Inorganic Nitrate Supplementation Lowers Blood Pressure in Humans
Role for Nitrite-Derived NO


Abstract—Ingestion of dietary (inorganic) nitrate elevates circulating and tissue levels of nitrite via bioconversion in the entero-salivary circulation. In addition, nitrite is a potent vasodilator in humans, an effect thought to underlie the blood pressure–lowering effects of dietary nitrate (in the form of beetroot juice) ingestion. Whether inorganic nitrate underlies these effects and whether the effects of either naturally occurring dietary nitrate or inorganic nitrate supplementation are dose dependent remain uncertain. Using a randomized crossover study design, we show that nitrate supplementation (KNO₃ capsules: 4 versus 12 mmol [n=6] or 24 mmol of KNO₃ (1488 mg of nitrate) versus 24 mmol of KCl [n=20]) or vegetable intake (250 mL of beetroot juice [5.5 mmol nitrate] versus 250 mL of water [n=9]) causes dose-dependent elevation in plasma nitrite concentration and elevation of cGMP concentration with a consequent decrease in blood pressure in healthy volunteers. In addition, post hoc analysis demonstrates a sex difference in sensitivity to nitrate supplementation dependent on resting baseline blood pressure and plasma nitrite concentration, whereby blood pressure is decreased in male volunteers, with higher baseline blood pressure and lower plasma nitrite concentration but not in female volunteers. Our findings demonstrate dose-dependent decreases in blood pressure and vasoprotection after inorganic nitrate ingestion in the form of either supplementation or by dietary ingestion. In addition, our post hoc analyses intimate sex differences in nitrate processing involving the entero-salivary circulation that are likely to be major contributing factors to the lower blood pressures and the vasoprotective phenotype of premenopausal women. (Hypertension. 2010;56:274-281.)

Key Words: clinical science ■ diet ■ NO ■ endothelium ■ blood pressure

Cardiovascular disease (CVD) is the biggest killer worldwide and is likely to increase in proportion as the non-Western world adopts a Western lifestyle (World Health Organization, fact sheet 317, www.who.int). Hypertension is a major risk factor for CVD and is predicted to reach a global prevalence of 30% by 2025. Because blood pressure (BP) remains elevated in ≈50% of all treated hypertensive patients, novel and cost-effective therapeutic strategies are urgently required for the treatment of this condition. In this regard, over the last decade, there has been a major initiative in the Western world to increase the public consumption of vegetables (Department of Health United Kingdom, 5 a day, www.nhs.uk/5aday) in part, as a strategy to prevent CVD. This approach has been taken because epidemiological cohort, and trial-based data demonstrate that increased consumption of a vegetable-rich diet confers protection from CVD, including hypertension. However, the exact mechanisms of the BP-lowering and protective effects of such a diet remain uncertain.

Large-scale clinical trials have failed to show a beneficial cardiovascular effect of several different nutrients found in vegetables, including antioxidant vitamins and folate. More recently, attention has been directed toward other possible elements in vegetables that may have a role, including inorganic nitrate. In 2006, Larsen et al demonstrated that supplementation of healthy volunteers with sodium nitrate resulted in a decrease in diastolic BP (DBP) but not systolic BP (SBP). More recently, we have shown that consumption of beetroot, which is a high nitrate-containing vegetable, also exerts a number of beneficial effects in healthy volunteers, including lowering of both SBP and DBP and protection of the endothelium from ischemia-reperfusion (IR)–induced endothelial damage.

The activity of orally ingested inorganic nitrate is thought to lie in its conversion to nitrite by facultative bacteria found...
on the dorsal surface of the tongue.15 The swallowing of this 
nitrite-rich saliva permits entry of nitrite into the circulation 
via the stomach, and then, once within the circulation, nitrite 
is thought to be converted to the potent vasodilator NO.16–18 
Evidence suggests that this circuit of bioactivation results in 
both vasodilator effects and protection against IR injury.19 
However, whether the beneficial cardiovascular effects of 
beetroot juice are specifically attributable to the dietary 
nitrate content of beetroot and whether the effects of either 
naturally occurring dietary nitrate or inorganic nitrate supple-
mentation are dose-dependent remain uncertain.

Herein, we have investigated whether the effects of dietary 
provision of inorganic nitrate is recapitulated using potassium 
nitrate capsules and whether the effect of inorganic nitrate on 
circulating nitrite/nitrate levels, BP, and endothelial function 
is dose and NO dependent in healthy volunteers.

**Methods**

**Volunteers**

The studies were granted full ethical approval by the local research 
ethics committee. All of the subjects gave informed consent after 
satisfying the inclusion criteria (please see the online Data Supple-
ment at http://hyper.ahajournals.org).

**BP Studies**

Volunteers were entered into 1 of 3 different studies. In the first 
study, 21 subjects were randomized to receive potassium nitrate 
capsules (KNO3; 24 mmol giving 1488 mg of nitrate; Martindale 
Pharmaceuticals) and an equivalent dose of potassium chloride (KCl, 
Martindale Pharmaceuticals) with 500 mL of low nitrate-containing 
water (nitrate: 61.2 ± 1.9 μmol/L; nitrite: 0.20 ± 0.03 μmol/L; Zep-
brook Ltd) in this double-blind, crossover study. In a separate study, 
6 additional individuals were randomized to receive either 4 or 
12 mmol (248 or 744 mg of nitrate, respectively) of KNO3, with 500 
ML of water in an open-label, crossover study. A further randomized, 
open-label, crossover study was performed in 9 healthy subjects to 
investigate dose dependency of the effects of beetroot juice–derived 
nitrate relative to our previous findings where 500 mL of juice were 
administered.14 Volunteers received either 250 mL of beetroot juice 
(James White Drinks Ltd) or 250 mL of water. In all of the groups, 
blood samples were taken and BP determined at baseline and then at 
specific intervals for ≤ 24 hours.

**Flow-Mediated Dilatation Study**

The impact of an IR insult on endothelial function was assessed in 12 
healthy subjects by measuring brachial artery diameter in the 
nondominant arm in response to reactive hyperemia (please see the 
online Data Supplement). Subjects were randomized in a double-
blind (for capsules only) crossover study design to receive either 
24 mmol of KNO3 or KCl with 500 mL of water and, on another 
occaision, 250 mL of beetroot juice 90 minutes before ischemia.

**BP Measurements**

All of the BP and heart rate (HR) measurements were taken in 
triplicate in the seated position using an Omron 715IT before and 
after capsule, beetroot juice, or water ingestion for ≤ 24 hours (please 
see the online Data Supplement).

**Blood Sampling**

Blood samples were taken at baseline; then after capsule, beetroot 
juice, or water ingestion, every 30 minutes up to 3 hours; then in 
some studies hourly from 3 to 6 hours; and then again at 24 hours 
(please see the online Data Supplement).

**Measurement of Plasma Nitrate/Nitrite and cGMP Concentration**

Plasma nitrite and nitrate (NOx) concentration were measured using 
zinc chemiluminescence (please see the online Data Supplement). 
cGMP was determined using an enzyme immunoassay (cGMP EIA 
Biotrak System, GE Healthcare UK Ltd) according to the manufac-
turer’s instructions.

**Statistical Analysis**

The data were analyzed by an individual who was blinded to the 
different interventions, using Graphpad Prism software version 5. All 
of the data are expressed as mean ±SEM, unless otherwise specified. 
For BP measurements and plasma nitrate and nitrite concentration, 
repeated-measures ANOVA was used, with Dunnett posttest for compar-
ison to baseline control and Bonferroni post test for compar-
ison between groups at individual time points. Unpaired t tests 
were used for comparisons of baseline statistics. For flow-mediated 
dilatation and cGMP responses, repeated-measures ANOVA fol-
lowed by Bonferroni posttests for individual group comparisons 
was used. Determinations of correlations between plasma nitrate or 
nitrite concentration with changes in SBP were completed using the 
Pearson correlation coefficient analysis.

**Results**

There were no significant differences in the general charac-
teristics of the individuals recruited for the separate phases of 
the BP study (Table S1, available in the online Data Supple-
ment). Beetroot juice was generally well tolerated by the 
subjects. The nitrate concentration in the beetroot juice was 
22.4 ± 3.8 mmol/L, whereas nitrite concentration was <50 
mmol/L. Capsules were well tolerated in general, although one 
volunteer, who had not taken toast with the capsules, was treated 
for gastritis after consumption of capsules. This individual was 
unblinded and withdrawn from the study. On unblinding, it was 
discovered that gastritis occurred after taking chloride capsules. 
All of the subsequent subjects were made to take toast with the 
capsules, and there were no further adverse effects.

**Dose-Dependent Increases in Circulating NO3−
and NO2− After Oral Inorganic Nitrate 
Capsule Ingestion**

After ingestion of KNO3 capsules (24 mmol), there was a 
rapid (within 30 minutes) increase in circulating plasma 
nitrate concentration, peaking at 3 hours and remaining 
significantly elevated at 24 hours (Figure 1A). In contrast, the 
rise in plasma nitrite concentration was moderate, followed by 
slow time course and significantly raised levels first 
evident at 1.5 hours, plateauing at ≈ 2.5 hours, sustained to 6 
hours, and remaining elevated at 24 hours (Figure 1B). These 
effects of KNO3 were dose dependent with plasma nitrate 
concentration elevated above baseline by ≈ 35−, 27−, and 
7-fold after administration of 24, 12, and 4 mmol of KNO3, 
respectively. The rises in plasma nitrite concentration also 
showed dose dependency, albeit with a more moderate rise of 
a 4.0−, 2.0−, and 1.3-fold increase, respectively (Figure 1).

**Inorganic Nitrate Supplementation Elevates 
Plasma cGMP Concentration**

Plasma cGMP concentration was significantly raised com-
pared with baseline at 3 and 24 hours after ingestion of KNO3 
capsules (24 mmol; Figure 1E).
Dose-Dependent Decreases in BP After Oral Inorganic Nitrate Capsule Ingestion

KNO₃ (24 mmol) ingestion caused reductions in both SBP and DBP over 24 hours compared with KCl control. The peak differences between the 2 limbs were 9.4±1.6 mm Hg (at 6 hours) and 6.0±1.1 mm Hg (at 2.75 hours) for SBP and DBP, respectively (Figure 2A and 2B). There was no significant difference in the HR response between the 2 groups (Figure 0).

Figure 1. Dose-dependent effects of orally administered inorganic nitrate supplementation on plasma NOx. The effects of KNO₃ (24 mmol) and KCl (24 mmol) control capsules on circulating plasma (A) nitrate, (B) nitrite, and (E) cGMP (n=20); and the effects of 4 and 12 mmol of KNO₃ on circulating plasma (C) nitrate and (D) nitrite (n=6). Data are expressed as mean±SEM. Significance shown for comparisons between groups as §§§P<0.001 for 2-way ANOVA; *P<0.01 and ***P<0.001 for Bonferroni post hoc tests; and †P<0.05, ††P<0.01, and †††P<0.001 for 1-way ANOVA followed by Dunnett posttest comparison with baseline (t=0).

Figure 2. Inorganic nitrate supplementation lowers BP. The effects of KNO₃ (24 mmol) and KCl (24 mmol) on (A) SBP and (B) DBP (n=20) and the effects of 4 and 12 mmol of KNO₃ on (C) SBP and (D) DBP (n=6). Data are expressed as mean±SEM. Significance shown for comparisons between groups as §§§P<0.001 for 2-way ANOVA; *P<0.05, **P<0.01, and ***P<0.001 for Bonferroni post hoc tests; and †P<0.05 for 1-way ANOVA followed by Dunnett posttest comparison with baseline (t=0).
S1). The effect of KNO3 on BP was dose dependent (Figure 2C and 2D).

**Changes in SBP Correlate With Baseline BP and Plasma Nitrite But Not Nitrate Concentration**

Post hoc analysis of the KNO3/KCl capsule study demonstrated that the decreases in BP after nitrate ingestion are not correlated with changes in plasma nitrate concentration ($P=0.95$, linear regression; Figure 3A) but are correlated with changes in plasma nitrite concentration ($r=-0.350$; $P<0.001$, linear regression; Figure 3B). In addition, the peak decreases in BP are also correlated negatively with baseline BP (SBP: $r=-0.728$, $P<0.001$; DBP: $r=-0.657$, $P<0.01$; Figure 3C and 3D). Finally, baseline BP is correlated negatively with baseline nitrite ($r=-0.373$; $P<0.05$) but not nitrate ($P=0.93$; Figure 3E and 3F).

**Sex Differences in Responses to Nitrate**

Interestingly, the above post hoc correlations exposed a prominent sex difference in the responses to nitrate. Separation of the KNO3/KCl capsule comparison study data by sex demonstrates that female volunteers had significantly lower baseline SBP, DBP, and body mass index (Table S2) compared with the male volunteers. In addition, whereas baseline plasma nitrate concentration was similar between the sexes, plasma nitrite concentration was significantly higher in the females (Table S2).

Additionally, the rise in plasma nitrate and nitrite concentration in males after KNO3 ingestion appeared significantly lower compared with females (Figure 4A and 4B). However, the fold increases in plasma nitrite concentration from baseline were similar ($\approx 3.3$- and $\approx 4.1$-fold for males and females, respectively). Conversely, KNO3-induced reduction in SBP and DBP was substantially greater in males compared with females (Figure 4C and 4D). There were no significant effects on HR (Figure S1).

No sex differences in the response to KCl with respect to SBP, DBP, or HR were found (Figure S2). The dose of nitrate per kilogram of body weight administered to females was $0.45\pm0.02$ mmol/kg and for males was $0.32\pm0.021$ mmol/kg (see Figure S3 for normalized plasma NOx relative to dose given).

**Inorganic Nitrate Prevents IR-Induced Endothelial Dysfunction**

In addition to the reduction in BP, nitrate capsules prevented IR-induced endothelial dysfunction (Figure S4). Moreover, this effect was not evident after chloride capsule ingestion.

**Dose-Dependent Effects of Beetroot Juice**

After juice ingestion (5.5 mmol nitrate dose), plasma nitrate rose rapidly and remained elevated over the 3-hour time course compared with water control (Figure 5A). Plasma nitrite concentration also increased, peaking at 2.5 hours with a $\approx 1.6$-fold rise above baseline levels and also remaining significantly elevated over the 3-hour time course compared with water control (Figure 5B). In addition, cGMP levels were elevated at 3 hours compared with baseline after beetroot juice ingestion (Figure 5E). Although SBP decreased with a peak reduction of $5.4\pm1.5$ mm Hg (SBP; Figure 5C) and endothelial dysfunction caused by IR injury prevented (Figure S3), there were no significant differences in DBP or HR between the limbs (Figures 5D and S1).

**Discussion**

Determining how vegetables confer protection against CVD and exploiting this to therapeutic advantage are likely to have considerable health and economic implications. Recently, it has been suggested that dietary nitrate found in high levels in vegetables might underlie some of the beneficial effects of vegetable-rich diets. In the present study we have shown that inorganic nitrate capsules or a dietary nitrate load, in the
form of beetroot juice, results in dose-dependent increases in plasma nitrite concentration via bioconversion in vivo. Stieg- 
litz postulated,20 >80 years ago, that the beneficial effects of 
inorganic nitrate (bismuth subnitrate) in hypertensive patients 
were because of conversion to nitrite in vivo, and our findings 
confirm that this bioactive nitrite, after reduction to NO, 
causes dose-dependent decreases in BP and prevents IR-
induced endothelial dysfunction in healthy volunteers.

Ingestion of KNO₃ capsules caused rises in circulating 
plasma nitrate and thence nitrite concentration that were dose 
and time dependent. Significant rises in plasma nitrate con-
centration were evident sooner than nitrite (30 minutes versus

Figure 4. Sex differences in circulating plasma (A) nitrate and (B) nitrite and (C) SBP and (D) DBP after administration of KNO₃ 
(24 mmol) capsules. Data are expressed as mean±SEM of males (n=8) and females (n=12). Significance shown for comparisons 
between groups as §§P<0.01 and §§§P<0.001 for 2-way ANOVA.

Figure 5. Dietary nitrate supplementation with beetroot juice raises plasma nitrite and lowers BP. The effects of beetroot juice (250 mL; 
5.5 mmol of nitrate) or water control on circulating plasma (A) nitrate, (B) nitrite, (E) cGMP, (C) SBP, and (D) DBP. Data are expressed 
as mean±SEM of n=9. Significance shown for comparisons between groups as §P<0.05 and §§§P<0.001 for 2-way ANOVA; 
*P<0.05, **P<0.01, and ***P<0.001 for Bonferroni post hoc tests; and †P<0.05 for paired Student t test.
1.5 hours for 24 mmol of KNO₃ reflecting the use of the entero-salivary pathway and lingual bacterial reduction of nitrate to nitrite.14,21 Approximately similar time courses for changes in both plasma nitrate and nitrite concentration were evident with lower doses of nitrate provided by either KNO₃ capsule or beetroot juice ingestion. Indeed, the dose of nitrate administered via beetroot juice of 5.5 mmol caused fold rises in plasma nitrate and nitrite concentration that fell between the effects of either 12 or 4 mmol provided via nitrate capsule. These findings indicate that, irrespective of source, that is, nitrate salt or in dietary form, the pharmacokinetics of nitrate and nitrite after an oral nitrate load remain largely unchanged and are dose dependent.

KNO₃ capsule ingestion substantially lowered SBP and DBP over 24 hours, whereas a similar dose of KCl did not alter BP over the same time period. These findings suggest that the BP changes were not attributed to the K⁺ content and, more likely, dependent on the endogenous conversion to nitrite and, thence, to NO, because the changes in plasma nitrite correlated closely with reductions in BP. Nitrite, within the realm of physiological concentrations, vasodilates both the arterial and venous sides of the forearm circulation of humans.17,22 and systemic nitrite application decreases BP in both primates18 and humans.17 In the main, it is thought that these effects of nitrite are because of its reduction to NO within the blood vessel wall23,24 and within the red blood cell,17,25 although there is some evidence that nitrite may exert direct effects independent of NO formation.26,27 The effects of inorganic nitrate were found to be dose dependent as reflected by the decreasing magnitude of response in SBP to a 24-, 12-, 5.5-, and 4-mmol dose. Importantly, as with plasma NOx, this dose dependency appeared to hold irrespective of whether the inorganic nitrate load was administered by KNO₃ capsules or beetroot juice. The similarities between the activity of these 2 distinct approaches to inorganic nitrate administration are further reflected by the demonstration that KNO₃ capsules protect against the endothelial dysfunction induced by an IR insult much in the same manner as shown previously for a matched nitrate dose in beetroot juice.14 This latter finding provides further support for our contention that inorganic nitrate underlies the beneficial effects of beetroot juice on the cardiovascular system.

Although it is largely accepted that NO underlies the bioactivity of nitrite, this has not been demonstrated clearly in humans in vivo. In the present study we demonstrate a temporal relationship between the rise in circulating nitrite concentration with a rise in cGMP levels. cGMP is the most sensitive indicator of NO bioactivity,28 and evidence of its elevation provides unequivocal evidence of the generation of bioactive NO.

Post hoc analyses of the KNO₃ capsule data demonstrate that the magnitude of the BP response is directly related to baseline BP (ie, the higher the baseline BP the greater the peak BP reduction achieved). This relationship is consistent with the observation that the effect of BP-lowering drugs in patients is also proportional to resting BP.29 Interestingly, in our cohort, baseline BP was closely correlated with baseline plasma nitrite but not nitrate concentration. Baseline plasma nitrite levels have been proposed to be an accurate reflection of endogenous NO generation via endothelial NO synthase–dependent conversion of l-arginine to NO,30 and our findings may simply be highlighting the known relationship between classic NO synthase–derived NO and BP. However, with the appreciation that nitrite is a bioactive molecule, our findings also support the possibility that the correlation of baseline plasma nitrite with BP is actually a reflection of the functional activity of physiological nitrite reduction as first proposed in 2000 by Gladwin et al.31 This, in turn, raises the possibility that intrinsic plasma nitrite concentration may be involved in “setting” the BP of healthy volunteers. Interpretation of plasma NOx is challenging because of the fact that multiple pathways for the generation and destruction of NOx and NO exist.19 Indeed, changes in plasma nitrite concentration may reflect endothelial NO synthase activity,30 NO oxidation,32 nitrate reduction,14,21 or all 3 at once. Nevertheless, plasma nitrite concentration correlated with BP at baseline and with changes in BP after nitrate supplementation, with corresponding increases in plasma cGMP concentration, suggesting that the measure of plasma nitrite does reflect, at least in part, nitrite bioactivity. Our data also appeared to suggest some clustering of responsiveness to nitrite into 2 groups, that is, although small changes in nitrite (≈<1 μmol/L) effected apparently substantial changes in BP, where the changes in nitrite were >1 μmol/L, little effect on BP was evident. Further post hoc analyses of our data suggest that, indeed, 2 distinct groups of responsiveness to inorganic nitrate dosing exist within our cohort according to sex.

A significant difference in baseline plasma nitrite concentration (but not plasma nitrate) associated with lower baseline BP was evident in our female volunteers compared with the male volunteers. This finding is supportive of the view that a close relationship between nitrite levels and BP exists in humans. This correlation has been demonstrated previously but attributed to differences in vascular endothelial NO synthase expression and activity,33 an effect that, in addition to endothelium-derived hyperpolarizing factor,34 has been proposed to mediate the prevalence of lower BP in premenopausal women compared with age-matched men.35 Our data herein also raise the further possibility that the association of circulating nitrite levels with lower BP evident in premenopausal women may relate, in part, to the bioactivity of the elevated levels of nitrite. This difference in basal nitrite levels may underlie the apparent decreased sensitivity to further elevations in plasma nitrite concentration. Dejam et al18 have demonstrated that, whereas low micromolar concentrations of nitrite produced substantial increases in blood flow in the forearm, a saturation of the vasodilatory effect was observed with higher micromolar levels. It is possible that the apparent lack of effect of nitrite in females relates to a similar “saturation” of its vasodilating effect.

In addition, our analyses intimate sex differences in the endogenous handling of nitrate. Indeed, a similar dietary nitrate load, whilst resulting in only subtle differences in nitrate levels between the sexes, caused a ≈2-fold higher plasma nitrite concentration in females compared with males. These data hint at intriguing sex differences in the processing of NOx. Although differences in absorption and excretion of NOx may underlie some of these differences, it is possible
that the differences in nitrite levels reflect different lingual bacterial loads or species responsible for nitrate reduction to nitrite. Currently, it is thought that the predominant lingual bacteria responsible for nitrate reduction is Gram-negative Veillonella spp and Gram-positive Actinomyces spp.36 Whether sex differences exist in the colonization of the tongue or nitrate reductase activities of these bacteria is currently unknown. It is also possible that the differences in plasma NOx levels simply reflect the differences in body weight between the 2 sexes, which were greater in the females compared with the males. However, normalization of plasma NOx concentrations to body weight did not alter the shape of the profiles seen, and significant differences in plasma nitrite concentration still persisted.

Taking all of the post hoc analyses together, we suggest that these apparent differences in processing of nitrate are likely to contribute to the prevalence of lower baseline BP in women compared with men.35 An important limitation of our findings is that the sex differences were exposed with post hoc analyses. Further investigation in a prospective fashion to corroborate these analyses is clearly warranted. In addition, we did not control for the stage of the menstrual cycle in our findings is that the sex differences were exposed with post hoc analyses. Further investigation in a prospective fashion to corroborate these analyses is clearly warranted. In addition, we did not control for the stage of the menstrual cycle in our female volunteers, and this may have some relevance, because resting BP is different throughout the menstrual cycle.37 Finally, in all of the measures of bioactivity, no significant changes were observed in the control limb using KCl capsules to match the 24-mmol KNO3 dose. The significance of this finding is 2-fold. First, this suggests that the effects on BP were attributable specifically to the activity of nitrate. Secondly, the lack of any BP effect of KCl also supports the view that, whilst potassium (dietary or supplementation38), known to exert a number of beneficial effects on the cardiovascular system, particularly decreases in BP, it is not responsible for the effects of KNO3 supplementation and is unlikely to underlie the effects of beetroot juice.

Perspectives

Although we acknowledge that our studies represent the responses of a healthy volunteer population, our evidence suggests that a dietary nitrate approach to CVD may have therapeutic use. This view is supported by the fact that the dose of 24 mmol administered in this study roughly approximates to the estimated nitrate content (≈20 mmol)39 in the Dietary Approaches to Stop Hypertension diet,9 a diet asso-

Sources of Funding

This work and V.K., A.B.M., and V.P. were funded by a British Heart Foundation Special Project grant.

Disclosures

A.A. and N.B. are directors of Heartbeet Ltd.

References


Acknowledgment

This work was conducted within the Barts & The London NIHR CV BRU.
Inorganic Nitrate Supplementation Lowers Blood Pressure in Humans: Role for Nitrite-Derived NO

Vikas Kapil, Alexandra B. Milsom, Michael Okorie, Sheiva Maleki-Toyserkani, Farihah Akram, Farkhanda Rehman, Shah Arghandawi, Vanessa Pearl, Nigel Benjamin, Stavros Loukogeorgakis, Raymond MacAllister, Adrian J. Hobbs, Andrew J. Webb and Amrita Ahluwalia

Hypertension. 2010;56:274-281; originally published online June 28, 2010; doi: 10.1161/HYPERTENSIONAHA.110.153536

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2010 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/56/2/274

An erratum has been published regarding this article. Please see the attached page for:
http://hyper.ahajournals.org/content/suppl/2010/06/25/HYPERTENSIONAHA.110.153536.DC1
http://hyper.ahajournals.org/content/suppl/2010/06/30/HYPERTENSIONAHA.110.153536.DC2

Data Supplement (unedited) at:
http://hyper.ahajournals.org/content/suppl/2010/06/25/HYPERTENSIONAHA.110.153536.DC1
http://hyper.ahajournals.org/content/suppl/2010/06/30/HYPERTENSIONAHA.110.153536.DC2

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/
In the version of the Hypertension article “Inorganic Nitrate Supplementation Lowers Blood Pressure in Humans: Role for Nitrite-Derived NO” by Kapil et al that was posted online on June 28, 2010 (DOI: 10.1161/HYPERTENSIONAHA.110.153536), an error occurred.

In the y-axis labels for Figures 1B and D, 3D and F, 4B, and 5B, “Nitrate” should be “Nitrite.”

These corrections have been included in the final print version of the article in the August 2010 issue of the journal (Hypertension, 2010;56:274–281) and in the current online version, which is available at http://hyper.ahajournals.org/cgi/content/full/56/2/274. The corrected figures appear below.
Figure 3.

Figure 4.
Hypertension regrets the error.
Correction to: Inorganic Nitrate Supplementation Lowers Blood Pressure in Humans: Role for Nitrite-Derived NO

In the article by Kapil et al, “Inorganic Nitrate Supplementation Lowers Blood Pressure in Humans: Role for Nitrite-Derived NO,” which published online on July 14, 2010, and appeared in the August 2010 issue of the journal (Hypertension. 2010;56:274–281. DOI: 10.1161/HYPERTENSIONAHA.110.153536), a correction is needed.

On page 277, Figure 3, the legend mislabeled figure panels A–D. The legend text that read “Correlation of changes in (A) nitrate and (B) nitrite to changes in SBP, correlation of peak changes in (C) SBP and (D) DBP to baseline SBP,” has been changed to read, “Correlation of peak changes in (A) SBP and (B) DBP to baseline SBP, correlation of changes in (C) nitrate and (D) nitrite to changes in SBP”.

This correction has been made to the current online version of the article, which is available at http://hyper.ahajournals.org/content/56/2/274.
Online supplement

Inorganic nitrate supplementation lowers blood pressure in humans: role for nitrite-derived nitric oxide

Vikas Kapil¹, Alexandra B Milsom¹, Michael Okorie²–³, Sheiva Maleki-Toyserkani⁴, Farihah Akram¹, Farkhanda Rehman¹, Shah Arghandawi¹, Vanessa Pearl¹, Nigel Benjamin⁵, Stavros Loukogeorgakis²–³, Raymond MacAllister², Adrian J Hobbs⁵, Andrew J Webb¹, Amrita Ahluwalia¹

¹Queen Mary University of London, Clinical Pharmacology, William Harvey Research Institute, Barts and The London School of Medicine and Dentistry, UK;
²Centre for Clinical Pharmacology, University College London, UK;
³University of Exeter and Plymouth, Exeter, UK;
⁴Vascular Physiology Unit, Institute of Child Health, University College London, London, UK;
⁵Neuroscience, Physiology and Pharmacology, University College London, UK.
Methods Supplement

Volunteers
The studies were granted full ethical approval by the Local Research Ethics Committee. All subjects gave informed consent after satisfying the inclusion criteria: healthy male or female adult of 18-45 years of age, a BMI of 18-40 kg/m², no systemic medication (other than the oral contraceptive pill) and non-smoker. Patients were instructed to keep to a low nitrate diet (i.e. no processed meat or green leafy vegetables) and to abstain from strenuous exercise on the day preceding the study and were fasted overnight. On arrival, volunteers were provided with a light breakfast (slice of dry wholemeal toast). There were 3 distinct phases of volunteer recruitment, each phase requiring between 2-4 visits with a minimum of 7 days between each visit.

Flow mediated dilatation (FMD)
In a temperature-controlled environment (24-26°C), endothelial function was assessed by measuring brachial artery diameter in response to reactive hyperemia as previously described. A B-mode scan of the brachial artery was obtained in longitudinal section above the antecubital fossa using a 7.0-MHz linear array transducer and a standard Acuson XP10 system (Acuson, Mountain View, California). Arterial diameter over a 1-2-cm section was determined for each image with the use of automatic edge-detection software (Brachial Tools, Iowa City, Iowa). Blood flow was manipulated in the brachial artery by a 7-cm-wide blood pressure cuff placed around the forearm immediately below the antecubital fossa. After 1 min of baseline flow, the cuff was inflated to 300 mm Hg for 5 min and released, resulting in a brief episode of reactive hyperemia. Brachial artery diameter changes in response to blood flow were assessed for a further 5 min. To determine the effect of IR on endothelial function, FMD was assessed before ischemia (induced by inflating a BP cuff placed around the upper part of the arm to a pressure of 200 mmHg for 20 min) and following 20 min reperfusion (achieved by cuff deflation). Brachial artery diameter was measured in millimeters and dilation expressed as percentage increase from baseline diameter.

Blood sampling
A 19-gauge butterfly needle, with extension set, was inserted prior to capsule or juice ingestion, and if required again, at 24h, and secured to skin. Blood samples were taken at baseline and then, following capsule, beetroot juice or water ingestion, every 30 min up to 3h, and then in some studies hourly from 3-6h and then again at 24h. Blood samples were taken atraumatically, via the butterfly needle, into pre-chilled lithium heparin tubes and immediately spun at 1300G at 4°C for 10 min. Plasma was separated and stored at -80°C until measurement of [nitrate] and [nitrite] were undertaken.

Blood pressure measurement
All BP and heart rate (HR) measurements were taken in triplicate in the seated position using an Omron 715IT. Subjects and investigators were blinded to the readings by means of laminated coverings for the machine and printer. The mean of the 2nd and 3rd readings were used for analysis purposes. BP was measured every 15 min for 1h to establish a baseline BP. Following capsule or beetroot juice ingestion BP measurements were taken every 15 min for
3h, then for those studies of duration longer than 3h, hourly for a further 3h and finally at 24h.

**Measurement of plasma nitrate/nitrite**

Prior to ozone chemiluminescence, plasma samples were filtered using Microcon® Ultracel YM-3 (3 kDa) filters (Millipore Corporation, Billerica, USA) and then [nitrate] and [nitrite] in the filtrate determined as previously described. Briefly, samples and standards containing nitrite and nitrate were first reduced to NO, which was then quantified using a NO analyzer (NOA 280, Sievers, Boulder, USA). To determine total [nitrite] and [nitrate] (NOx), samples were added to 0.1 mol/L vanadium (III) chloride in 1M hydrochloric acid refluxing at 90°C under nitrogen. Nitrite concentrations were determined by addition of samples to 1.5 % potassium iodide in glacial acetic acid under nitrogen at room temperature. Concentrations of nitrate were calculated by subtraction of [nitrite] from NOx values.
Reference List


Tables

**Table S1** Volunteer demographics and baseline hemodynamic parameters for the 3 distinct BP studies and FMD study. Data are shown as mean ± SEM values. Baseline BP = mean of readings in 1st hour. ND = not determined.

<table>
<thead>
<tr>
<th>Study</th>
<th>KNO₃ vs. KCl</th>
<th>KNO₃ dose-response</th>
<th>Beetroot juice</th>
<th>FMD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects (n)</td>
<td>20</td>
<td>6</td>
<td>9</td>
<td>12</td>
</tr>
<tr>
<td>Age (years)</td>
<td>22.5±0.9</td>
<td>28.8±1.7</td>
<td>25.1±1.1</td>
<td>24.7±1.4</td>
</tr>
<tr>
<td>BMI (kg/m²) (range 19.6-30.0)</td>
<td>22.5±0.6</td>
<td>24.5±1.6</td>
<td>26.5±0.9</td>
<td>ND</td>
</tr>
<tr>
<td>Baseline SBP (mmHg)</td>
<td>110.1±3.4</td>
<td>114.5±4.6</td>
<td>120.6±4.1</td>
<td>103.6±0.8</td>
</tr>
<tr>
<td>Baseline DBP (mmHg)</td>
<td>70.1±2.3</td>
<td>71.0±2.2</td>
<td>70.9±2.5</td>
<td>62.6±0.7</td>
</tr>
</tbody>
</table>

**Table S2** Sex differences in demographics, baseline hemodynamic characteristics and baseline plasma [nitrate] / [nitrite] for 24mmol inorganic nitrate vs. chloride control study. Significance values for unpaired Student t-test shown in last column.

<table>
<thead>
<tr>
<th>Baseline Characteristics</th>
<th>Male (n=8)</th>
<th>Female (n=12)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>23.0±1.4</td>
<td>22.3±1.2</td>
<td>p=0.70</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.2±1.0</td>
<td>21.4±0.7</td>
<td>p&lt;0.05*</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>126.4±2.5</td>
<td>101.5±2.3</td>
<td>p&lt;0.001***</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>73.3±1.4</td>
<td>66.7±2.2</td>
<td>p&lt;0.05*</td>
</tr>
<tr>
<td>Plasma [Nitrate] µM</td>
<td>35.0±6.9</td>
<td>33.8±7.9</td>
<td>p=0.91</td>
</tr>
<tr>
<td>Plasma [Nitrite] µM</td>
<td>0.362±0.03</td>
<td>0.536±0.05</td>
<td>p&lt;0.01**</td>
</tr>
</tbody>
</table>

Statistical significance shown as * for p<0.05, ** p<0.01 and *** for p<0.001 using unpaired students t-test.
Supplementary Figures
Figure S1
Inorganic or dietary nitrate supplementation does not significantly alter heart rate (HR). The effects on change in HR after administration of (A) 24mmol KNO₃ and KCl (n=20), (B) 4mmol and 12mmol KNO₃ (n=6), (D) beetroot juice (250 ml; 5.5 mmol nitrate) (n=9); and (C) sex-differences in change in HR from baseline after administration of 24mmol KNO₃ (males n=8, females n=12). Data are expressed mean ± SEM. No significant differences between groups following 2-way ANOVA.
Figure S2
Sex differences in change in (A) SBP, (B) DBP and (C) HR from after administration of KCl (24mmol) capsules. Data are expressed as mean ± SEM of males n=8 and females n=12. No significant differences between groups following 2-way ANOVA.
**Figure S3**

Plasma [nitrate]/[nitrite] relative to dose administered following 24mmol KNO₃ administration. Data are expressed mean ± SEM. Significance shown for comparisons between groups as §p<0.05, §§§p<0.001 for 2-way ANOVA.
Figure S4
Inorganic nitrate ingestion protects against IR-induced endothelial dysfunction. FMD (%) after IR injury (control pre- and post-) and after administration of KCl capsules (24mmol), KNO₃ capsules (24mmol) or 250ml beetroot juice (5.5mmol). Data are expressed mean ± SEM of n=12. Significance shown as *p<0.05 and ***p<0.001 for Bonferroni post-hoc tests, following 1-way ANOVA, for comparisons between groups.
Correction

In the version of the *Hypertension* article “Inorganic Nitrate Supplementation Lowers Blood Pressure in Humans: Role for Nitrite-Derived NO” by Kapil et al that was posted online on June 28, 2010 (DOI: 10.1161/HYPERTENSIONAHA.110.153536), an error occurred.

In the $y$-axis labels for Figures 1B and D, 3D and F, 4B, and 5B, “Nitrate” should be “Nitrite.”

The corrections have been made in the current online version and will be made in the final print version of the article in the August 2010 issue of the journal.

*Hypertension* regrets the error.