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Increased nitrate intake from beetroot juice does not alter soluble cellular adhesion molecules and circulating inflammatory cytokines in individuals with treated hypertension: a randomised, controlled trial

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ABSTRACT

Dietary nitrate, found predominantly in green leafy vegetables and other vegetables such as radish, celery, and beetroot, has been shown to beneficially modulate inflammatory processes and immune cell function in animals and healthy individuals. The impact of increased nitrate intake on soluble inflammatory mediators in individuals with hypertension is unclear. We assessed whether the daily consumption of dietary nitrate via beetroot juice for 1-week lowered levels of circulating inflammatory markers in men and women with treated hypertension. Twenty-seven male and female participants were recruited to a randomized, placebo-controlled, double-blind crossover trial. The effects of 1-week intake of nitrate-rich beetroot juice versus 1-week intake of nitrate-depleted beetroot juice (placebo) were investigated. Plasma concentrations of circulating soluble adhesion molecules (ICAM-1, VCAM-1, CD62E, CD62P), inflammatory cytokines (IL-1β, IL-6, IL-10, IL-12p70, TNF-α) and chemokines (IL-8, MCP-1) were measured by multiplex flow cytometric bead array in samples collected on day 7 of each intervention period. Other outcomes included alterations in nitrate metabolism assessed by measuring nitrate and nitrite concentrations in plasma, saliva, and urine. One week of beetroot juice did not alter levels of the soluble adhesion markers or cytokines assessed. A 7-fold increase in salivary nitrite, an 8-fold increase in salivary nitrate, a 3-fold increase in plasma nitrate and nitrite, and a 4-fold increase in urinary nitrate and nitrite compared to placebo was observed ($p<0.001$ for all comparisons). Increasing dietary nitrate consumption over 7 days is not effective in reducing soluble inflammatory mediators in individuals with treated hypertension. This trial was registered at anzctr.org.au as ACTRN 12613000116729.

KEYWORDS: nitrate; nitrite; nitric oxide; beetroot; inflammation

INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of noncommunicable mortality and morbidity globally[1]. The World Health Organization advocates for primary prevention of CVD through lifestyle interventions, with diet being a cornerstone of these strategies[2]. Chronic low-grade inflammation has been identified as a significant contributor to noncommunicable diseases, including CVD[3]. Accumulating evidence strongly suggests that diet composition modulates inflammatory processes, with certain nutrients and plant bioactive compounds being identified as being particularly effective[3]. Thus, dietary modification represents a highly efficacious and cost-effective lifestyle intervention to prevent CVD, potentially mediated through modulation of chronic low-grade inflammation[4]. One promising dietary component that has garnered emerging research interest is nitrate, an inorganic molecule found in abundance in green leafy vegetables and other vegetables such as radish, celery, and beetroot[5-7].

Dietary nitrate is metabolized in humans through the enterosalivary nitrate-nitrite-nitric oxide (NO) pathway[8]. Since nitrite acts as a physiological reservoir for NO in hypoxic conditions, dietary nitrate, metabolized through the nitrate-nitrite-NO pathway, may be an important exogenous source of NO[9]. Nitric oxide is a well-recognised, ubiquitous signaling molecule in mammalian physiology with a myriad of essential functions including vasodilation, platelet inhibition, and immunomodulation[8]. Dietary nitrate ingestion has been shown in studies to have beneficial effects on markers of vascular health in human participants including increased flow-mediated dilatation and decreased blood pressure[10-12]. In the last few years, numerous *in vitro* and animal studies have reported the ability of dietary nitrate to modulate inflammation and the immune system, with human studies now emerging[13]. These anti-inflammatory and immunomodulatory effects may contribute to the cardiovascular health benefits of dietary nitrate and nitrite. Previous studies have described reductions in circulating soluble adhesion molecules (including ICAM-1, VCAM-1, CD62E, and CD62P) and inflammatory biomarkers (including CRP, IL-1β, IL-6, TNF-α) following increases in dietary nitrate levels[11, 12, 14-25]. Furthermore, studies assessing the effects of increases in dietary nitrate and nitrite in both animal and human studies have demonstrated immunomodulatory effects including reduced leucocyte vascular adhesion, reduced leucocyte tissue infiltration, and reduced numbers of pro-inflammatory CD11b+ granulocytes[13, 18, 19, 24-27]. Given the strong evidence for a role of inflammation and immune system activation in the development and aggravation of CVD, especially hypertension, an increased consumption of nitrate-rich vegetables may prove useful in countering the burden of inflammation on CVD[3, 28]. Nevertheless, little is known about the effects of dietary nitrate on inflammatory processes in individuals with hypertension.

The objective of this study was to investigate whether increased dietary nitrate intake for one week lowers soluble cellular adhesion molecules and circulating inflammatory cytokines in men and women with treated hypertension. Dietary nitrate was supplemented in participants via the intake of beetroot juice and compared to a nitrate-depleted beetroot juice. The effect of increased dietary nitrate intake for one week on blood pressure in this study has previously been reported[29].

SUBJECTS AND METHODS

The methods and design of the overall study have been published previously[29] but are described here in brief.

Participants

Men and women, taking between 1 and 3 antihypertensive medications were recruited from the general population in Perth, Western Australia, via newspaper advertisements, between February 2013 and August 2013. These participants were aged between 53 and 70 years; had a body mass index (BMI) 21 kg/m^2 to 34 kg/m^2; had a systolic blood pressure greater than 120 mmHg and less than 160 mmHg; a diastolic blood pressure less than 94 mmHg; were not diabetic; were non-smokers; did not have a history of any major illness such as CVD or cancer; had no change of antihypertensive medication within the previous month; did not use more than 3 antihypertensive medications (one antihypertensive medication, n = 8; two antihypertensive medications, n = 14; three antihypertensive medications, n = 5); and had not used antibiotic medication within the previous month.

Trial design

This study was a randomized, placebo-controlled, double-blind crossover study, with two 1-week intervention periods, conducted over a total of 5-weeks for each participant. A low-nitrate background diet was adhered to by all participants for the entire 5-week study period. This 5-week period was separated into a 1-week lead-in period, a 1-week intervention period, a 2-week washout period, followed by a second 1-week intervention period. Medication and lifestyle factors (including physical activity and alcohol intake) were not altered throughout the 5-week study period. Each participant completed a total of 4 visits to the University of Western Australia, School of Medicine and Pharmacology, located at the Royal Perth Hospital Research Foundation. These visits were scheduled at day 0 (start of intervention) and day 7 (end of the intervention) for each of the two intervention periods. Saliva, fasting plasma, and a 24-hour urine collection were obtained on day 7 of each intervention period. Blood samples were collected into tubes containing EDTA (BD Vacutainer® K2 EDTA Tube) and immediately centrifuged (3000 x g; 15 min, 4°C). The plasma was stored at -80°C until measurement.

This study was conducted in accordance with the Declaration of Helsinki of 1975 and approved by the University of Western Australia Human Research Ethics Committee. All participants provided written consent prior to inclusion into the study. This study was registered with the Australian New Zealand Clinical Trials Registry as ACTRN 12613000116729.

Interventions

The interventions for this study were an active (nitrate) intervention and a control (placebo) intervention, both on a background of a low-nitrate diet and an unaltered lifestyle. The active intervention consisted of 2 x 70 ml nitrate-rich beetroot juice, with 70 mL consumed with breakfast and 70 ml consumed with dinner (Beet It; James White Drinks, Ltd., Ipswich, UK). The total nitrate intake from each 70 ml nitrate-rich beetroot juice was 217 mg/day (range: 161 – 273 mg/day), thus participants increased their nitrate intake by 322 – 546 mg/day. The control intervention consisted of 2 x 70 ml nitrate-depleted beetroot juice, with 70 mL consumed with breakfast and 70 mL consumed with dinner (nitrate-depleted beetroot juice prepared and supplied by Beet It; James White Drinks, Ltd., Ipswich, UK). The total nitrate intake from each 70 ml nitrate-depleted beetroot juice was 21 mg/day (range: 3 – 35 mg/day), thus participants increased their nitrate intake by 6 - 70 mg/day.

Low nitrate background diet

A list of foods to limit or avoid were given to each participant. Participants were directed to avoid intake of beetroot and green leafy vegetables high in nitrate (including lettuce, celery, spinach, Chinese greens, other leafy greens, parsley, and related herbs).

Biochemical analyses

Nitrate and nitrite analysis

Concentrations of nitrate and nitrite in saliva, plasma and urine samples were determined by gas chromatography-mass spectrometry (GC-MS) using [^{15}N] sodium nitrate and [^{15}N] sodium nitrite as internal standards as previously described[30].

Soluble cellular adhesion molecule and circulating inflammatory cytokine analysis

Cytometric bead array was used to determine concentrations of ICAM-1, VCAM-1, P-selectin, E-selectin, IL-1β, IL-6, IL-8, TNF-α, IL-10 and IL12-p70 in plasma samples collected from participants during each phase of the study (Human Inflammatory Cytokine kit and assorted flex sets, BD Biosciences, San Jose, CA). Briefly, analyte-specific capture beads were combined to form a multiplex assay mixture. Plasma samples and standards were then added to the analyte-specific beads with distinct fluorescence intensity for allophycocyanin (APC) and allophycocyanin-Cy7 (APC-Cy7). Following incubation of plasma and standards with the analyte-specific capture beads (1 hour), samples were mixed with phycoerythrin(PE)-conjugated detection antibodies and incubated for an additional 2 hours. Plasma was diluted 1:4 for all assays except for the analysis of plasma levels of ICAM-1 and VCAM-1, the latter both of which were analysed at a plasma dilution 1:200. After incubation and washing, sample data was acquired using an Attune NxT flow cytometer (Thermo Fisher Scientific). APC and APC-Cy7 were used to separate and identify bead populations for each analyte, while PE fluorescence was measured to calculate analyte concentration from an analyte-specific standard curve. Data was analysed using FCAP Array III Software (BD Biosciences). Limits of detection for IL-8, IL-1β, IL-6, TNF-α, IL-10 and IL12-p70 were 3.6, 7.2, 2.5, 3.3, 3.7, and 1.9 pg/ml respectively.

Other biochemical analyses

A series of biochemical analyses were performed at the PathWest commercial pathology laboratory at Royal Perth Hospital, Perth, Western Australia on samples collected during screening appointments. Total serum cholesterol, HDL cholesterol, triglycerides and serum glucose were measured using fully automated, routine protocols (Roche Hitachi 917, Roche Diagnostics Australia Pty. Ltd., Castle Hill, New South Wales, Australia). LDL cholesterol concentrations were calculated using the Friedewald formula[31].

Statistics

Baseline participant characteristics are presented as mean ± SDs and ranges. Non-normally distributed values are presented as median [interquartile range]. Treatment effects for post-intervention outcomes were obtained using linear mixed models with adjustment for treatment order with the subject identifier included as a random intercept. For outcomes with values below the lower limit of detection, treatment effects were analysed using a tobit model with the ‘censReg’ R package and the ‘BHHH’ method with the subject identifier included as a random intercept[32].

Statistical analyses were performed using IBM SPSS Statistics for Windows, version 25 (IBM), STATA/IC 15.1 (StataCorp LLC) and R statistics (R Core Team, 2021)[33].

RESULTS

Baseline and descriptive data

Of the 93 volunteers screened for the study, 17 women and 10 men (n = 27 total) were recruited, with all participants completing the trial (**Figure 1**). Baseline demographic and clinical data for all participants are shown in **Table 1**.

Saliva, plasma and urinary nitrate and nitrite

Compared to the placebo group, plasma, salivary and urinary nitrate levels were significantly higher ($P < 0.001$ for all) after the nitrate intervention [β (95% CI); plasma: 87.3 μmol/l (69.2, 105.3); saliva: 1984.0 μmol/ l (1605.1, 2362.9); urine: 1079.9 μmol/ l (912.8, 1246.9)]. Similarly, plasma, salivary and urinary nitrite levels were also significantly higher ($P < 0.001$ for all) after the nitrate intervention [β (95% CI); plasma: 3.8 μmol/ l (2.9, 4.8); saliva: 752.4 μmol/ l (643.1, 861.7); urine: 383.9 μmol/ l (326.3, 441.5)]. These results are presented graphically in **Figure 2** with complete results presented in[29].

Soluble cellular adhesion molecules, circulating cytokine and chemokines

There were no significant differences observed between the intervention groups in measurements of the soluble cellular adhesion molecules ICAM-1, VCAM-1, P-selectin, and E-selectin (**Table 2**, **Figure 3**). There were no significant differences in the circulating cytokines IL-1β, IL-6, TNF-α, IL-10 or IL12-p70 (Table 2, **Figure 4**). There were no significant differences observed in the chemokines MCP-1 or IL-8 between the intervention groups (Table 2, Figures 3 and 4). The outliers depicted in Figure 4 are from 2 different participants with one participant demonstrating increases in IL-6 and IL-8, whilst the other participant demonstrated marked increases in IL-1β and TNF-α.

DISCUSSION

In this 1-week randomised, placebo-controlled, double-blind crossover study in men and women with hypertension, we observed a significant increase in saliva, plasma and urine nitrate and nitrite after the nitrate-rich beetroot juice (~434 mg/day nitrate from beetroot juice on a background low nitrate diet) compared to the nitrate-depleted beetroot juice. This confirms that the increased nitrate intake was effective in increasing endogenous nitrate and nitrite levels through the enterosalivary nitrate-nitrite-NO pathway. Despite observed increases in circulating nitrate/nitrite, no significant change in soluble cellular adhesion molecules and circulating inflammatory cytokines were observed.

The concept of inflammation as a contributor to hypertension is supported by numerous epidemiological, animal, and human studies[28, 34, 35]. Of interest is the low-grade chronic inflammatory state associated with ageing and many non-communicable diseases including metabolic syndrome and CVD[3, 7]. The exact mechanism behind the link between inflammation and hypertension is yet to be fully elucidated, however evidence exists for a role of endothelial dysfunction, hyperactive sympathetic nervous dysregulation, and inflammatory cell infiltration into the renal tubulointerstitial space[7, 28]. Multiple studies have described raised levels of certain inflammatory biomarkers in individuals with hypertension as compared to healthy populations[36-40]. While values have been reported for other disease populations, including CVD and hypertension, pathological threshold levels have yet to be defined. Reference ranges for inflammatory biomarkers from healthy populations have been described[41].

In the present study, the plasma levels of soluble adhesion markers ICAM-1 (~170 – 180 ng/ml) and VCAM (~590 – 605 ng/ml) were similar to those recorded in other studies in patients with hypertension (ICAM-1: 235 – 315 ng/ml; VCAM: 327 – 684 ng/ml)[37-39, 42]. Other studies that have compared cytokines in individuals with hypertension to normotensive controls report up to ~1.2 fold and ~1.1-fold higher levels respectively[37-39]. Soluble P-selectin (~33-35 ng/ml) and E-selectin (~11 ng/ml) plasma levels observed in the present study are slightly lower than those previously reported for healthy individuals (P-selectin: 50-60 ng/ml; E-selectin: 43-80 ng/ml)[41] and individuals with hypertension (P-selectin: ~157 -169 ng/ml; E-selectin: 30-40 ng/ml)[39, 42]. Monocyte chemoattractant protein-1 (MCP-1) levels (~0.04 ng/ml) were lower than those published in other studies (0.09-0.4 ng/ml)[37, 38, 41, 42]. While not detectable in all individuals, changes in circulating inflammatory cytokines between individuals were observed. Additionally, where detected, levels were within ranges previously reported (IL-8) or higher than values reported for individuals with a similar age (IL-1β, IL-6, IL-10, IL-12p70, TNFα)[43]. However, it should be noted that there is no current agreement on the inflammatory states (acute, chronic, or low-grade inflammation) that biomarkers of inflammation represent[41]. Additionally, there are several factors that modify the concentration of inflammatory biomarkers including age, diet, sex, body fat, physical activity, genetics, and gut microbiota composition[41]. Furthermore, due to different technologies used to quantitate these biomarkers, sample preparation and storage variations, comparing levels across different studies is difficult. In particular, the accurate measure of blood cytokines is recognised to be problematic[44]. It is considered more informative to look at change in concentration of inflammatory biomarkers in response to a challenge as opposed to basal levels[41].

A number of studies in both animal and humans (predominantly healthy individuals) have shown that dietary nitrate beneficially modulates soluble inflammatory markers, phenotypic and functional characteristics of circulating leukocytes, leukocyte–vasculature interactions, and leukocyte–platelet interactions[7-9, 45]. In animal models, water with added nitrate or nitrite has been shown to reduce levels of circulating, and cellular expression of, inflammatory biomarkers including CRP, IL-1β, IL-6, TNF-α[15, 19, 20, 22-25, 46]. The doses required for these anti-inflammatory effects are between 15 and 73 mg/l of nitrate-infused water, and between 33.5 and 100.5 mg/l of nitrite-infused water[15, 18, 22-25, 46]. Anti-inflammatory effects in healthy mice have also been noted with spinach-derived nitrate ingestion of between 15 to 60 mg/kg of nitrate[20]. In human participants, few *in vivo* studies have investigated the effects of dietary nitrate on inflammatory markers, and the results have been mixed. Doses of 426 mg per day of dietary nitrate have been shown to reduce the vascular adhesion markers E-Selectin and P-Selectin in obese patients without significant changes to endothelial function[47]. In patients with prehypertension, beetroot juice made from 250 g of either raw or cooked beetroot reduced blood pressure, improved vascular flow-mediated dilation and reduced levels of ICAM-1, VCAM-1, E-selectin, IL-6, hsCRP, and TNF-α[48]. Conversely, Velmurugan and colleagues showed that 375 mg per day of dietary nitrate from beetroot juice improved vascular flow-mediated dilation and reduced platelet-monocyte aggregates without changes to the serum hsCRP[26] .

We hypothesised that there may be a difference in circulating inflammatory markers after nitrate intake indicating a less inflammatory environment which occurs independently of blood pressure changes. Lower levels of adhesion markers have been demonstrated after nitrate ingestion without concurrent improvements in endothelial function[47.] There are several possible reasons why no effect on inflammatory biomarkers were observed in the current study. The participants in the study were taking between 1 and 3 medications for hypertension. It is unclear whether these medications influence levels of inflammatory markers. Another possible explanation is the length of the nitrate intervention. The chronicity of low-grade inflammation is likely a contributor to endothelial dysfunction and therefore reductions in inflammatory markers that are sustained over longer periods of time are more likely to offer more clinical benefit[3]. Whilst changes to immune cells and inflammatory markers have been noted in multiple acute intervention studies[13, 47, 48], only one human study thus far has investigated the effects of dietary nitrate on inflammatory indicators in longer intervention periods of a 6-week duration[26]. Future studies may consider including temporality as an independent variable in the study design.

In conclusion, we observed that ingestion of nitrate-rich beetroot juice regularly for 1 week had no effect on soluble inflammatory mediators in individuals with treated hypertension. Given the evidence in the literature contrary to these findings, clinical trials of longer duration, with measurements at more frequent time points and observational studies looking at associations of habitual nitrate with markers of inflammation are required.

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Statement of contributions

Author contribution's to manuscript

1. **Designed research (project conception, development of overall research plan, and study oversight):** KR, AHL, EK, VM, KDC, RJW, JMH, CPB

2. **Conducted research (hands-on conduct of the experiments and data collection):** HK, AHL, KDC, JMH, CPB

3. **Analyzed data or performed statistical analysis:** NPB, AHL, KM, JMH, CPB

4. **Wrote manuscript:** KR, CPB

5. **Contributed to manuscript revisions:** KR, AHL, HK, EB, NPB, VM, MS, LB, RJW, KM, KDC, ON, JMH, CPB

6. **Had primary responsibility for final content:** KR, CPB

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Figure captions

Figure 1: Consort flow diagram showing participant flow from recruitment through screening and randomisation to trial completion.

Figure 2: Post-intervention concentrations of (A) plasma nitrite, (B) plasma nitrate, (C) salivary nitrite, (D) salivary nitrate, (E) urinary nitrite and (F) urinary nitrate for the placebo and nitrate interventions.

Figure 3: Plasma levels of the soluble cellular adhesion molecules (A) ICAM-1, (B) VCAM-1, (C) E-selectin, (D) P-selectin, and the chemoattractant (E) MCP-1 in 27 men and women with treated hypertension. P-values for treatment effects were obtained using linear mixed models, with no significant difference between nitrate treatment and placebo observed for any outcome (P>0.1 for all).

Figure 4: Plasma levels of the circulating inflammatory cytokines (A) IL-1β, (B) IL-6, (C) IL-8, (D) IL-10, (E) IL-12p70, and (F) TNF-α in 27 men and women with treated hypertension. P-values for treatment effects were obtained using tobit models accounting for values below the lower limit of detection, with no significant difference between nitrate treatment and placebo observed for any outcome (P>0.1 for all).

Table 1. Baseline characteristics of 27 men and women with treated hypertension (males n=10; females n=17) according to intervention order.

	Placebo - Nitrate (n=13)		Nitrate - Placebo (n=14)	
	Mean ± SD	**Range**	**Mean ± SD**	**Range**
Age (years)	64 ± 3	55 - 69	62 ± 5	53 - 70
Height (cm)	169 ± 8	155 - 180	171 ± 9	156 - 184
Weight (kg)	78 ± 8	64 - 91	78 ± 13	57 - 97
Body mass index (kg/m^2)	27 ± 3	25 - 34	27 ± 4	21 - 33
Systolic blood pressure (mm Hg)	132 ± 11	119 - 150	134 ± 12	119 - 160
Diastolic blood pressure (mm Hg)	76 ± 9	67 - 94	76 ± 12	48 - 93
Heart rate (bpm)	63 ± 12	48 - 96	65 ± 6	56 - 74
Total cholesterol (mmol/l)	5.3 ± 0.8	4.0 – 6.7	5.1 ± 0.9	3.8 – 6.6
LDL cholesterol (mmol/l)	3.5 ± 0.8	2.4 – 4.8	3.4 ± 0.7	2.1 – 4.8
HDL cholesterol (mmol/l)	1.4 ± 0.2	1.2 – 1.7	1.2 ± 0.3	0.7 – 1.8
Triglycerides (mmol/l)	1.1 ± 0.3	0.6 – 1.6	1.2 ± 0.6	0.5 – 2.7
Glucose (mmol/l)	5.6 ± 0.3	5.0 – 6.2	5.4 ± 0.4	4.7 – 6.0

Table 2. Plasma levels of the soluble cellular adhesion molecules, circulating cytokine and chemokines in 27 men and women with treated hypertension.

	Placebo[a]	Nitrate[a]	Estimated treatment effect (95% CI)	P-value
ICAM-1 ng/ml	169.2 [144.4 – 207.3]	179.7 [144.3 – 211.2]	10.3 (-4.3, 25.0)	0.17
VCAM-1[b] ng/ml	591.6 [486.6 – 674.4]	604.0 [475.2 – 641.7]	27.3 (-41.0, 95.6)	0.43
E-selectin ng/ml	11.3 [9.9 – 12.8]	11.3 [9.5 – 12.6]	0.1 (-0.1, 0.3)	0.44
P-selectin ng/ml	33.4 [28.6 – 37.2]	34.6 [30.3 – 37.0]	0.8 (-0.1, 1.6)	0.09
MCP-1 ng/ml	0.04 [0.02 – 0.06]	0.03 [0.02 – 0.06]	0.01 (-0.01, 0.03)	0.27
IL-1β [c] pg/ml	7.2 [7.2 – 7.2]	7.2 [7.2 – 10.5]	-44.3 (-102.1, 13.5)	0.13
IL-6[c] pg/ml	2.5 [2.5 – 2.5]	2.5 [2.5 – 3.5]	-4.4 (-13.3, 4.5)	0.34
IL-8[c] pg/ml	3.6 [3.6 – 6.3]	3.6 [3.6 – 11.6]	-11.6 (-36.3, 13.1)	0.36
IL-10[c] pg/ml	3.3 [3.3 – 4.6]	3.3 [3.3 – 6.6]	-4.6 (-10.9, 1.7)	0.15
IL-12p70 pg/ml	15.3 [7.9 – 27.7]	26.9 [4.9 – 37.2]	-7.4 (-16.1, 1.3)	0.09
TNFα[c] pg/ml	3.7 [3.7 – 3.7]	3.7 [3.7 – 3.7]	-12.0 (-86.1, 62.1)	0.75

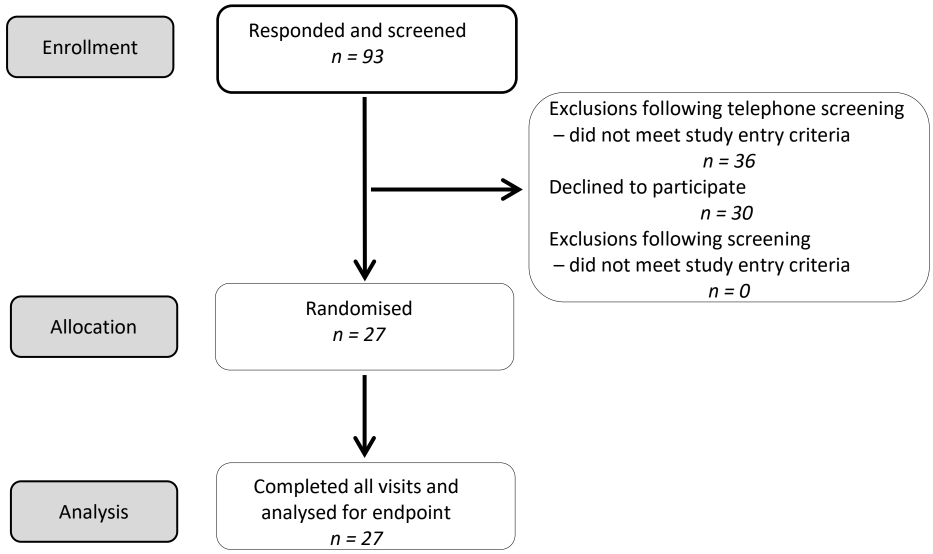
Estimated treatment effects were obtained using linear mixed models (ICAM, VCAM, E-selectin, P-selectin and MCP-1) and tobit models (IL-1β, IL-6, IL-8, IL-10, IL-12p70 and TNFα).

[a]Median [IQR]

[b]Outlier removed

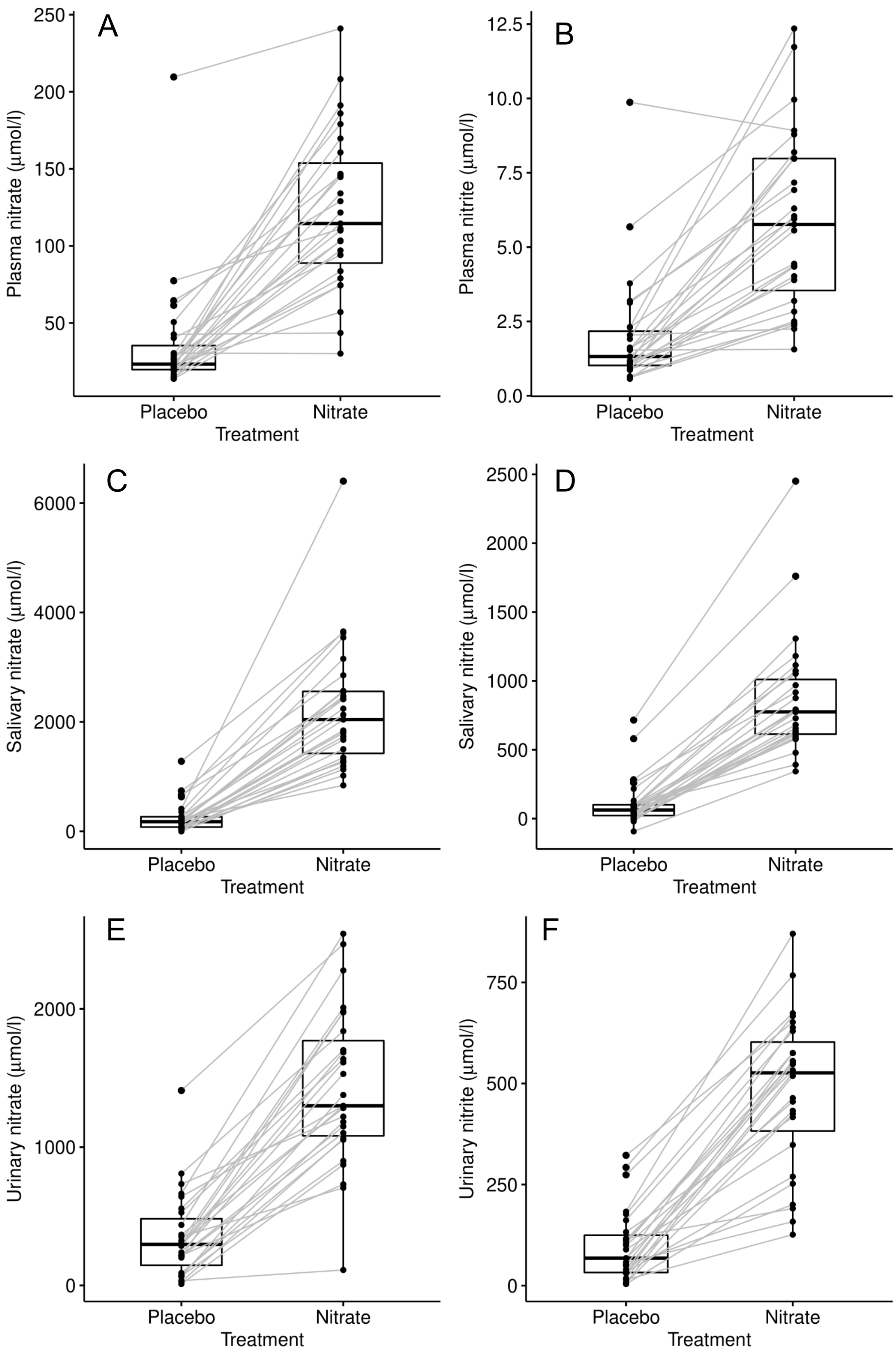
[c]p25 and median are the lower limit of detection

Figure 1.



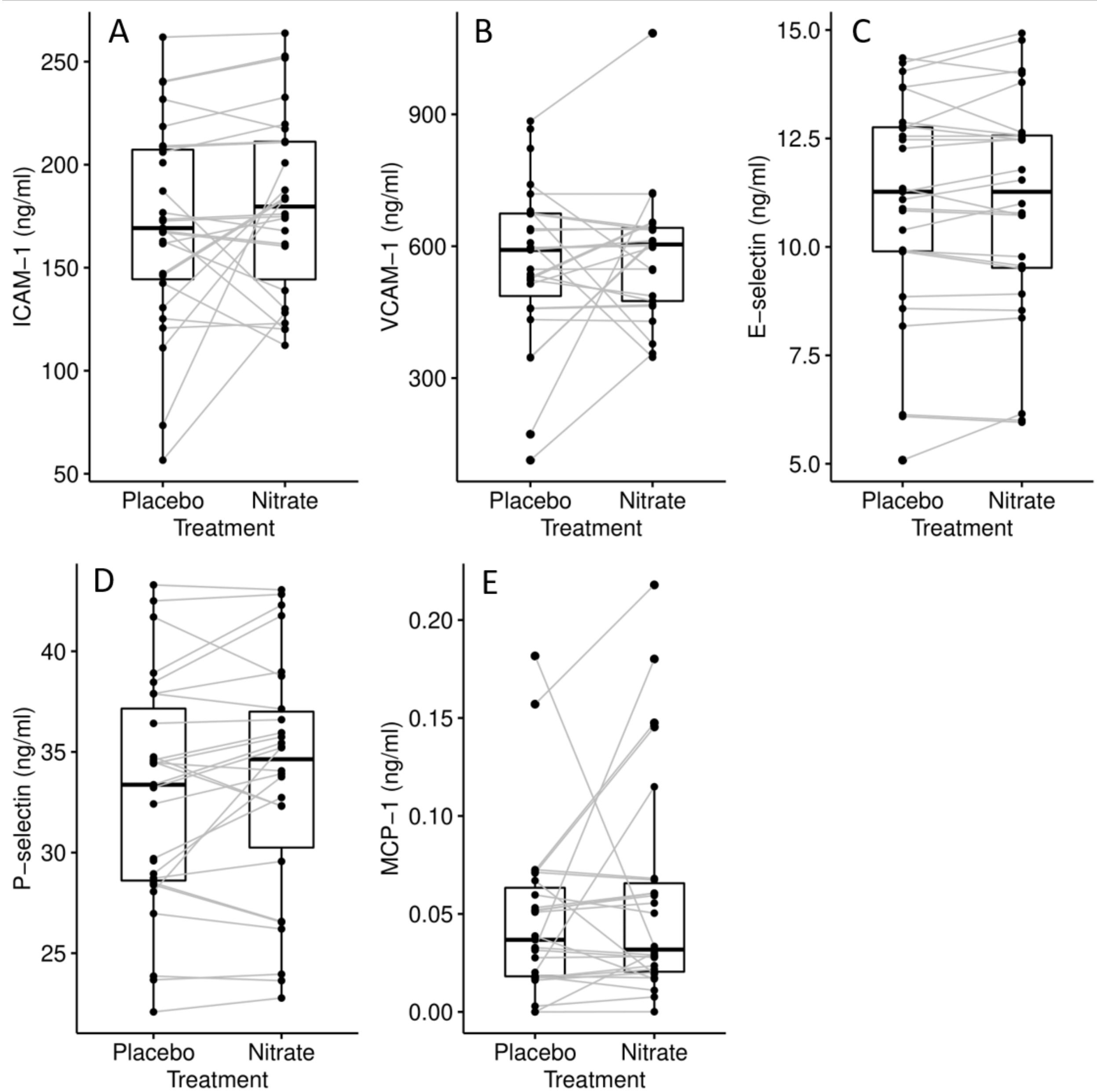
Enrollment
Responded and screened
n = 93
Exclusions following telephone screening
– did not meet study entry criteria
n = 36
Declined to participate
n = 30
Exclusions following screening
– did not meet study entry criteria
n = 0
Allocation
Randomised
n = 27
Analysis
Completed all visits and
analysed for endpoint
n = 27

Figure 2.



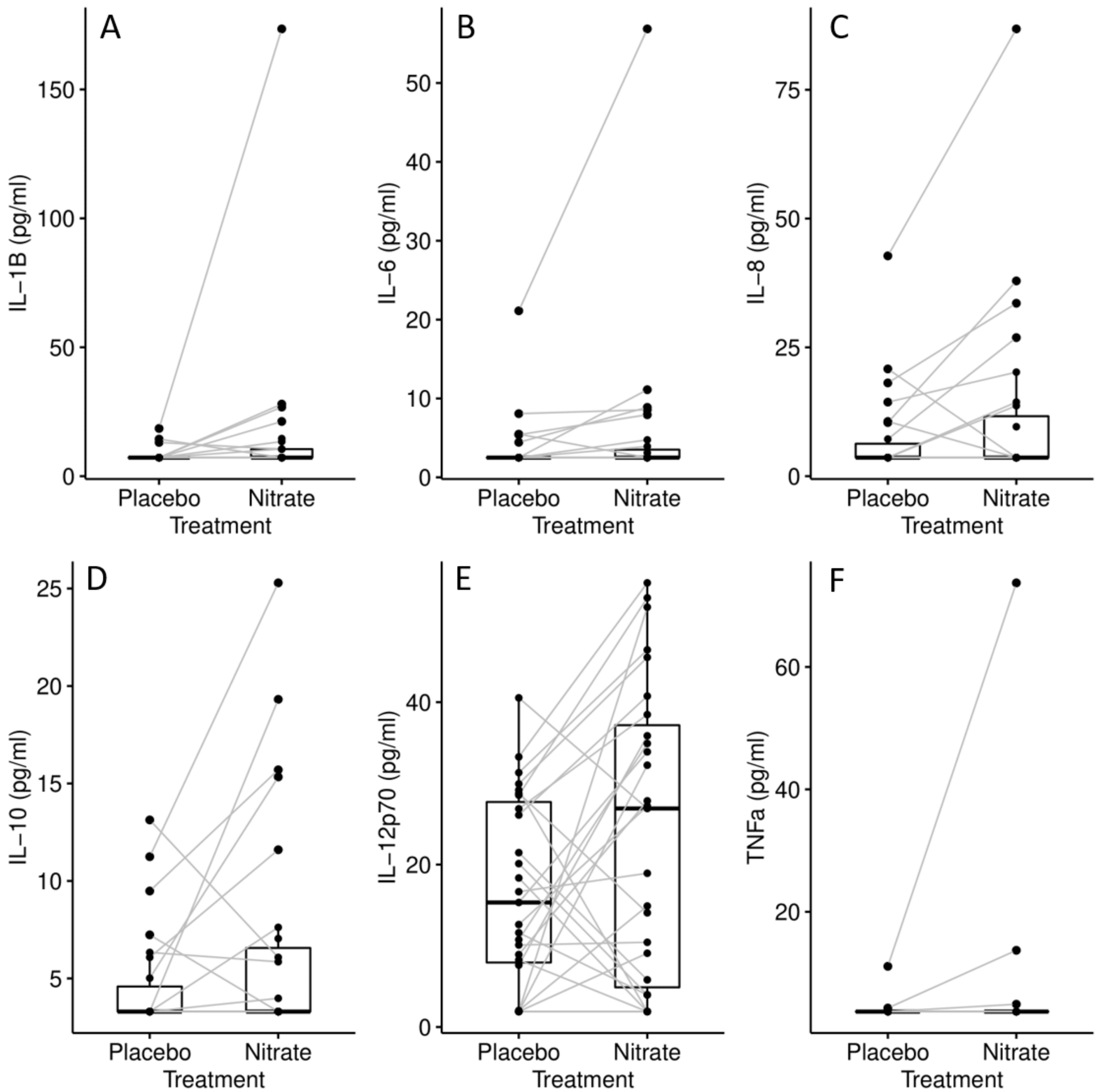
A
Plasma nitrate (μmol/l)
250
200
150
100
50
Placebo
Nitrate
Treatment
B
Plasma nitrite (μmol/l)
12.5
10.0
7.5
5.0
2.5
0.0
Placebo
Nitrate
Treatment
C
Salivary nitrate (μmol/l)
6000
4000
2000
0
Placebo
Nitrate
Treatment
D
Salivary nitrite (μmol/l)
2500
2000
1500
1000
500
0
Placebo
Nitrate
Treatment
E
Urinary nitrate (μmol/l)
2000
1000
0
Placebo
Nitrate
Treatment
F
Urinary nitrite (μmol/l)
750
500
250
0
Placebo
Nitrate
Treatment

Figure 3.



A
ICAM−1 (ng/ml)
250
200
150
100
50
Placebo
Nitrate
Treatment
B
VCAM−1 (ng/ml)
900
600
300
Placebo
Nitrate
Treatment
C
E−selectin (ng/ml)
15.0
12.5
10.0
7.5
5.0
Placebo
Nitrate
Treatment
D
P−selectin (ng/ml)
40
35
30
25
Placebo
Nitrate
Treatment
E
MCP−1 (ng/ml)
0.20
0.15
0.10
0.05
0.00
Placebo
Nitrate
Treatment

Figure 4.



A
B
C
D
E
F
IL−1B (pg/ml)
IL−6 (pg/ml)
IL−8 (pg/ml)
IL−10 (pg/ml)
IL−12p70 (pg/ml)
TNFa (pg/ml)
Placebo
Nitrate
Treatment