been adjusted as required. Through the process of addressing the reviewers' comments, we feel the manuscript is much improved and hope that you deem it of a standard acceptable for publication in Nitric Oxide.

-Reviewer 1

  - Bailey and colleagues investigated whether dietary iodide intake altered handling, metabolism and clinical effect on blood pressure of concomitant dietary nitrate ingestion in 9 healthy volunteers

They demonstrated small effects from supra-ADI iodide intake on plasma nitrate but not nitrite levels, and no effects on blood pressure, suggesting that nitrate uptake/secretion by salivary glands is not rate limiting.

The manuscript is well presented and referenced

Thank you for your thorough review of the manuscript and the positive comment.

Minor comments:

1. The authors should distinguish processes that occur in salivary glands [nitrate uptake] from saliva [perhaps some nitrate reduction and carriage of nitrite to stomach] to make it easier for non-experts to understand the processes referred to in this manuscript

We have added text in the introduction to distinguish between processes that occur in the salivary glands from the role that saliva plays in dietary nitrate metabolism, as requested.

2. I think the conclusions of this manuscript could be altered to say that at these doses investigated, iodide competition reveals that salivary gland processes are not rate limiting for the physiological effects of nitrate supplementation at doses studied

The conclusion has been amended to more clearly indicate that the supra-ADI dose of iodide administered in this study is unlikely to interfere with the increases in salivary and plasma [nitrite] and the lowering of blood pressure after dietary nitrate supplementation. However, while our findings in this study suggest that the salivary glands might not be limiting for dietary nitrate metabolism and some of its vascular benefits, our recent study (Bailey et al., 2016, Nitric Oxide) showed that when plasma and salivary [thiocyanate] [a more potent competitive inhibitor of salivary nitrate uptake (Edwards et al., 1954, Lancet)] is greater, as occurs in cigarette smokers, salivary [nitrate] and plasma [nitrite] increase less and the lowering of blood pressure is attenuated after nitrate supplementation in smokers compared to non-smoking controls. Therefore, the uptake of nitrate into the salivary gland can rate-limit dietary

nitrate metabolism. As a result, we cannot state that salivary glands are not rate-limiting to dietary nitrate metabolism on the balance of the existing evidence on the topic.

3. Why were different molar amounts of KI and K-gluconate used [300-fold difference between treatments] ? This should be explained and commented on in a limitation section. Could there be contamination of BP results as one treatment had more K supplementation that may lower BP in short term intervention studies [see any number of McGregor and He reviews]

This is a good point and we have acknowledged this as a potential limitation in the revised discussion. These doses were selected based on the supplements the company was able to provide. However, while we concede that inter-trial differences in potassium ingestion might have impacted the blood pressure responses somewhat, a recent Cochrane review concluded that potassium supplementation does not alter blood pressure in primary hypertensives (Dickinson et al., 2006) with additional evidence that potassium supplementation does not impact blood pressure in normotensive adults (e.g., Barden et al., 1986, Hypertension; Miller et al., 1987, Hypertension). Therefore, based on this evidence, we are confident that the differences in potassium content between the nitrate and nitrate + iodide trials would not have impacted our conclusion that blood pressure was not different between these two experimental conditions.

What is the half life of KI and K-gluconate? is a 7 day washout adequate? How is gluconate handled by the body, could itself impact on gastric/salivary handling of NOx species?

We are not aware of any evidence to suggest that gluconate could impact any of the key processes responsible for the stepwise reduction of nitrate to nitrite and then nitric oxide, which was why it was selected as a placebo in the current study. The half-life for iodide has been reported as ~ 8 hours (Delgado et al., 2015, Thyroid). Therefore, the 7 day washout should have been sufficient for plasma [iodide] to return to baseline between conditions.

4. Why did the protocol have double supplementation on the final morning? lack of steady state kinetics would abound perhaps and should be commented on in a limitation section

The total daily supplementation dose was consumed 2 hours prior to testing to coincide with the peak plasma [NO$_2^-$] attained following ingestion of 8.4 mmol NO$_3^-$ (Wylie et al., 2013, JAP). We have acknowledged in the revised discussion that is it unclear if these results would be reproduced on days 1-5 of supplementation when the plasma and salivary [nitrate] and [nitrite] pharmacokinetics were likely different.

5. What were the basal nitrate levels in CON in plasma as only change from baseline is showed in figure 3. Is 40uM plasma nitrate difference physiologically relevant. Based on the known conversion of plasma nitrite via ES circulation to plasma nitrite being on a few %, perhaps not at all surprising that no diff in plasma nitrite noted. This should be commented on.

The revised results section provides plasma [nitrate] in the control trial as requested. Based on our previous dose-response study (Wylie et al., 2013, JAP), the difference in plasma [nitrate] between the nitrate and nitrate + iodide trials, although statistically

significant, did not meaningfully impact the changes in plasma [nitrite] and blood pressure in this study. We have added text in the discussion section to make this clear.

6. What was the sample size based on. A priori power calculations should be shown.

The effect of nitrate and iodide co-ingestion was not known so it was not possible to provide a direct power calculation. The sample size was based on the effect size for the change in plasma [nitrite] in smokers, who had a higher plasma [thiocyanate] (another competitive inhibitor of salivary nitrate uptake) than non-smokers, after the same dietary nitrate supplementation procedures as used in this study. The effect size for the increase in plasma [nitrite] after dietary nitrate supplementation in smokers was 1.7. For a high statistical power (0.80) with an alpha error probability of 0.05, a sample of 5 would be required to detect this effect. Although the sample was small in this study, the effect size for the change in plasma [nitrite] from the nitrate and nitrate + iodide conditions was -0.03. Therefore, a larger sample would not influence the conclusions of our study.

-Reviewer 2

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The paper by Stephen Bailey and co-authors tested the hypothesis that I- ingestion would interfere with nitrate- metabolism and blunt blood pressure reductions after dietary nitrate - supplementation. In a small randomized, cross-over trial, nine healthy adults were enrolled. After an initial baseline assessment (CON), the volunteers received six-day supplementation with NO_3-rich beetroot juice (8.4 mmol NO_3/day) plus 198 mg potassium gluconate (NIT) or NO_3-rich beetroot juice and 450 μg potassium iodide/day (NIT + I). In summary:

a) Salivary [I-] was higher in NIT + I compared to CON and NIT.

b) Salivary and plasma [NO_3-] and [NO_2-] were higher in NIT and NIT + I compared to CON

c) 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) in NIT + I compared to NIT ($P<0.05$), suggesting of a lower salivary NO_3- uptake.

d) Plasma and salivary [NO_2-] were not different between NIT and NIT + I ($P>0.05$).

e) Compared to control, Systolic blood pressure and MAP were lower in NIT and in NIT + I arm with no differences between them ($P>0.05$).

Based in their results, the authors argue that increased dietary I- intake, which is recommended in several countries to prevent iodine deficiency, does not compromise the blood pressure-lowering effect of dietary nitrate.

Positive points

- The paper is well written in overall, the figures are ok, legends are adequate, and the statistic is adequate.

- This is an interesting study and potentially important in a growing field

Thank you for your thorough review of the manuscript and the positive comments.

However, while the topic is quite interesting, there is no clear explanations why authors would expect major differences induced by the chosen dose of Iodine. The nitrate plasma concentration is quite high (40-60 micro molar) compared to iodide plasma concentration reported in the literature.

This is a good point. Plasma [iodide] is lower than plasma [nitrate] based on the literature. There is evidence to suggest that competitive inhibition of the thyroid gland anion transporter is ~ 8 times greater for iodide compared to nitrate (Tonacchera et al., 2004, Thyroid). However, while iodide has been shown to competitively inhibit nitrate uptake into the salivary gland (Edwards et al., 1954, Lancet) the magnitude of this effect is not clear. We have included this additional information to provide a more clear rationale for the current study in line with your suggestion.

It makes complicate to explain an interesting increase in plasma nitrate in described in Figure 3A for NIT + I group, which suggest that in some way Iodide supplementation is interfering with nitrate bioavailability. And; still, the lower salivary I- reported in NIT group (Fig 1) – Would be nitrate supplementation interfering in iodine transportation?

This is an interesting point. Perchlorate, thiocyanate, iodide and nitrate compete for transport into the salivary glands such that increasing iodide intake would be hypothesised to lower salivary nitrate uptake at a given nitrate dose, which is confirmed by our finding of a higher plasma [nitrate] and a lower salivary-plasma [nitrate] ratio with concurrent nitrate and iodide supplementation compared to nitrate supplementation alone. On the other hand, increasing nitrate intake would be hypothesised to lower salivary iodide uptake at a given iodide dose, which is confirmed by our finding of a lower salivary [iodide] after nitrate supplementation with no iodide supplementation compared to the control trial with no iodide or nitrate supplementation. This concept is covered in the second paragraph of our discussion.

Major points

- The missing CON + I arm (control + iodide supplementation) does not invalidate their findings but it would greatly   improve the MS

We agree that this additional experimental arm would improve the inferences that can be drawn from our data. Unfortunately, it is not possible to complete these additional experiments since too much time has elapsed since the original experiments were conducted and the participants are no longer students at the University of Exeter. However, since the purpose of this study was to assess the impact of iodide ingestion on

dietary nitrate metabolism and blood pressure after nitrate supplementation, rather than the effects of iodide ingestion on basal dietary nitrate metabolism and blood pressure, we feel our study provides sufficient data to address the experimental hypotheses. Nonetheless, we have conceded in the revised discussion that the effects of iodide ingestion on basal dietary nitrate metabolism and blood pressure were not assessed in this study which is an area for further research.

- The abstract should be improved; the paper is much better that the abstract report

We have made some improvements to the abstract as requested.

- The authors must report the absolute values, for all groups, including the control group. The delta values reported makes complicated to evaluate the baseline conditions.

Absolute values for all data are now either stated in the results section or presented in Tables 1-2.

- Could the authors explain why potassium gluconate was used as placebo? In addition, the abstract reports 198 mg potassium gluconate (NIT) and the methods section 99 mg potassium gluconate. Which dose was used?

The daily potassium gluconate dose was 198 mg. On days 1-5 of supplementation, subjects consumed 1 x 99 mg potassium gluconate capsule in the morning and evening, whereas on day 6 of supplementation, subjects consumed 2 x 99 mg potassium gluconate capsules 2 hours prior to testing. This section has been rewritten and a figure of the experimental protocol (figure 1) has been provided to improve clarity.

- In the methods section, maybe would be better to improve the description of experimental protocol used, maybe a figure would make it simple and didactical.

We agree and have provided a figure (Figure 1) to illustrate the experimental protocol employed more clearly.

- Discussion section, page 10, line 6: rephrase this paragraph or add a suitable reference

This sentence has been deleted and we have rephrased this paragraph as requested.

Minor points

-    Maybe would be interesting to the authors read a recent paper in hypertension (PMID: 27802417) and update the discussion in 5th paragraph, page 10, as well another one that evaluate the differences in plasma/saliva nitrate/nitrite ratios (PMID: 27810735) after a dietary dose of nitrate

Thank you for pointing out these excellent recent papers. We were not able to reference these in our original submission as they had not been published. These have been referenced in our revised discussion section.

- Maybe would be interesting to the authors report the regular name "Nitrate" instead "NIT", Control instead CON, and Nitrate + I instead "NIT + I". The "NIT" is quite simple to be confounded by nitrite

This has been changed in line with your suggestion.

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

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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 (~~CON~~ 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} (~~NIT~~ nitrate), and 140 ml·day^{-1} NO_3^--rich beetroot juice and 450 μg potassium iodide·day^{-1} (~~NIT + I~~ nitrate + iodide) in a randomized, cross-over experiment. Salivary [I^-] was higher in the ~~NIT + I~~ nitrate + iodide compared to ~~CON~~ the control and NIT trials ($P<0.05$). Salivary and plasma [NO_3^-] and [NO_2^-] were higher in ~~NIT~~ the nitrate and ~~NIT + I~~ nitrate + iodide trials compared to ~~CON~~ 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 ~~NIT + I~~ nitrate + iodide trial compared to ~~NIT~~ the nitrate trial ($P<0.05$)~~, indicative of a lower salivary NO_3^- uptake~~. Plasma and salivary [NO_2^-] were not different between ~~NIT~~ the nitrate and ~~NIT + I~~ nitrate + iodide trials ($P>0.05$). Systolic blood pressure was lower than ~~CON~~ control (112 ± 13 mmHg) in ~~NIT~~ the nitrate (106 ± 13 mmHg) and ~~NIT + I~~ nitrate + iodide (106 ± 11 mmHg) trials ($P<0.05$), with no differences between ~~NIT~~ the nitrate and ~~NIT + I~~ 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.

Key Words: Entero-salivary circulation; nitrite; nitric oxide; vascular health; nutrition

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^- is then transported in saliva to the mouth for second-pass metabolism via the so-called entero-salivary circulation [15-19]. passes into the entero-salivary circulation where it is delivered to the mouth for second-pass metabolism [15]. 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 hours).

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 control test and for the 2 days preceding this test and to replicate this prior to the subsequent trials. After completing the CON 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 (NIT nitrate), or 2 × 70 ml NO_3^--rich beetroot juice and 2 × 225 µg potassium iodide capsules (NIT + I nitrate + iodide), per day as part of a double-blind, cross-over experimental design (Figure 1). Subjects consumed 1 × 70 ml NO_3^--rich beetroot juice and 1 × 99 mg potassium gluconate placebo capsule (NIT nitrate) or 1 × 225 µg potassium iodide capsule (NIT + I 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 (NIT nitrate) or 2 × 225 µg potassium iodide capsules (NIT + I nitrate + iodide) 2 hours 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 were drawn into 6 mL lithium-heparin tubes (Sarstedt, Leicester, UK). Samples were centrifuged at 4,000 rpm and 4°C for 10 min, within 1-min of collection. Plasma was subsequently extracted and immediately frozen at -80°C for later analysis of [NO_3^-] and [NO_2^-]. Unstimulated saliva samples (~ 5 mL) were collected into 30 mL universal containers and 1.5 mL aliquots were frozen at -80°C for later analysis of [I^-], [NO_3^-] and [NO_2^-].

2.4 Data analysis procedures

2.4.1 *[I^-] determination*

After thawing at room temperature, saliva samples were centrifuged at 1600 g for 10 min, and the supernatant was removed for subsequent analysis. 1 mL of saliva supernatant, 1 mL of deionised water and 40 μL of ionic strength adjustor were added to a 30 mL universal container for assessment of salivary [I^-]. Salivary [I^-] was determined by plotting the mV signal derived from an iodide-selective electrode (PerfectION™, Mettler-Toledo AG, Switzerland) against a calibration plot of I^- standards. All measures were completed at 22°C.

2.4.2 $[NO_3^-]$ and $[NO_2^-]$ determination

All glassware, utensils, and surfaces were rinsed with deionized water to remove residual NO intermediates prior to $[NO_2^-]$ and $[NO_3^-]$ analysis. Plasma samples were deproteinized using zinc sulfate/sodium hydroxide precipitation prior to determination of $[NO_3^-]$. Firstly, 500 µL of 0.18 N NaOH was added to 100 µL of sample followed by 5 min incubation at room temperature. Subsequently, samples were treated with 300 µL aqueous ZnSO4 (5% w/v) and vortexed for 30 s before undergoing an additional 10 min incubation period at room temperature. Samples were then centrifuged at 4,000 rpm for 5 min, and the supernatant was removed for subsequent analysis. The $[NO_3^-]$ of the deproteinized plasma sample was determined by its reduction to NO in the presence of 0.8 % (w/v) VCl_3 in 1M HCl within an air-tight purging vessel. Plasma samples were introduced to the vessel via 50 uL injections into the septum at the top of the vessel. The spectral emission of electronically excited nitrogen dioxide, derived from the reaction of NO with ozone, was detected by a thermoelectrically cooled, red-sensitive photomultiplier tube housed in a Sievers gas-phase chemiluminescence nitric oxide analyzer (Sievers NOA 280i. Analytix Ltd, Durham, UK). The $[NO_3^-]$ was determined by plotting signal (mV) area against a calibration plot of sodium nitrate standards. The $[NO_2^-]$ of the undiluted (non-deproteinized) plasma was determined by its reduction to NO in the presence of glacial acetic acid and aqueous NaI (4% w/v) from sodium nitrite standards. 100 uL injections were used for plasma $[NO_2^-]$ determination. After thawing at room temperature, saliva samples were centrifuged for 10 min at 14000 rpm and the supernatant was removed for subsequent analysis. The supernatant was diluted 100 fold with deionized water and $[NO_3^-]$ and $[NO_2^-]$ were determined from 50 uL injections using the same reagents describe above for the plasma analyses.

2.4.3 [TSH] and [T4] determination

Plasma thyroid stimulating hormone ([TSH]) and thyroxine ([T4]) concentrations were assessed in duplicate using ELISA kits purchased from DRG Diagnostics (DRG Instruments GmbH, Germany).

2.5 Statistics

A one-way repeated-measures ANOVA was employed to determine the effects of the different dietary interventions (CON control, NIT nitrate and NIT + I nitrate + iodide) on the relevant outcome variables. Where the analysis revealed a significant main effect for supplement, Fishers Least Significant Difference tests were employed to determine the origin

of such effects. All data are presented as mean ± SD unless otherwise indicated. Statistical significance was accepted when $P<0.05$.

3. RESULTS

The NO_3^- and I^- supplements administered in this study were well tolerated by all subjects with no negative side effects reported. Subjects consumed all doses of the supplements for each experimental condition and self-reported that their diet was consistent across all the dietary interventions.

3.1 *Salivary [I^-]*

Salivary [I^-] responses in the ~~CON~~ control, ~~NIT~~ nitrate and ~~NIT + I~~ nitrate + iodide conditions are illustrated in Figure 2. There was a main effect for supplement on salivary [I^-] ($P<0.05$), with salivary [I^-] being lower in the ~~NIT~~ nitrate condition (384 ± 245 µg·L^{-1}) compared to the ~~CON~~ control (487 ± 305 µg·L^{-1}) and ~~NIT + I~~ nitrate + iodide (794 ± 269 µg·L^{-1}) conditions ($P<0.05$), and higher than both the ~~NIT~~ nitrate and ~~CON~~ control conditions compared to the ~~NIT + I~~ nitrate + iodide condition ($P<0.05$).

3.2 *Plasma [TSH] and [T4]*

Plasma [TSH] and [T4] responses in the ~~CON~~ control, ~~NIT~~ nitrate and ~~NIT + I~~ nitrate + iodide conditions are presented in Table 1. There were no differences in [TSH] or [T4] between the ~~CON~~ control, ~~NIT~~ nitrate and ~~NIT + I~~ nitrate + iodide conditions ($P>0.05$).

3.3 *Salivary [NO_3^-] and [NO_2^-]*

The changes in salivary [NO_3^-] and [NO_2^-] in the ~~NIT~~ nitrate and ~~NIT + I~~ nitrate + iodide conditions relative to ~~CON~~ control are illustrated in Figure 3. There were main effects for supplement for salivary [NO_3^-] and [NO_2^-] ($P<0.05$). Salivary [NO_3^-] and [NO_2^-] were greater in ~~NIT~~ the nitrate (8318 ± 2399 and 2168 ± 1302 µM) and ~~NIT + I~~ nitrate + iodide (6630 ± 3516 and 1952 ± 1316 µM) trials compared to ~~CON~~ control (168 ± 177 and 170 ± 107 µM; $P<0.05$). There were no differences between ~~NIT~~ the nitrate ~~(2168 ± 1302 µM)~~ and ~~NIT + I~~ nitrate + iodide ~~(1952 ± 1316 µM)~~ trials for salivary [NO_2^-] ($P>0.05$), but there was a trend ($P=0.07$) for a higher salivary [NO_3^-] in ~~NIT~~ the nitrate trial ~~(8318 ± 2399 µM)~~ compared to ~~NIT + I~~ the nitrate + iodide trial ~~(6630 ± 3516 µM)~~.

3.4 Plasma [NO$_3^-$] and [NO$_2^-$]

The changes in plasma [NO$_3^-$] and [NO$_2^-$] in the ~~NIT~~ nitrate and ~~NIT + I~~ nitrate + iodide conditions relative to ~~CON~~ control are illustrated in Figure 4. There were main effects for supplement for plasma [NO$_3^-$] and [NO$_2^-$] ($P<0.05$). Plasma [NO$_3^-$] and [NO$_2^-$] were greater in ~~NIT~~ the nitrate (438 ± 117 µM and 404 ± 142 nM) and ~~NIT + I~~ nitrate + iodide (474 ± 127 µM and 407 ± 145 nM) trials compared to ~~CON~~ control (26 ± 7 µM and 86 ± 22 nM $P<0.05$). Plasma [NO$_3^-$] was higher in ~~NIT + I~~ the nitrate + iodide ~~(474 ± 127 µM)~~ trial compared to ~~NIT~~ the nitrate trial ~~(438 ± 117 µM;~~ $P<0.05$), while plasma [NO$_2^-$] was not different between ~~NIT~~ the nitrate ~~(404 ± 142 nM)~~ and ~~NIT + I~~ nitrate + iodide trials ~~(407 ± 145 nM;~~ $P>0.05$).

3.5 Salivary-plasma [NO$_3^-$] and [NO$_2^-$] ratios

The salivary-plasma [NO$_3^-$] ratio was lower in the ~~NIT + I~~ nitrate + iodide trial (14 ± 6 µM) compared to ~~NIT~~ nitrate trial (20 ± 6 µM; $P<0.05$; Figure 5). The salivary-plasma [NO$_2^-$] ratio was not different between the ~~NIT + I~~ nitrate + iodide (5184 ± 3481 µM) and ~~NIT~~ nitrate conditions (5552 ± 3145 µM; $P>0.05$; Figure 4).

3.6 Blood pressure responses

Systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial pressure (MAP) responses in the ~~CON~~ control, ~~NIT~~ nitrate and ~~NIT + I~~ nitrate + iodide conditions are presented in Table 2. There were main effects for supplement for SBP, DBP and MAP ($P<0.05$). SBP and MAP were lower than ~~CON~~ control in both ~~NIT~~ the nitrate and ~~NIT + I~~ nitrate + iodide trials ($P<0.05$), while DBP was only lower than ~~CON~~ control in ~~NIT~~ the nitrate trial ($P<0.05$; Table 2). There were no differences in any of the blood pressure variables between ~~NIT~~ the nitrate and ~~NIT + I~~ nitrate + iodide trials ($P>0.05$). The change in plasma [NO$_2^-$] tended to be negatively correlated with the change in SBP between the ~~CON~~ control and ~~NIT~~ nitrate trials ($r = -0.65$; $P=0.06$).

4. DISCUSSION

The main original findings from this study were: 1) salivary NO$_3^-$ uptake was lower after co-ingesting NO$_3^-$ and I$^-$ compared to NO$_3^-$ ingested alone (as reflected by a higher plasma [NO$_3^-$] and a lower salivary-plasma [NO$_3^-$] ratio in the former compared to the latter); and 2) circulating plasma [NO$_2^-$] was increased and blood pressure was lowered to a similar extent

after ingesting NO_3^- with and without I^- co-ingestion. Therefore, while increased dietary I^- has the potential to impede salivary NO_3^- uptake, in the present study this did not blunt the increase in plasma $[NO_2^-]$ and the lowering of blood pressure after short-term dietary NO_3^- supplementation. These findings suggest that conforming to global initiatives to increase dietary I^- intake is unlikely to compromise the lowering of blood pressure after dietary NO_3^- ingestion, at least at the NO_3^- dose administered in this study.

Salivary $[I^-]$ was increased above the un-supplemented control by 63% in the ~~NIT + I~~ nitrate + iodide condition in this study. Interestingly, ingestion of NO_3^- alone lowered salivary $[I^-]$ to 79% of that observed in the un-supplemented control condition. This observation complements our previous finding of a lower salivary $[SCN^-]$ after NO_3^- ingestion [30]. Collectively, these findings substantiate notion that SCN^-, I^- and NO_3^- compete for a common salivary transporter [27], with recent evidence pointing to sialin as the key transport protein for salivary NO_3^- uptake [49]. Importantly, although salivary $[I^-]$ was lower in ~~NIT~~ the nitrate compared to the ~~CON~~ control trial, TSH and T4 were not different between the ~~CON~~ control, ~~NIT~~ nitrate and ~~NIT + I~~ nitrate + iodide trials. These findings corroborate a previous study which reported no differences in thyroid hormones following short-term NO_3^- supplementation [40]. Therefore, while short-term supplementation with 8 mmol NO_3^- (a dose that could be achieved through ingestion of 200-300 g of NO_3^--rich vegetables [41-43]) can lower salivary $[I^-]$, this was not sufficient to perturb thyroid gland function. However, chronic supplementation with large ~~> 8 mmol~~ NO_3^- doses might have the potential to ~~daily is not recommended as physiological effects on blood pressure and exercise capacity are not always greater [8, but see 44], and there could be a risk of compromising~~ compromise thyroid gland function [44]. ~~In particular, individuals at risk from, or being treated for, hypothyroidism are not recommended to consume excessive NO_3^-.~~ Further research is required to assess the effects of dietary NO_3^- supplementation on thyroid function to optimise supplementation guidelines for different populations.

The short-term dietary NO_3^- supplementation regime employed in this study increased salivary and plasma $[NO_3^-]$ and $[NO_2^-]$, consistent with several previous reports [6,18,33,37,38,45]. However, when the same absolute NO_3^- dose was co-ingested with I^-, plasma $[NO_3^-]$ was 8% higher, salivary $[NO_3^-]$ was 20% lower (P=0.07) and the salivary-plasma $[NO_3^-]$ ratio was 30% lower. These findings support the notion of an antagonistic effect of I^- on salivary NO_3^- uptake, a key rate-limiting step for dietary NO_3^- metabolism in mammals [26], in line

with previous observations [27]. However, despite a lower salivary NO_3^- uptake, salivary $[NO_2^-]$ was not different between the ~~NIT + I~~ nitrate + iodide and ~~NIT~~ nitrate trials. This observation is similar to our recent study in smokers who, compared to non-smokers, exhibited a smaller increase in salivary $[NO_3^-]$, but a similar increase in salivary $[NO_2^-]$, after ingesting the same NO_3^- dose [30]. In line with the salivary $[NO_2^-]$ results, and in spite of the potential for increased NO generation in the stomach with NO_3^- and I^- co-ingestion [32,33], plasma $[NO_2^-]$ was not different between the ~~NIT + I~~ nitrate + iodide and ~~NIT~~ nitrate trials. Therefore, while salivary NO_3^- uptake was compromised in ~~NIT + I~~ the nitrate + iodide trial compared to ~~NIT~~ the nitrate trial, salivary and plasma $[NO_2^-]$ were similarly increased in ~~NIT~~ the nitrate and ~~NIT + I~~ nitrate + iodide trials. ~~We cannot, however, exclude the possibility that I⁻ ingestion might impact salivary NO₃⁻ uptake, and its subsequent metabolism, at lower NO₃⁻ doses.~~

Consistent with numerous previous reports [6,8,30,42,43,46], SBP was lowered by 6 mmHg in the ~~NIT~~ nitrate trial in this study compared to ~~CON~~ control. Likewise, a 6 mmHg lowering in SBP was observed in the ~~NIT + I~~ nitrate + iodide trial compared to ~~CON~~ control. In keeping with previous reports [8,47], the change in SBP following dietary NO_3^- supplementation tended (P=0.06) to be negatively correlated with the change in plasma $[NO_2^-]$. The association between the increase in circulating plasma $[NO_2^-]$ and lower SBP after NO_3^- supplementation may be the result of vasodilation evoked from a direct effect of NO_2^- on the vasculature [22,23] and/or NO_2^- reduction to NO [24]. These changes might be mediated by increased plasma [cGMP] [47], or altered renal physiology [48]. Therefore, the similar reduction in SBP in the ~~NIT~~ nitrate and ~~NIT + I~~ nitrate + iodide groups is likely to be a function of the similar increase in plasma $[NO_2^-]$ in these groups. ~~These findings suggest that increasing dietary NO₃⁻ intake concomitant with increased dietary I⁻ is unlikely to compromise the increase in circulating plasma [NO₂⁻] and associated lowering of blood pressure.~~ Taken together our results suggest that consuming a supra-ADI dose of I^- perturbed dietary NO_3^- uptake, but this was not a physiologically relevant change as the increase in circulating plasma $[NO_2^-]$ and the lowering of blood pressure were similar after ingesting NO_3^- with or without I^- co-ingestion. However, we acknowledge that potassium intake, which has the potential to independently lower blood pressure, particularly in hypertensive individuals [49], was higher in the nitrate trial (NO_3^--rich beetroot juice and 198 mg potassium gluconate) compared to the nitrate + iodide trial (NO_3^--rich beetroot juice and 450 µg potassium iodide), and this might have impacted on the blood pressure findings in this

study. However, since our subjects were all normotensive, and since there is evidence that potassium supplementation is unlikely to measurably impact blood pressure in normotensive adults [50-52], the inter-trial differences in potassium intake is unlikely to have confounded interpretation of the blood pressure results in this study.

The impetus for increased population dietary I^- intake originated from Switzerland and the USA, where salt iodisation was mandated in the 1920s in an attempt to alleviate hypothyroidism [34]. These initiatives have proven effective at increasing I^- exposure and lowering the incidence of hypothyroidism [36]. Consequently, numerous countries have imposed salt iodisation programmes or the manufacture of certain food products with iodised salt [35,36]. The recommended adequate daily intake (ADI) for I^- is 150 μg for adult males and females, and 200 μg during pregnancy and lactation [53]. These doses could readily be achieved through a diet rich in seafood and dairy products [34], but the I^- dose administered in this study (450 μg·day^{-1}) exceeded the upper range of average daily I^- in the USA where iodised salt is mandated [54]. However, despite administering a large I^- dose in this study (i.e., an I^- dose that is not likely to be achieved by most individuals through the diet), the increase in plasma $[NO_2^-]$ and the lowering of blood pressure after NO_3^- supplementation were not different with or without co-ingestion of I^-. Therefore, our results imply that conforming to global initiatives to increase dietary I^- intake is unlikely to impede the lowering of blood pressure after ingesting a NO_3^- dose equivalent to a few hundred grams of NO_3^--rich vegetables such as spinach, lettuce or rocket [41-43]. However, it is unclear whether dietary I^- supplementation can interfere with NO_3^- metabolism and vascular function in the absence of dietary NO_3^- supplementation or at a NO_3^- dose lower than administered in the current study. Further research is required to address these questions.

5. CONCLUSION

In conclusion, concurrent I^- and NO_3^- ingestion lowered salivary NO_3^- uptake, as indicated by a higher plasma $[NO_3^-]$ and a lower salivary-plasma $[NO_3^-]$ ratio, compared to the same dose of NO_3^- consumed without I^- co-ingestion. However, despite a lower salivary NO_3^- uptake with I^- and NO_3^- co-ingestion, salivary and plasma $[NO_2^-]$ were elevated and blood pressure was lowered to a similar extent when NO_3^- was consumed with or without I^- co-ingestion. These observations are important because they suggest that increasing dietary I^-, which is

encouraged in several countries worldwide through salt iodisation programmes, does not interfere with the lowering of blood pressure after NO_3^- supplementation, even when a supra-ADI I^- dose is administered. Therefore, our results suggest that conforming to global initiatives to increase dietary I^- is unlikely to compromise the ability of a NO_3^- enriched diet to improve blood pressure.

Acknowledgment

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Figure Legends

Figure 1: A schematic of the experimental design.

Figure 2: Salivary iodide concentration ([I$^-$]) following no dietary supplementation (~~CON~~ control), supplementation with nitrate-rich beetroot juice (~~NIT~~ nitrate) and co-supplementation with ~~NIT~~ nitrate and potassium iodide (~~NIT + I~~ nitrate + iodide). The filled bars represent the group mean ± SEM responses in the ~~CON~~ control, ~~NIT~~ nitrate and ~~NIT + I~~ nitrate + iodide conditions. The solid grey lines represent the individual responses in the ~~CON~~ control, ~~NIT~~ nitrate and ~~NIT + I~~ nitrate + iodide conditions. * indicates significantly different from ~~CON~~ control and ~~NIT + I~~ nitrate + iodide. # indicates significantly different from ~~CON~~ control and ~~NIT~~ nitrate.

Figure 3: Salivary nitrate concentration ([NO$_3^-$]) (upper panel) and nitrite concentration ([NO$_2^-$]) (lower panel) following supplementation with nitrate-rich beetroot juice (~~NIT~~ nitrate) and co-supplementation with ~~NIT~~ nitrate and potassium iodide (~~NIT + I~~ nitrate + iodide). Data are expressed as the change from the control condition without NO$_3^-$ or I$^-$ supplementation. The filled bars represent the group mean ± SEM responses while the solid grey lines represent the individual responses in the ~~NIT~~ nitrate and ~~NIT + I~~ nitrate + iodide conditions. There was a trend for a lower salivary [NO$_3^-$] in the ~~NIT + I~~ nitrate + iodide trial compared to the ~~NIT~~ nitrate trial (*P*=0.07).

Figure 4: Plasma nitrate concentration ([NO$_3^-$]) (upper panel) and nitrite concentration ([NO$_2^-$]) (lower panel) following supplementation with nitrate-rich beetroot juice (~~NIT~~ nitrate) and co-supplementation with NIT and potassium iodide (NIT + I). Data are expressed as the change from the control condition without NO$_3^-$ or I$^-$ supplementation. The filled bars represent the group mean ± SEM responses while the solid grey lines represent the individual responses in the ~~NIT~~ nitrate and ~~NIT + I~~ nitrate + iodide conditions. * indicates significantly different from ~~NIT~~ nitrate.

Figure 5: Salivary-plasma nitrate [NO$_3^-$] (upper panel) and nitrite [NO$_2^-$] (lower panel) ratios following supplementation with nitrate-rich beetroot juice (~~NIT~~ nitrate) and co-supplementation with ~~NIT~~ nitrate and potassium iodide (~~NIT + I~~ nitrate + iodide). The filled bars represent the group mean ± SEM responses while the solid grey lines represent the individual responses in the ~~NIT~~ nitrate and ~~NIT + I~~ nitrate + iodide conditions. * indicates significantly different from ~~NIT~~ nitrate.

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

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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.

Key Words: Entero-salivary circulation; nitrite; nitric oxide; vascular health; nutrition

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^- 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 hours).

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 (Figure 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 hours 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 were drawn into 6 mL lithium-heparin tubes (Sarstedt, Leicester, UK). Samples were centrifuged at 4,000 rpm and 4°C for 10 min, within 1-min of collection. Plasma was subsequently extracted and immediately frozen at -80°C for later analysis of $[NO_3^-]$ and $[NO_2^-]$. Unstimulated saliva samples (~ 5 mL) were collected into 30 mL universal containers and 1.5 mL aliquots were frozen at -80°C for later analysis of $[I^-]$, $[NO_3^-]$ and $[NO_2^-]$.

2.4 Data analysis procedures

2.4.1 *$[I^-]$ determination*

After thawing at room temperature, saliva samples were centrifuged at 1600 g for 10 min, and the supernatant was removed for subsequent analysis. 1 mL of saliva supernatant, 1 mL of deionised water and 40 μL of ionic strength adjustor were added to a 30 mL universal container for assessment of salivary $[I^-]$. Salivary $[I^-]$ was determined by plotting the mV signal derived from an iodide-selective electrode (PerfectION™, Mettler-Toledo AG, Switzerland) against a calibration plot of I^- standards. All measures were completed at 22°C.

2.4.2 $[NO_3^-]$ and $[NO_2^-]$ determination

All glassware, utensils, and surfaces were rinsed with deionized water to remove residual NO intermediates prior to $[NO_2^-]$ and $[NO_3^-]$ analysis. Plasma samples were deproteinized using zinc sulfate/sodium hydroxide precipitation prior to determination of $[NO_3^-]$. Firstly, 500 μL of 0.18 N NaOH was added to 100 μL of sample followed by 5 min incubation at room temperature. Subsequently, samples were treated with 300 μL aqueous ZnSO4 (5% w/v) and vortexed for 30 s before undergoing an additional 10 min incubation period at room temperature. Samples were then centrifuged at 4,000 rpm for 5 min, and the supernatant was removed for subsequent analysis. The $[NO_3^-]$ of the deproteinized plasma sample was determined by its reduction to NO in the presence of 0.8 % (w/v) VCl_3 in 1M HCl within an air-tight purging vessel. Plasma samples were introduced to the vessel via 50 uL injections into the septum at the top of the vessel. The spectral emission of electronically excited nitrogen dioxide, derived from the reaction of NO with ozone, was detected by a thermoelectrically cooled, red-sensitive photomultiplier tube housed in a Sievers gas-phase chemiluminescence nitric oxide analyzer (Sievers NOA 280i. Analytix Ltd, Durham, UK). The $[NO_3^-]$ was determined by plotting signal (mV) area against a calibration plot of sodium nitrate standards. The $[NO_2^-]$ of the undiluted (non-deproteinized) plasma was determined by its reduction to NO in the presence of glacial acetic acid and aqueous NaI (4% w/v) from sodium nitrite standards. 100 uL injections were used for plasma $[NO_2^-]$ determination. After thawing at room temperature, saliva samples were centrifuged for 10 min at 14000 rpm and the supernatant was removed for subsequent analysis. The supernatant was diluted 100 fold with deionized water and $[NO_3^-]$ and $[NO_2^-]$ were determined from 50 uL injections using the same reagents describe above for the plasma analyses.

2.4.3 [TSH] and [T4] determination

Plasma thyroid stimulating hormone ([TSH]) and thyroxine ([T4]) concentrations were assessed in duplicate using ELISA kits purchased from DRG Diagnostics (DRG Instruments GmbH, Germany).

2.5 Statistics

A one-way repeated-measures ANOVA was employed to determine the effects of the different dietary interventions (control, nitrate and nitrate + iodide) on the relevant outcome variables. Where the analysis revealed a significant main effect for supplement, Fishers Least Significant Difference tests were employed to determine the origin of such effects. All

data are presented as mean $\pm$ SD unless otherwise indicated. Statistical significance was accepted when $P<0.05$.

3. RESULTS

The NO_3^- and I^- supplements administered in this study were well tolerated by all subjects with no negative side effects reported. Subjects consumed all doses of the supplements for each experimental condition and self-reported that their diet was consistent across all the dietary interventions.

3.1 *Salivary [I^-]*
Salivary [I^-] responses in the control, nitrate and nitrate + iodide conditions are illustrated in Figure 2. There was a main effect for supplement on salivary [I^-] ($P<0.05$), with salivary [I^-] being lower in the nitrate condition (384 ± 245 $\mu g \cdot L^{-1}$) compared to the control (487 ± 305 $\mu g \cdot L^{-1}$) and nitrate + iodide (794 ± 269 $\mu g \cdot L^{-1}$) conditions ($P<0.05$), and higher than both the nitrate and control conditions compared to the nitrate + iodide condition ($P<0.05$).

3.2 *Plasma [TSH] and [T4]*
Plasma [TSH] and [T4] responses in the control, nitrate and nitrate + iodide conditions are presented in Table 1. There were no differences in [TSH] or [T4] between the control, nitrate and nitrate + iodide conditions ($P>0.05$).

3.3 *Salivary [NO_3^-] and [NO_2^-]*
The changes in salivary [NO_3^-] and [NO_2^-] in the nitrate and nitrate + iodide conditions relative to control are illustrated in Figure 3. There were main effects for supplement for salivary [NO_3^-] and [NO_2^-] ($P<0.05$). Salivary [NO_3^-] and [NO_2^-] were greater in the nitrate (8318 ± 2399 and 2168 ± 1302 μM) and nitrate + iodide (6630 ± 3516 and 1952 ± 1316 μM) trials compared to control (168 ± 177 and 170 ± 107 μM; $P<0.05$). There were no differences between the nitrate and nitrate + iodide trials for salivary [NO_2^-] ($P>0.05$), but there was a trend ($P=0.07$) for a higher salivary [NO_3^-] in the nitrate trial compared to the nitrate + iodide trial.

3.4 *Plasma [NO$_3^-$] and [NO$_2^-$]*

The changes in plasma [NO$_3^-$] and [NO$_2^-$] in the nitrate and nitrate + iodide conditions relative to control are illustrated in Figure 4. There were main effects for supplement for plasma [NO$_3^-$] and [NO$_2^-$] ($P<0.05$). Plasma [NO$_3^-$] and [NO$_2^-$] were greater in the nitrate (438 ± 117 µM and 404 ± 142 nM) and nitrate + iodide (474 ± 127 µM and 407 ± 145 nM) trials compared to control (26 ± 7 µM and 86 ± 22 nM $P<0.05$). Plasma [NO$_3^-$] was higher in the nitrate + iodide trial compared to the nitrate trial ($P<0.05$), while plasma [NO$_2^-$] was not different between the nitrate and nitrate + iodide trials ($P>0.05$).

3.5 *Salivary-plasma [NO$_3^-$] and [NO$_2^-$] ratios*

The salivary-plasma [NO$_3^-$] ratio was lower in the nitrate + iodide trial (14 ± 6 µM) compared to nitrate trial (20 ± 6 µM; $P<0.05$; Figure 5). The salivary-plasma [NO$_2^-$] ratio was not different between the nitrate + iodide (5184 ± 3481 µM) and nitrate conditions (5552 ± 3145 µM; $P>0.05$; Figure 4).

3.6 *Blood pressure responses*

Systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial pressure (MAP) responses in the control, nitrate and nitrate + iodide conditions are presented in Table 2. There were main effects for supplement for SBP, DBP and MAP ($P<0.05$). SBP and MAP were lower than control in both the nitrate and nitrate + iodide trials ($P<0.05$), while DBP was only lower than control in the nitrate trial ($P<0.05$; Table 2). There were no differences in any of the blood pressure variables between the nitrate and nitrate + iodide trials ($P>0.05$). The change in plasma [NO$_2^-$] tended to be negatively correlated with the change in SBP between the control and nitrate trials ($r =-0.65$; $P=0.06$).

4. DISCUSSION

The main original findings from this study were: 1) salivary NO$_3^-$ uptake was lower after co-ingesting NO$_3^-$ and I$^-$ compared to NO$_3^-$ ingested alone (as reflected by a higher plasma [NO$_3^-$] and a lower salivary-plasma [NO$_3^-$] ratio in the former compared to the latter); and 2) circulating plasma [NO$_2^-$] was increased and blood pressure was lowered to a similar extent after ingesting NO$_3^-$ with and without I$^-$ co-ingestion. Therefore, while increased dietary I$^-$ has the potential to impede salivary NO$_3^-$ uptake, in the present study this did not blunt the

increase in plasma [NO_2^-] and the lowering of blood pressure after short-term dietary NO_3^- supplementation. These findings suggest that conforming to global initiatives to increase dietary I^- intake is unlikely to compromise the lowering of blood pressure after dietary NO_3^- ingestion, at least at the NO_3^- dose administered in this study.

Salivary [I^-] was increased above the un-supplemented control by 63% in the nitrate + iodide condition in this study. Interestingly, ingestion of NO_3^- alone lowered salivary [I^-] to 79% of that observed in the un-supplemented control condition. This observation complements our previous finding of a lower salivary [SCN^-] after NO_3^- ingestion [30]. Collectively, these findings substantiate notion that SCN^-, I^- and NO_3^- compete for a common salivary transporter [27], with recent evidence pointing to sialin as the key transport protein for salivary NO_3^- uptake [49]. Importantly, although salivary [I^-] was lower in the nitrate compared to the control trial, TSH and T4 were not different between the control, nitrate and nitrate + iodide trials. These findings corroborate a previous study which reported no differences in thyroid hormones following short-term NO_3^- supplementation [40]. Therefore, while short-term supplementation with 8 mmol NO_3^- (a dose that could be achieved through ingestion of 200-300 g of NO_3^--rich vegetables [41-43]) can lower salivary [I^-], this was not sufficient to perturb thyroid gland function. However, chronic supplementation with large NO_3^- doses might have the potential to compromise thyroid gland function [44]. Further research is required to assess the effects of dietary NO_3^- supplementation on thyroid function to optimise supplementation guidelines for different populations.

The short-term dietary NO_3^- supplementation regime employed in this study increased salivary and plasma [NO_3^-] and [NO_2^-], consistent with several previous reports [6,18,33,37, 38,45]. However, when the same absolute NO_3^- dose was co-ingested with I^-, plasma [NO_3^-] was 8% higher, salivary [NO_3^-] was 20% lower (P=0.07) and the salivary-plasma [NO_3^-] ratio was 30% lower. These findings support the notion of an antagonistic effect of I^- on salivary NO_3^- uptake, a key rate-limiting step for dietary NO_3^- metabolism in mammals [26], in line with previous observations [27]. However, despite a lower salivary NO_3^- uptake, salivary [NO_2^-] was not different between the nitrate + iodide and nitrate trials. This observation is similar to our recent study in smokers who, compared to non-smokers, exhibited a smaller increase in salivary [NO_3^-], but a similar increase in salivary [NO_2^-], after ingesting the same NO_3^- dose [30]. In line with the salivary [NO_2^-] results, and in spite of the potential for increased NO generation in the stomach with NO_3^- and I^- co-ingestion [32,33], plasma [NO_2^-]

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was not different between the nitrate + iodide and nitrate trials. Therefore, while salivary NO_3^- uptake was compromised in the nitrate + iodide trial compared to the nitrate trial, salivary and plasma $[NO_2^-]$ were similarly increased in the nitrate and nitrate + iodide trials.

Consistent with numerous previous reports [6,8,30,42,43,46], SBP was lowered by 6 mmHg in the nitrate trial in this study compared to control. Likewise, a 6 mmHg lowering in SBP was observed in the nitrate + iodide trial compared to control. In keeping with previous reports [8,47], the change in SBP following dietary NO_3^- supplementation tended (P=0.06) to be negatively correlated with the change in plasma $[NO_2^-]$. The association between the increase in circulating plasma $[NO_2^-]$ and lower SBP after NO_3^- supplementation may be the result of vasodilation evoked from a direct effect of NO_2^- on the vasculature [22,23] and/or NO_2^- reduction to NO [24]. These changes might be mediated by increased plasma [cGMP] [47], or altered renal physiology [48]. Therefore, the similar reduction in SBP in the nitrate and nitrate + iodide groups is likely to be a function of the similar increase in plasma $[NO_2^-]$ in these groups. Taken together our results suggest that consuming a supra-ADI dose of I^- perturbed dietary NO_3^- uptake, but this was not a physiologically relevant change as the increase in circulating plasma $[NO_2^-]$ and the lowering of blood pressure were similar after ingesting NO_3^- with or without I^- co-ingestion. However, we acknowledge that potassium intake, which has the potential to independently lower blood pressure, particularly in hypertensive individuals [49], was higher in the nitrate trial (NO_3^--rich beetroot juice and 198 mg potassium gluconate) compared to the nitrate + iodide trial (NO_3^--rich beetroot juice and 450 µg potassium iodide), and this might have impacted on the blood pressure findings in this study. However, since our subjects were all normotensive, and since there is evidence that potassium supplementation is unlikely to measurably impact blood pressure in normotensive adults [50-52], the inter-trial differences in potassium intake is unlikely to have confounded interpretation of the blood pressure results in this study.

The impetus for increased population dietary I^- intake originated from Switzerland and the USA, where salt iodisation was mandated in the 1920s in an attempt to alleviate hypothyroidism [34]. These initiatives have proven effective at increasing I^- exposure and lowering the incidence of hypothyroidism [36]. Consequently, numerous countries have imposed salt iodisation programmes or the manufacture of certain food products with iodised salt [35,36]. The recommended adequate daily intake (ADI) for I^- is 150 µg for adult males and females, and 200 µg during pregnancy and lactation [53]. These doses could readily be

achieved through a diet rich in seafood and dairy products [34], but the I^- dose administered in this study (450 µg·day^{-1}) exceeded the upper range of average daily I^- in the USA where iodised salt is mandated [54]. However, despite administering a large I^- dose in this study (i.e., an I^- dose that is not likely to be achieved by most individuals through the diet), the increase in plasma $[NO_2^-]$ and the lowering of blood pressure after NO_3^- supplementation were not different with or without co-ingestion of I^-. Therefore, our results imply that conforming to global initiatives to increase dietary I^- intake is unlikely to impede the lowering of blood pressure after ingesting a NO_3^- dose equivalent to a few hundred grams of NO_3^--rich vegetables such as spinach, lettuce or rocket [41-43]. However, it is unclear whether dietary I^- supplementation can interfere with NO_3^- metabolism and vascular function in the absence of dietary NO_3^- supplementation or at a NO_3^- dose lower than administered in the current study. Further research is required to address these questions.

5. CONCLUSION

In conclusion, concurrent I^- and NO_3^- ingestion lowered salivary NO_3^- uptake, as indicated by a higher plasma $[NO_3^-]$ and a lower salivary-plasma $[NO_3^-]$ ratio, compared to the same dose of NO_3^- consumed without I^- co-ingestion. However, despite a lower salivary NO_3^- uptake with I^- and NO_3^- co-ingestion, salivary and plasma $[NO_2^-]$ were elevated and blood pressure was lowered to a similar extent when NO_3^- was consumed with or without I^- co-ingestion. These observations are important because they suggest that increasing dietary I^-, which is encouraged in several countries worldwide through salt iodisation programmes, does not interfere with the lowering of blood pressure after NO_3^- supplementation, even when a supra-ADI I^- dose is administered. Therefore, our results suggest that conforming to global initiatives to increase dietary I^- is unlikely to compromise the ability of a NO_3^- enriched diet to improve blood pressure.

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Figure Legends

Figure 1: A schematic of the experimental design.

Figure 2: Salivary iodide concentration ([I⁻]) following no dietary supplementation (control), supplementation with nitrate-rich beetroot juice (nitrate) and co-supplementation with nitrate and potassium iodide (nitrate + iodide). The filled bars represent the group mean ± SEM responses in the control, nitrate and nitrate + iodide conditions. The solid grey lines represent the individual responses in the control, nitrate and nitrate + iodide conditions. * indicates significantly different from control and nitrate + iodide. # indicates significantly different from control and nitrate.

Figure 3: Salivary nitrate concentration ([NO$_3$⁻]) (upper panel) and nitrite concentration ([NO$_2$⁻]) (lower panel) following supplementation with nitrate-rich beetroot juice (nitrate) and co-supplementation with nitrate and potassium iodide (nitrate + iodide). Data are expressed as the change from the control condition without NO$_3$⁻ or I⁻ supplementation. The filled bars represent the group mean ± SEM responses while the solid grey lines represent the individual responses in the nitrate and nitrate + iodide conditions. There was a trend for a lower salivary [NO$_3$⁻] in the nitrate + iodide trial compared to the nitrate trial (*P*=0.07).

Figure 4: Plasma nitrate concentration ([NO$_3$⁻]) (upper panel) and nitrite concentration ([NO$_2$⁻]) (lower panel) following supplementation with nitrate-rich beetroot juice (nitrate) and co-supplementation with NIT and potassium iodide (NIT + I). Data are expressed as the change from the control condition without NO$_3$⁻ or I⁻ supplementation. The filled bars represent the group mean ± SEM responses while the solid grey lines represent the individual responses in the nitrate and nitrate + iodide conditions. * indicates significantly different from nitrate.

Figure 5: Salivary-plasma nitrate [NO$_3$⁻] (upper panel) and nitrite [NO$_2$⁻] (lower panel) ratios following supplementation with nitrate-rich beetroot juice (nitrate) and co-supplementation with nitrate and potassium iodide (nitrate + iodide). The filled bars represent the group mean ± SEM responses while the solid grey lines represent the individual responses in the nitrate and nitrate + iodide conditions. * indicates significantly different from nitrate.
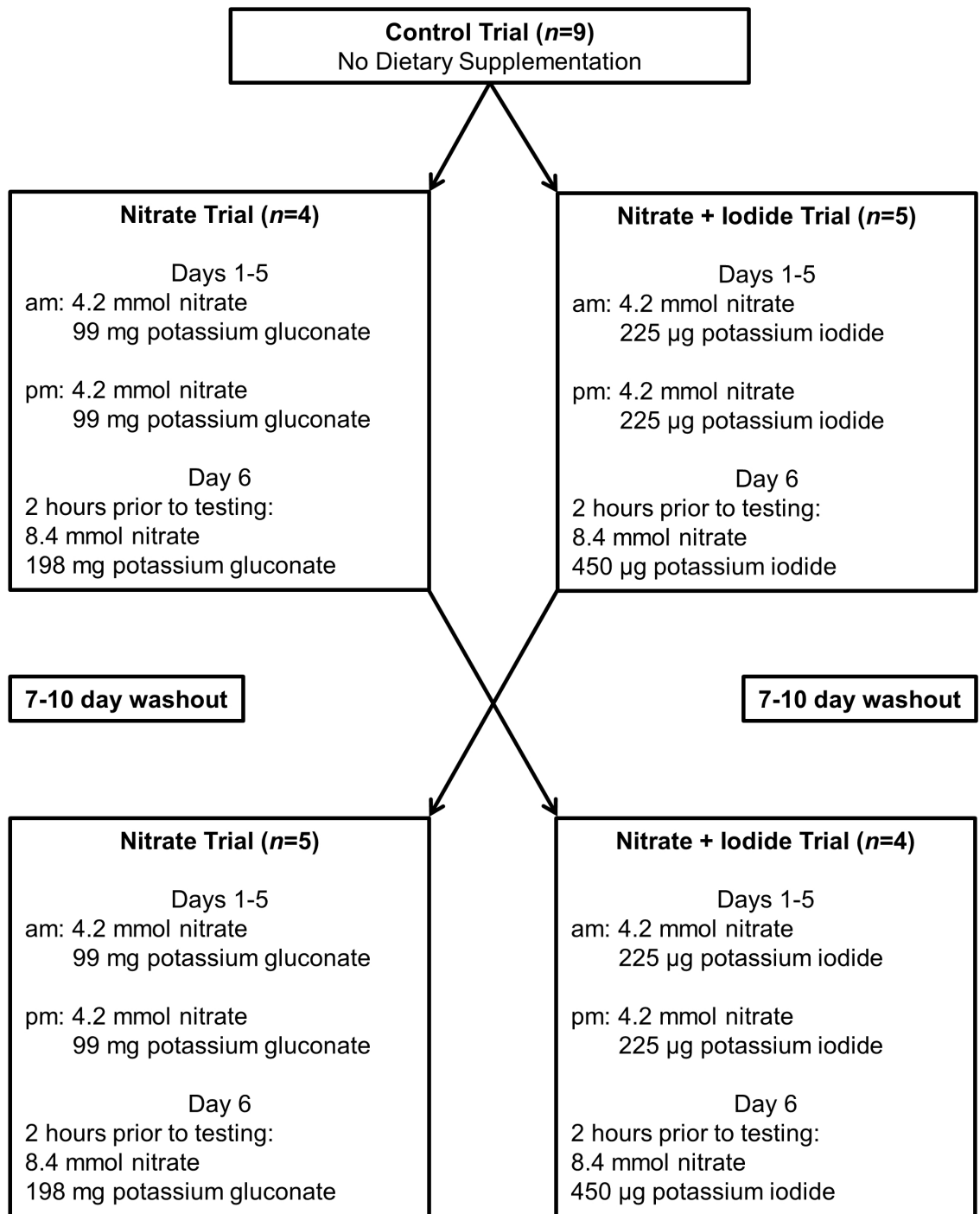
Control Trial (*n*=9)
No Dietary Supplementation

Nitrate Trial (*n*=4)

Days 1-5
am: 4.2 mmol nitrate
        99 mg potassium gluconate

pm: 4.2 mmol nitrate
        99 mg potassium gluconate

Day 6
2 hours prior to testing:
8.4 mmol nitrate
198 mg potassium gluconate

Nitrate + Iodide Trial (*n*=5)

Days 1-5
am: 4.2 mmol nitrate
        225 µg potassium iodide

pm: 4.2 mmol nitrate
        225 µg potassium iodide

Day 6
2 hours prior to testing:
8.4 mmol nitrate
450 µg potassium iodide

7-10 day washout

7-10 day washout

Nitrate Trial (*n*=5)

Days 1-5
am: 4.2 mmol nitrate
        99 mg potassium gluconate

pm: 4.2 mmol nitrate
        99 mg potassium gluconate

Day 6
2 hours prior to testing:
8.4 mmol nitrate
198 mg potassium gluconate

Nitrate + Iodide Trial (*n*=4)

Days 1-5
am: 4.2 mmol nitrate
        225 µg potassium iodide

pm: 4.2 mmol nitrate
        225 µg potassium iodide
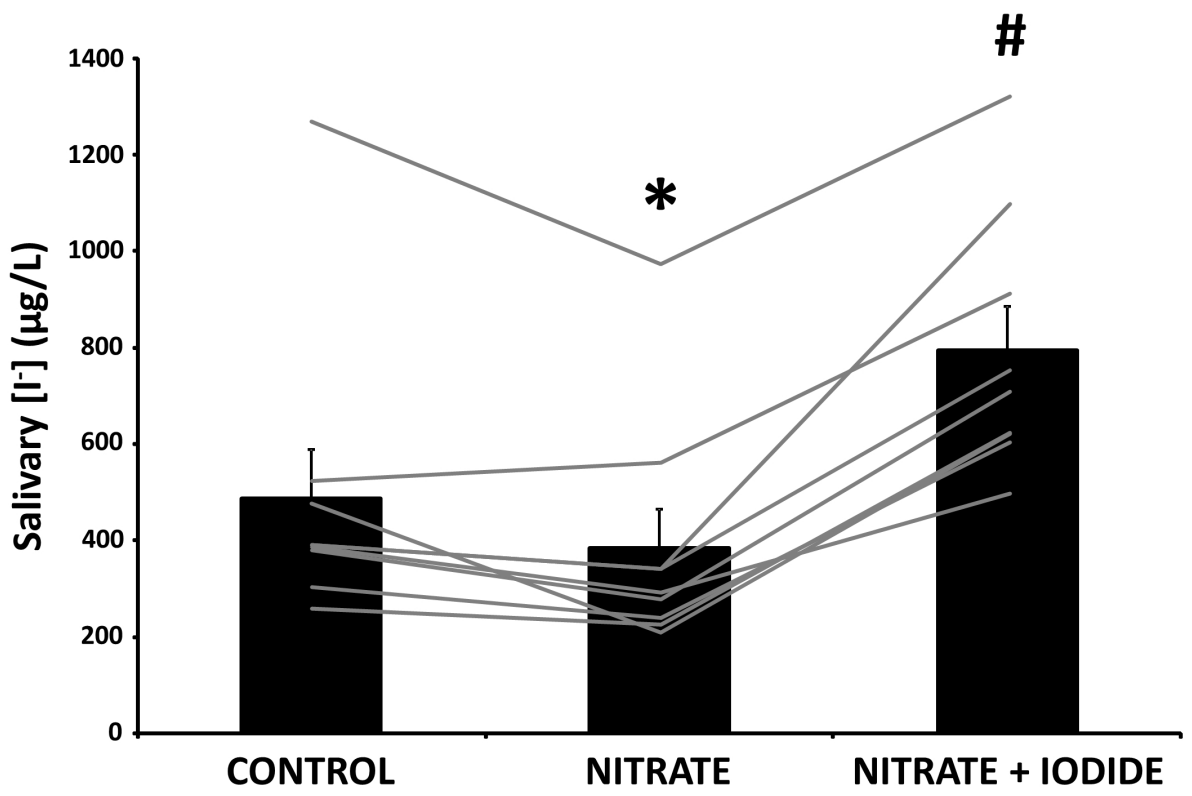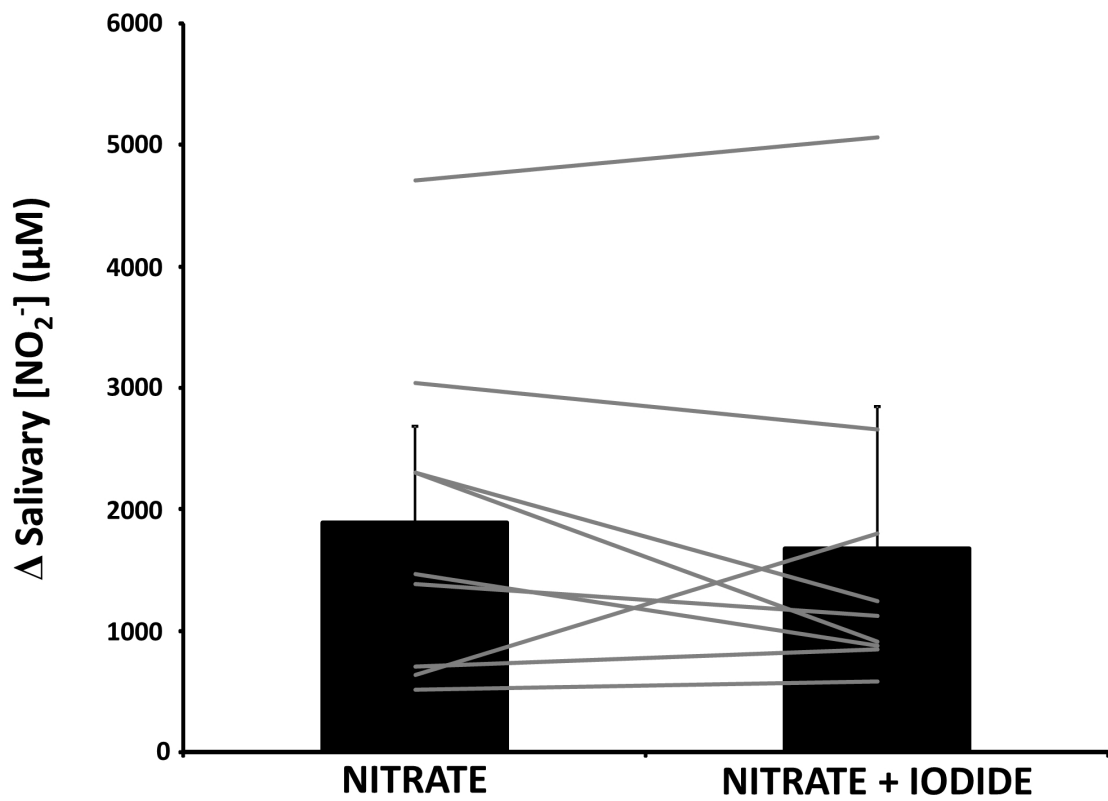
Day 6
2 hours prior to testing:
8.4 mmol nitrate
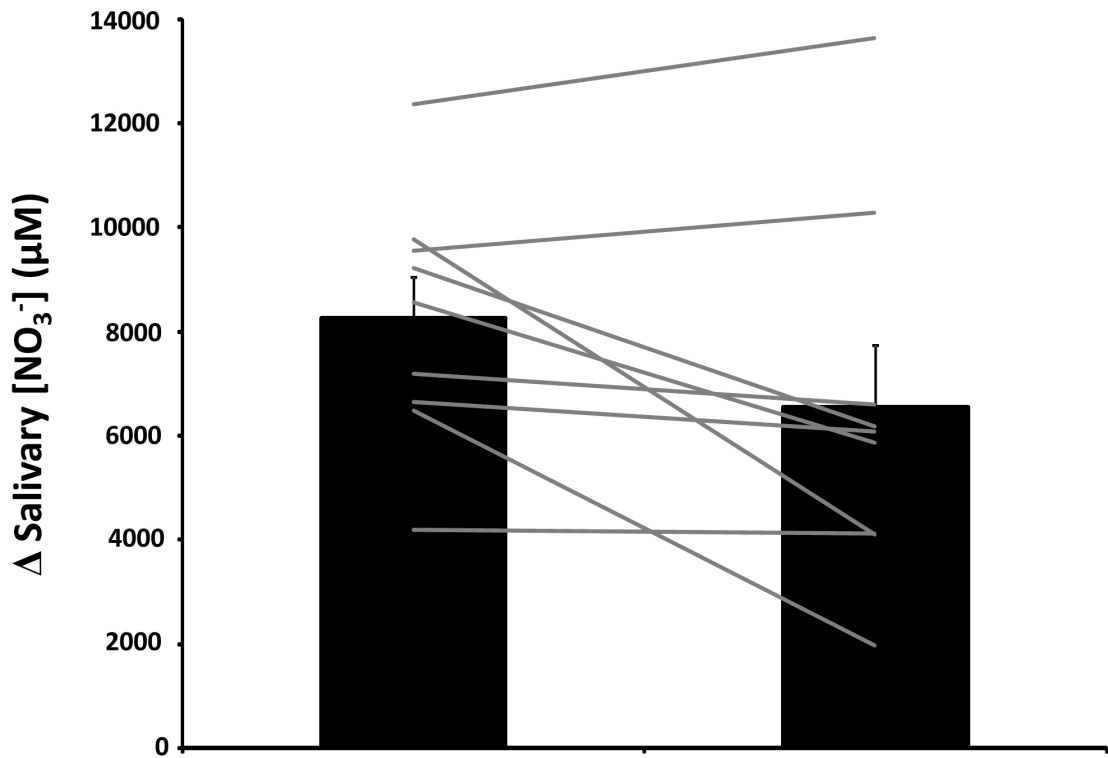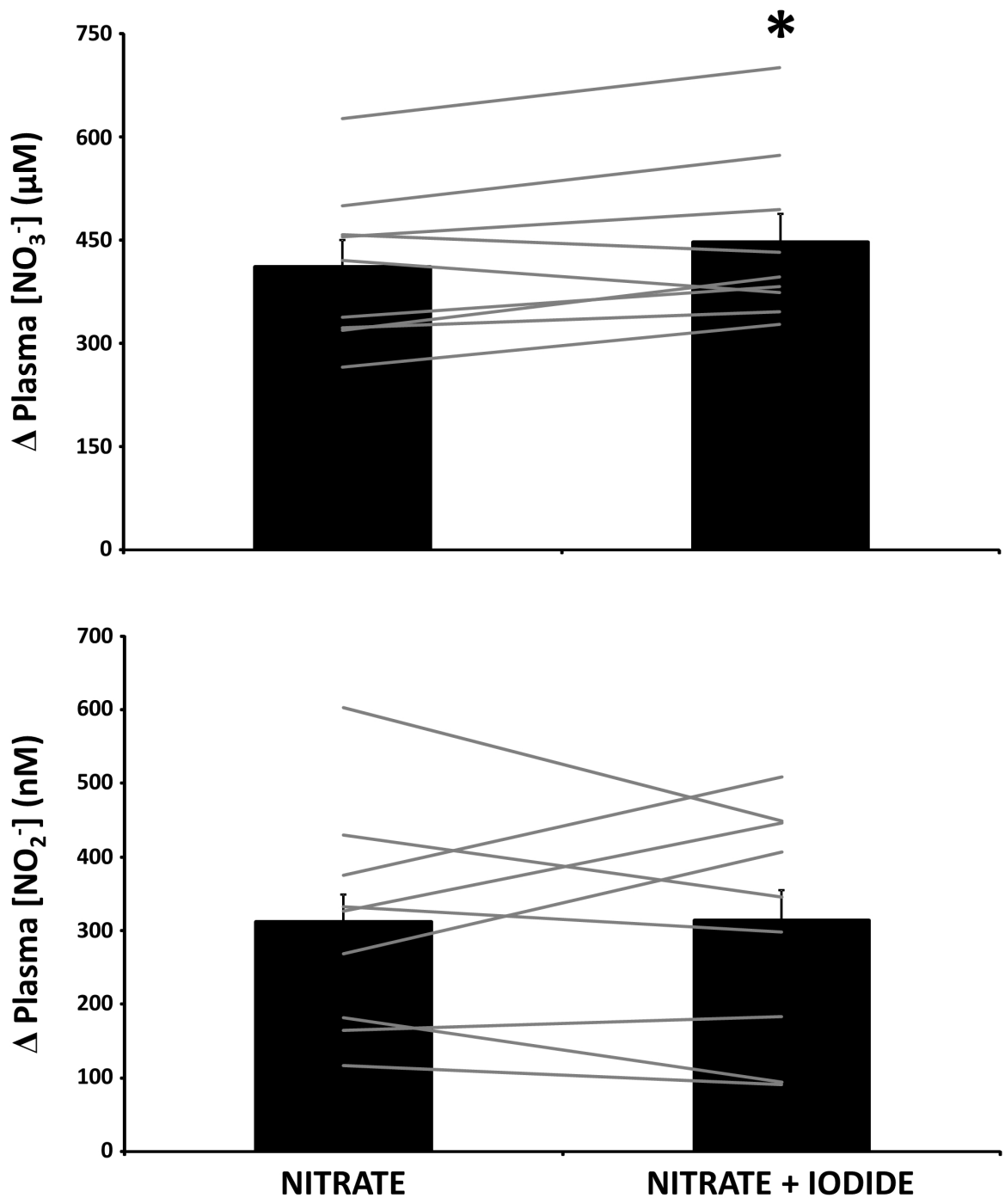450 µg potassium iodide

Figure 1
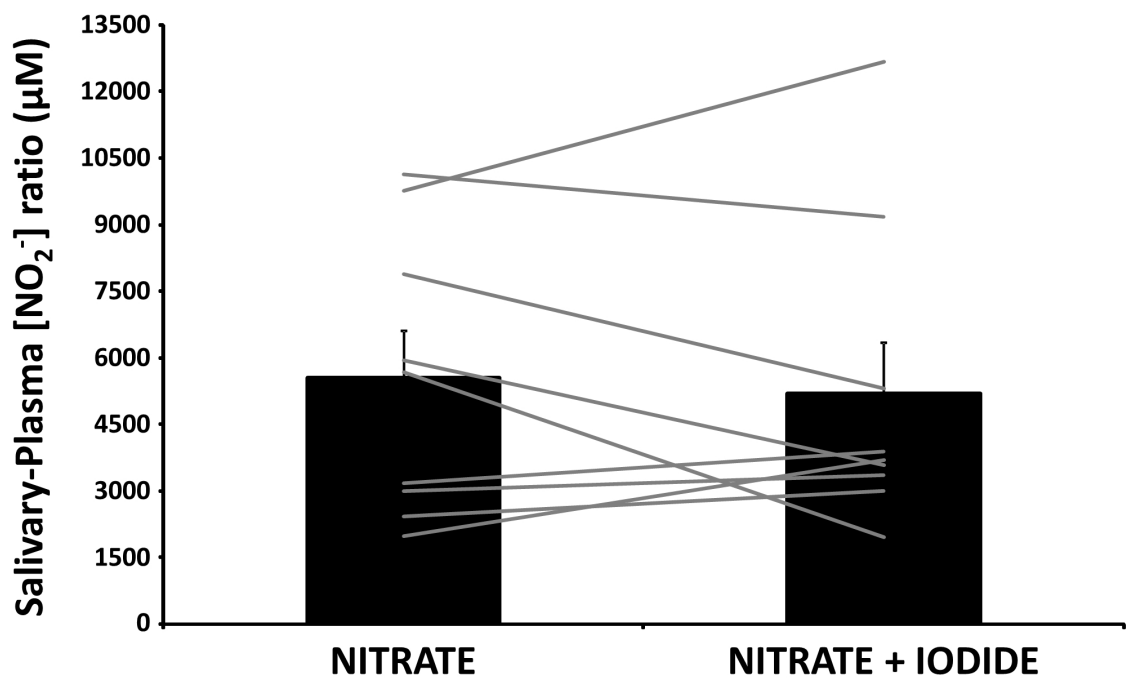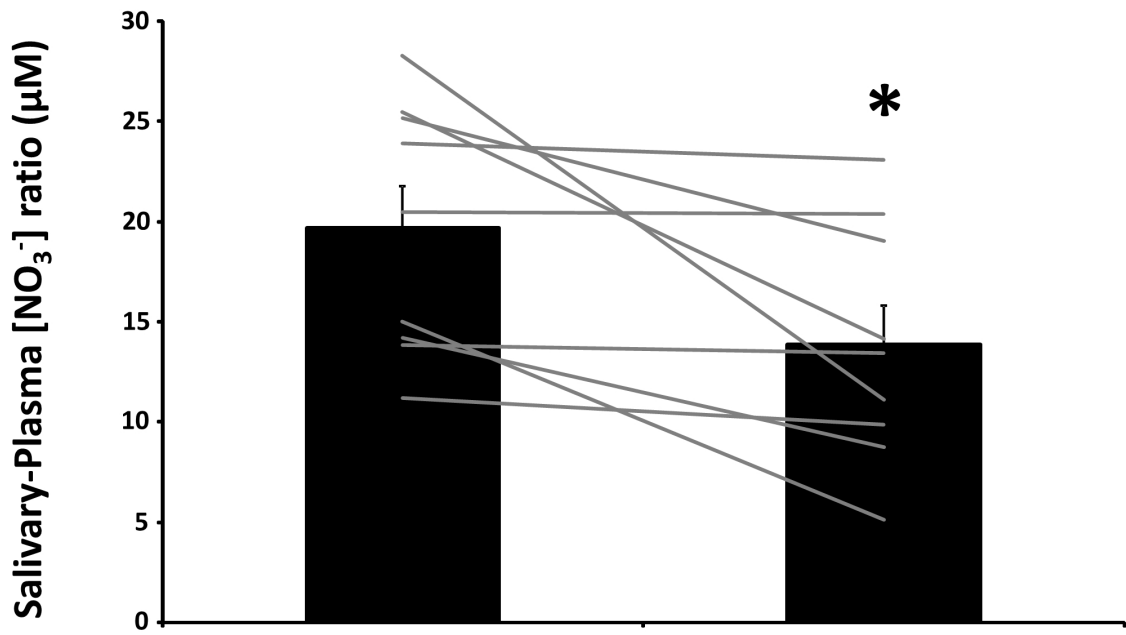
Figure 2

Figure 3

Figure 4

Figure 5

Table 1. Plasma thyroid stimulating hormone ([TSH]) and thyroxine ([T$_4$]) concentrations following no supplementation (CON), nitrate-rich beetroot juice supplementation (NIT) and co-supplementation with nitrate-rich beetroot juice supplementation and potassium iodide (NIT + I).

	CON	NIT	NIT + I
Plasma [TSH] (mIU/L)	1.2 ± 0.6	1.1 ± 0.6	1.5 ± 0.6
Plasma [T$_4$] (µg/dl)	4.2 ± 0.7	3.8 ± 0.4	3.8 ± 0.9

Values are presented as the mean ± SD.

Table 2. Resting supine blood pressure measures following no supplementation (CON), nitrate-rich beetroot juice supplementation (NIT) and co-supplementation with nitrate-rich beetroot juice supplementation and potassium iodide (NIT + I).

	CON	NIT	NIT + I
Systolic blood pressure (mmHg)	112 ± 13	106 ± 13*	106 ± 11*
Diastolic blood pressure (mmHg)	59 ± 6	56 ± 4*	57 ± 5
Mean arterial pressure (mmHg)	80 ± 8	75 ± 5*	76 ± 7*

Values are presented as the mean ± SD. * = significantly different from CON ($P<0.05$).

HIGHLIGHTS

- Inorganic iodide interfered with salivary nitrate uptake
- The change in plasma [nitrite] was not adversely impacted by iodide supplementation
- Nitrate supplementation lowered blood pressure with and without iodide co-ingestion
- Iodide supplementation did not compromise the hypotensive effects of nitrate supplementation