Influence of iodide ingestion on dietary nitrate metabolism and blood pressure following short-term 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^-), but it has yet to be determined whether I^- ingestion compromises NO_3^- metabolism and blood pressure reductions after dietary NO_3^- supplementation. Therefore, this study tested the hypothesis that I^- ingestion would interfere with NO_3^- metabolism and blunt blood pressure reductions after dietary NO_3^- supplementation. Nine healthy adults (mean ± SD, age 20 ± 1 yr, body mass 71 ± 16 kg) reported to the laboratory for initial baseline assessment (CON) 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), and 140 ml·day^{-1} NO_3^--rich beetroot juice and 450 µg potassium iodide·day^{-1} (NIT + I) in a randomized, cross-over experiment. Salivary [I^-] was higher in NIT + I compared to CON and NIT (P<0.05). Salivary and plasma [NO_3^-] and [NO_2^-] were higher in NIT and NIT + I compared to CON (P<0.05). Plasma [NO_3^-] was higher (474 ± 127 vs. 438 ± 117 µM), salivary [NO_3^-] + [NO_2^-] was lower (8582 ± 3450 vs. 10487 ± 2382 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 NIT + I compared to NIT (P<0.05). Plasma and salivary [NO_2^-] were not different between NIT and NIT + I (P>0.05). Systolic blood pressure was lower than CON (112 ± 13 mmHg) in NIT (106 ± 13 mmHg) and NIT + I (106 ± 11 mmHg; P<0.05), with no differences between NIT and NIT + I (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 imposed 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

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 or without iodide co-ingestion
- Iodide supplementation did not compromise the hypotensive effects of nitrate supplementation

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 compliment 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^-) in association with a reduction in blood pressure and arterial stiffness [6-8], important predictors of future cardiovascular events [9,10]. In addition, NO_3^- supplementation can improve vascular function in healthy older adults [11] and some patient populations including peripheral artery disease [12] and heart failure [13] patients. Therefore, increasing dietary NO_3^- intake appears to confer cardioprotective effects and might hold promise as a nutritional intervention to lower the epidemiological and economic burden of cardiovascular diseases on society [14].

Approximately 25% of NO_3^- consumed through the diet passes into the entero-salivary circulation where it is delivered to the mouth for second-pass metabolism [15]. Upon arrival at the oral cavity, microflora on the tongue reduce NO_3^- to nitrite (NO_2^-) [15-19]. After ingestion, NO_2^- is chemically reduced to nitric oxide (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 exert a beneficial effect on the vasculature either through direct NO_2^- action [22,23] or through its subsequent reduction to nitric oxide (NO) via numerous NO_2^- reductatses [24]. While mammalian tissue is capable of reducing NO_3^- to NO_2^- [25], the rate limiting steps for the chemical 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.

Vegetable consumption provides the largest source of dietary NO_3^- intake [30]. A diet rich in fruit and vegetables is accompanied by a lower cardiovascular disease morbidity and incidence of future cardiovascular events [31,32]. This evidence has prompted government initiatives in numerous countries to increase fruit and vegetable consumption, including the Dietary Approaches to Stop Hypertension (DASH) diet in the United States of America [33], the 5-A-Day diet in the United Kingdom [34] and variations of this latter diet in countries within the European Union [35]. However, it has been suggested that the cardioprotective effect of NO_3^--rich vegetables outweighs that afforded by vegetables low in NO_3^- [36-38]. Therefore, while adhering to global initiatives to increase fruit and vegetable consumption affords cardioprotection, incorporating a greater proportion of NO_3^--rich vegetables into the diet appears to hold greater potential as a dietary approach to increase cardioprotection, and is currently being actively encouraged [36,39,40].

We have recently reported that cigarette smoking [41], 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. However, while SCN^- can interfere with dietary NO_3^- metabolism and its lowering of blood pressure, it has yet to be determined whether this is also the case when NO_3^- and I^- are co-ingested. Although I^- has the potential to interfere with salivary NO_3^- uptake (27), and therefore its subsequent reduction to NO_2^- in the oral cavity, this potential perturbation to dietary NO_3^- metabolism might be offset by a compensatory increase in NO generation in the stomach. Indeed, nitrous acid (HNO_2), which is formed from the deprotonation of ingested salivary NO_2^- in the stomach [42], can react with I^- to form NO at an acidic pH [43]. Accordingly, further research is required to assess the extent to which dietary I^- enrichment impacts dietary NO_3^- metabolism and associated vascular health benefits. This is important because I^- is present in numerous food sources, with seafood and dairy products, particularly seaweed, white fish, yogurt and milk, abundant in I^- [44]. 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 [45,46]. These government initiatives have been successful at increasing I^- exposure [46], but it is unclear if this might be to the detriment of dietary NO_3^- metabolism and its beneficial effect on blood pressure.

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 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 [47], subjects were required to refrain from mouthwash use for the duration of the study. Each subject was also asked to avoid consumption of NO_3^--rich and SCN^--rich foods for the duration of the study, and 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 test and for the 5 days preceding this test and to replicate this prior to the subsequent trials. After completing the CON trial, subjects were randomly assigned to receive six days of supplementation with NO_3^--rich beetroot juice and a placebo of potassium gluconate (NIT), or NO_3^--rich beetroot juice and potassium iodide (NIT + I) as part of a double-blind, cross-over experimental design. Over the first five days of supplementation, subjects ingested 4.2 mmol NO_3^- (as 70 ml of concentrated beetroot juice) and a single 99 mg potassium gluconate capsule in the NIT condition, and 4.2 mmol NO_3^- and a single 225 μg potassium iodide capsule in the NIT + I condition, in the morning and

evening. Subjects ingested 140 ml of beetroot juice and two capsules two hours before reporting to the laboratory on day six of NIT and NIT + I 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 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*

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*

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 seconds 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 [T_4] determination*

Plasma thyroid stimulating hormone ([TSH]) and thyroxine ([T_4]) 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, NIT and NIT + I) 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, NIT and NIT + I conditions are illustrated in figure 1. There was a significant main effect for supplement on salivary [I^-] ($P<0.05$), with salivary [I^-] being lower in the NIT condition (384 ± 245 $\mu g \cdot L^{-1}$) compared to the CON (487 ± 305 $\mu g \cdot L^{-1}$) and NIT + I (794 ± 269 $\mu g \cdot L^{-1}$) conditions ($P<0.05$), and higher than both the NIT and CON conditions in the NIT + I condition ($P<0.05$).

3.2 *Plasma [TSH] and [T4]*

Plasma [TSH] and [T_4] responses in the CON, NIT and NIT + I conditions are presented in table 1. There were no difference in [TSH] or [T_4] between the CON, NIT and NIT + I conditions ($P>0.05$).

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

The changes in salivary [NO_3^-], [NO_2^-] and [NOx] in the NIT and NIT + I conditions relative to CON are illustrated in figure 2. There were significantly main effects for supplement for salivary [NO_3^-], [NO_2^-] and [NOx] ($P<0.05$). Salivary [NO_3^-], [NO_2^-] and [NOx] were greater in NIT and NIT + I compared to CON ($P<0.05$). There were no differences between NIT (2168 ± 1302 µM) and NIT + I (1952 ± 1316 µM) for salivary [NO_2^-] ($P>0.05$), there was a trend ($P=0.07$) for a higher salivary [NO_3^-] in NIT (8318 ± 2399 µM) compared to NIT + I (6630 ± 3516 µM) and salivary [NOx] was higher in NIT (10487 ± 2382 µM) compared to NIT + I (8582 ± 3450 µM; $P<0.05$).

3.4 *Plasma [NO_3^-] and [NO_2^-]*

The changes in plasma [NO_3^-] and [NO_2^-] in the NIT and NIT + I conditions relative to CON are illustrated in figure 3. There were significantly main effects for supplement for plasma [NO_3^-] and [NO_2^-] (P<0.05). Plasma [NO_3^-] and [NO_2^-] were greater in NIT and NIT + I compared to CON (P<0.05). Plasma [NO_3^-] was higher in NIT + I (474 ± 127 µM) compared to NIT (438 ± 117 µM; P<0.05), while plasma [NO_2^-] was not different between NIT (404 ± 142 nM) and NIT + I (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 (14 ± 6 µM) compared to NIT (20 ± 6 µM; P<0.05; Figure 4). The salivary-plasma [NO_2^-] ratio was not different between the NIT + I (5184 ± 3481 µM) and NIT conditions (5552 ± 3145 µM; P>0.05; Figure 4).

3.6 *Blood pressure responses*

SBP, DBP and MAP responses in the CON, NIT and NIT + I conditions are presented in table 2. There were significantly main effects for supplement for SBP, DBP and MAP (P<0.05). SBP and MAP were lower than CON in both NIT and NIT + I (P<0.05), while DBP was only lower than CON in NIT (P<0.05; table 2). There were no differences in any of the blood pressure variables between NIT and NIT + I (P>0.05). The change in plasma [NO_2^-] tended to be negatively correlated with the change in SBP between the CON and NIT groups (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^-], lower salivary [NO_3^-] + [NO_2^-] and a lower salivary-plasma [NO_3^-] ratio in the former compared to the latter), 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, 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^- supplementation, 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 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 parallels our previous finding of a lower salivary [SCN^-] after NO_3^-ingestion [41]. 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 [48]. Importantly, although salivary [I^-] was lower in NIT compared to the CON trial, TSH and T4 were not different between the CON, NIT and NIT + I trials. These findings corroborate a previous study which reported no differences in thyroid hormones following short-term NO_3^- supplementation [49]. Therefore, while short-term supplementation with 8 mmol NO_3^- (a dose that could be achieved through ingestion 200-300 g of NO_3^--rich vegetables [36-38]) can lower salivary [I^-], this was not sufficient to perturb thyroid gland function. However, chronic supplementation with > 8 mmol NO_3^- daily is not recommended as blood pressure is not lowered further and exercise capacity is not increased more [8], but there could be a risk of compromising thyroid gland function [50]. 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, 41,47, 51]. 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 [NO_3^-] + [NO_2^-] was 18% lower. The increase in plasma [NO_3^-] and lowering of salivary [NO_3^-] + [NO_2^-] in the NIT + I condition is indicative of an antagonistic effect of I^- on salivary NO_3^- uptake, in line with previous observations [27]. Moreover, the salivary-plasma [NO_3^-] ratio was 30% lower in the NIT + I condition compared to the NIT condition. Therefore, for a given plasma [NO_3^-], salivary NO_3^- uptake was ~ 30% lower when NO_3^- and I^- were co-ingested compared to NO_3^- ingestion alone. Taken together these findings indicate that, compared to NO_3^- ingested independently of I^-, combined dietary supplementation with NO_3^- and I^-

compromised salivary NO_3^- uptake, which is a key rate-limiting step for dietary NO_3^- supplementation in mammals [26]. However, despite a lower salivary NO_3^- uptake, salivary [NO_2^-] was not significantly different between the NIT + I and NIT trials. This observation is similar to our recent study in smokers who exhibited a smaller increase in salivary [NO_3^-], but a similar increase in salivary [NO_2^-], compared to non-smokers after ingesting the same NO_3^- dose (41). 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 [42,43], plasma [NO_2^-] was not different between the NIT + I and NIT trials. Therefore, while salivary NO_3^- uptake was compromised in NIT + I compared to NIT, salivary and plasma [NO_2^-] were similarly increased in NIT and NIT + I. Nevertheless, we cannot exclude the possibility that I^- ingestion might impact salivary NO_3^- uptake, and its subsequent metabolism, at lower NO_3^- doses.

Consistent with numerous previous reports [6,8,37,38,41], SBP was lowered by 6 mmHg in the NIT trial in this study compared to CON. Likewise, a 6 mmHg lowering in SBP was observed in the NIT + I group compared to CON. In keeping with previous reports [8,52], 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 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; 52], or altered renal physiology [53]. Therefore, the similar reduction in SBP in the NIT and NIT + I groups is likely to be a function of a similar increase in plasma [NO_2^-] in these groups. These findings suggest that increasing dietary NO_3^- intake concomitant with increased dietary I^- is unlikely to compromise the increase in circulating plasma [NO_2^-] and associated lowering of blood pressure.

The impetus for increased dietary I^- originated from Switzerland and the USA, where salt iodisation was mandated in the 1920s in an attempt to alleviate hypothyroidism [44]. These initiatives have proved effective at increasing I^- exposure and lowering the incidence of hypothyroidism [46]. Consequently, numerous countries have imposed salt iodisation programmes or the manufacture of certain food products with iodised salt [45,46]. The recommended adequate daily intake for I^- is 150 μg for adult males and females, and 200 μg during pregnancy and lactation [54]. These doses could readily be achieved through a diet

rich in seafood and dairy products [44], but the I^- dose administered in this study (450 $\mu g \cdot day^{-1}$) exceeded the upper range of average daily I^- in the US where iodised salt is mandated [55]. 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 data 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 [36-38].

5. CONCLUSION

In conclusion, concurrent I^- and NO_3^- ingestion lowered salivary NO_3^- uptake, as indicated by a higher plasma [NO_3^-], a lower salivary [NO_3^-] + [NO_2^-], and a lower salivary-plasma [NO_3^-] ratio, compared to the same dose of NO_3^- consumed without I^- co-ingestion. However, in spite of 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 imposed in several countries worldwide through salt iodisation programmes, does not interfere with the lowering of blood pressure after NO_3^- supplementation. 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: Salivary iodide concentration ($[I^-]$) following no dietary supplementation (CON), supplementation with nitrate-rich beetroot juice (NIT) and co-supplementation with NIT and potassium iodide (NIT + I). The filled bars represent the group mean ± SEM responses in the CON, NIT and NIT + I conditions. The solid grey lines represent the individual responses in the CON, NIT and NIT + I conditions. * indicates significantly different from CON and NIT + I. # indicates significantly different from CON and NIT.

Figure 2: Salivary nitrate concentration ($[NO_3^-]$) (upper panel), nitrite concentration ($[NO_2^-]$) (middle panel) and nitrate + nitrite concentration ([NOx]) (lower panel) following supplementation with nitrate-rich beetroot juice (NIT) 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 and NIT + I conditions. * indicates significantly different from NIT.

Figure 3: Plasma nitrate concentration ($[NO_3^-]$) (upper panel) and nitrite concentration ($[NO_2^-]$) (lower panel) following supplementation with nitrate-rich beetroot juice (NIT) 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 and NIT + I conditions. * indicates significantly different from NIT.

Figure 4: Salivary-plasma nitrate $[NO_3^-]$ (upper panel) and nitrite $[NO_2^-]$ (lower panel) ratios following supplementation with nitrate-rich beetroot juice (NIT) and co-supplementation with NIT and potassium iodide (NIT + I). The filled bars represent the group mean ± SEM responses while the solid grey lines represent the individual responses in the NIT and NIT + I conditions. * indicates significantly different from NIT.

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).

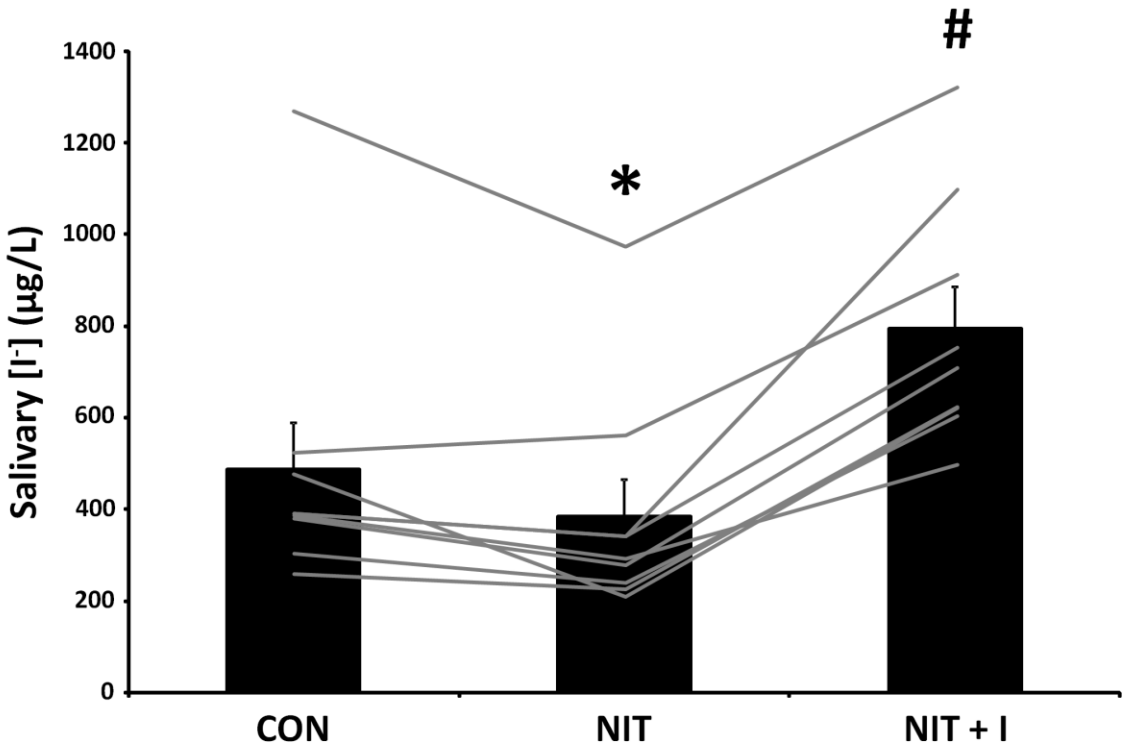

Figure 1

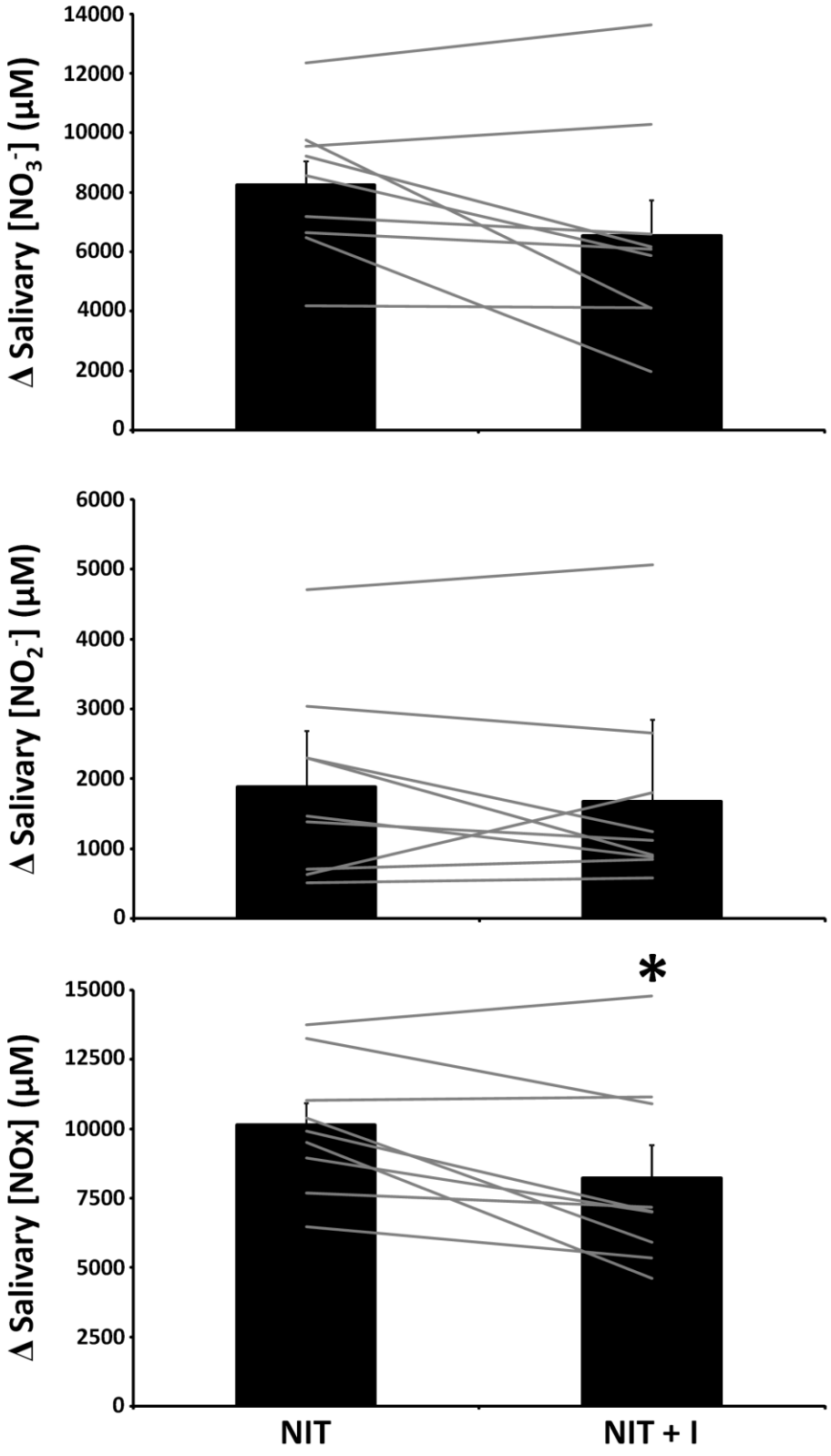
Δ Salivary [NO3-] (µM)
14000
12000
10000
8000
6000
4000
2000
0
Δ Salivary [NO2-] (µM)
6000
5000
4000
3000
2000
1000
0
Δ Salivary [NOx] (µM)
*
15000
12500
10000
7500
5000
2500
0
NIT
NIT + I

Figure 2

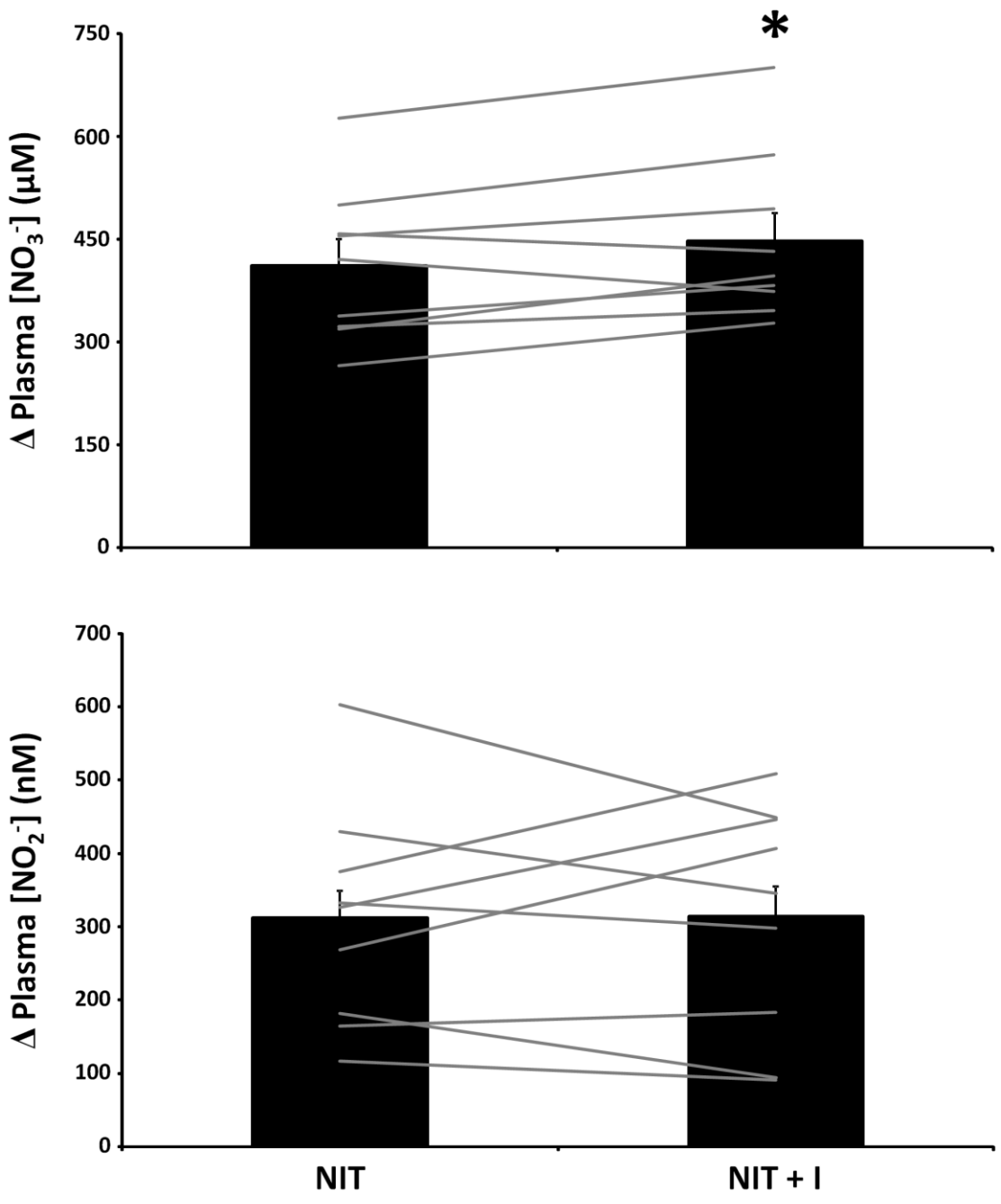
*
750
600
450
300
150
0
Δ Plasma [NO3-] (μM)
700
600
500
400
300
200
100
0
Δ Plasma [NO2-] (nM)
NIT
NIT + I

Figure 3

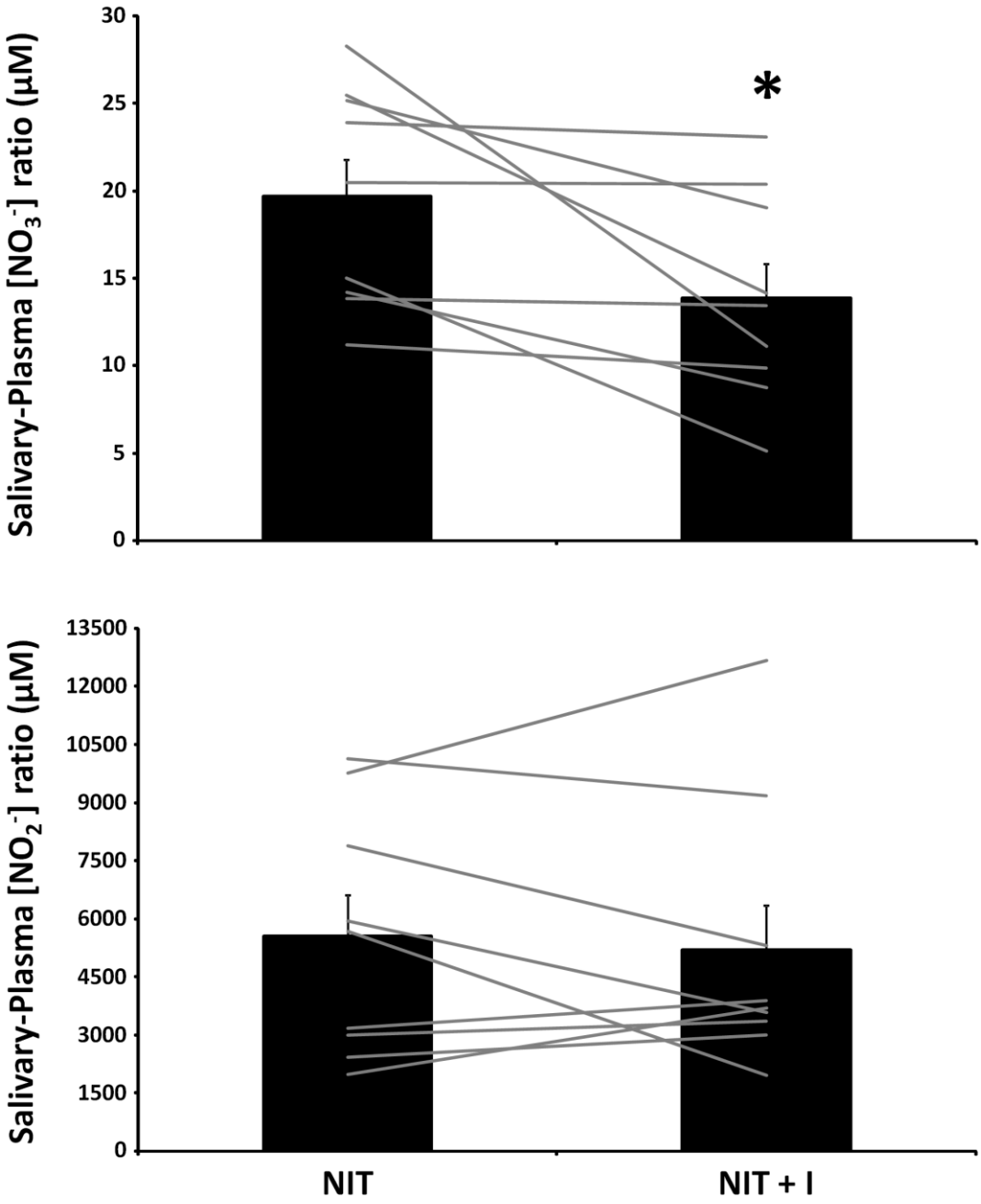
Salivary-Plasma [NO3-] ratio (µM)
30
25
20
15
10
5
0
*
Salivary-Plasma [NO2-] ratio (µM)
13500
12000
10500
9000
7500
6000
4500
3000
1500
0
NIT
NIT + I

Figure 4