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Effects of sodium and potassium supplementation on endothelial function: a fully controlled dietary intervention study

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Abstract

High Na and low K intakes have adverse effects on blood pressure, which increases the risk for CVD. The role of endothelial dysfunction and inflammation in this pathophysiological process is not yet clear. In a randomised placebo-controlled cross-over study in untreated (pre) hypertensives, we examined the effects of Na and K supplementation on endothelial function and inflammation. During the study period, subjects were provided with a diet that contained 2.4 g/d of Na and 2.3 g/d of K for a 10 460 kJ (2500 kcal) intake. After 1-week run-in, subjects received capsules with supplemental Na (3.0 g/d), supplemental K (2.8 g/d) or placebo, for 4 weeks each, in random order. After each intervention, circulating biomarkers of endothelial function and inflammation were measured. Brachial artery flow-mediated dilation (FMD) and skin microvascular vasomotion were assessed in sub-groups of twenty-two to twenty-four subjects. Of thirty-seven randomised subjects, thirty-six completed the study. Following Na supplementation, serum endothelin-1 was increased by 0.24 pg/ml (95% CI 0.03, 0.45), but no change was seen in other endothelial or inflammatory biomarkers. FMD and microvascular vasomotion were unaffected by Na supplementation. K supplementation reduced IL-8 levels by 0.28 pg/ml (95% CI 0.03, 0.53), without affecting other circulating biomarkers. FMD was 1.16% (95% CI 0.37, 1.96) higher after K supplementation than after placebo. Microvascular vasomotion was unaffected. In conclusion, a 4-week increase in Na intake increased endothelin-1, but had no effect on other endothelial or inflammatory markers. Increased K intake had a beneficial effect on FMD and possibly IL-8, without affecting other circulating endothelial or inflammatory biomarkers.

Key words: Sodium: Potassium: Endothelial function: Inflammation: Flow-mediated dilation: Randomised controlled trials

Excess Na intake and low K intake have been associated with detrimental effects on blood pressure (BP) and CVD risk^(1,2), as recently confirmed by us in thirty-six adults who had a 7.5/2.7 mmHg higher 24 h BP after Na supplementation and 3.9/1.6 mmHg lower 24 h BP after K supplementation⁽³⁾. The vascular endothelium has been suggested to play a key role in BP homeostasis⁽⁴⁾. However, limited well-controlled studies have examined the effects of Na and K intake on endothelial function.

Endothelial function can be measured by circulating blood biomarkers that are expressed by activation of the endothelium⁽⁵⁾. For Na intake, randomised controlled trials with an intervention duration of 4 weeks or more mainly focused on the vasoconstrictor endothelin-1 and showed inconsistent results^(6–10). Similar studies on K intake showed no effect on endothelin-1⁽¹¹⁾ or on soluble adhesion molecules^(12,13). Effects of Na and K intake

on low-grade inflammation, closely related to endothelial function⁽¹⁴⁾, are largely unknown. High-sensitivity C-reactive protein (CRP) did not respond to the intake of Na^(8–10) or K^(12,13) in randomized controlled trials.

Flow-mediated dilation (FMD), the dilation of conduit arteries in response to blood flow-induced increases in shear stress, is a functional biomarker of endothelial function⁽¹⁵⁾. In randomized controlled trials, modest Na reductions ranging from 1.4 to 2.3 g/d for 2–6 weeks improved brachial artery FMD by 1.5–2.4%^(6,8,16). FMD has also been shown to improve after increased K intake of 2.5 g/d for 4 weeks⁽¹⁷⁾. A randomized controlled trial with a lower dose of K (i.e. 1.6 g/d) for 6 weeks demonstrated no effect⁽¹²⁾.

Microvascular vasomotion, the periodic oscillations of microvessel diameter, is thought to be partly dependent on

Abbreviations: BP, blood pressure; CRP, C-reactive protein; EID, endothelium-independent dilation; FMD, flow-mediated dilation; LDF, laser Doppler flowmetry; NO, nitric oxide; SBP, systolic blood pressure; vWf, von Willebrand factor.

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endothelial function⁽¹⁸⁾. Microvascular vasomotion, assessed via spectral analysis of skin laser Doppler flowmetry (LDF) tracing, has not yet been studied in relation to Na and K intakes.

In a double-blind placebo-controlled cross-over study, we performed a comprehensive assessment of Na and K supplementation on endothelial function and low-grade inflammation in subjects with untreated elevated BP. In addition, we assessed microvascular vasomotion as an exploratory secondary outcome.

Methods

Study population

The details of this study have been published previously⁽³⁾. Potential subjects were recruited from within a 10 km radius of the research centre through subject email databases and advertisements. Non-smoking men and women, aged between 40 and 80 years, with a fasting supine systolic BP (SBP) between 130 and 159 mmHg were eligible to participate. Exclusion criteria included a history of diabetes mellitus or cardiovascular, gastrointestinal, liver or renal diseases based on questionnaire data and laboratory parameters; BMI > 40 kg/m²; use of medication known to affect the cardiovascular system; use of nutritional supplements; an energy-restricted or medically prescribed diet; unstable body weight in the past 2 months; alcohol use over 21 units for women and 28 units for men/week (1 unit equalling 10–15 g of ethanol); and pregnant or lactating women.

Of the thirty-nine subjects who started the 1-week run-in, thirty-seven were randomised. One randomised subject dropped out because of gastrointestinal complaints due to the capsules, leaving thirty-six subjects who completed the study. This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all the procedures involving human subjects were approved by the Medical Ethics Committee of Wageningen University. Written informed consent was obtained from all the subjects. The study was registered at ClinicalTrials.gov (registration no. NCT01575041).

Study design

The study was a randomised, double-blind, placebo-controlled cross-over trial in which diet was fully controlled, as described previously⁽³⁾. In brief, during the 1-week run-in and three consecutive intervention periods of 4 weeks, not separated by washout, subjects were provided with a relatively low-Na, low-K diet that contained 2.4 g/d of Na and 2.3 g/d of K for a 10 460 kJ (2500 kcal) intake. At the end of the run-in (baseline), subjects were randomly allocated to one of the six possible treatment orders, based on sex and SBP (130–139 and \geq 140 mmHg). Treatments were daily consumption of eight sodium chloride capsules (Na: 3.0 g), eight potassium chloride capsules (K: 2.8 g) and eight placebo (cellulose) capsules (Microz), for 4 weeks each, in random order. Body weight was kept constant during the study period through adjustments in energy intake, and subjects were asked to maintain their usual level of physical activity.

At baseline and at the end of each intervention period, subjects underwent 24 h ambulatory BP monitoring and collected 24 h urine by discarding the first morning urine and collecting all urine secretions for the next 24 h. Subjects also underwent anthropometric measurements, blood sampling, and office BP, FMD and microvascular vasomotion assessment. The measurements were taken following an overnight fast (from 20.00 hours) at fixed time points in the morning, in a temperature-controlled (20–24°C) quiet room at the research centre.

Measurements

Biochemical analysis. Fasting blood samples collected in EDTA- and sodium citrate-containing tubes were centrifuged at 1550 **g** for 15 min at 4 and 20°C, respectively. Blood samples collected in heparin-coated tubes were centrifuged at 800 **g** for 20 min at 4°C. Aliquots were stored at –80°C until the end of the study for analysis.

Concentrations of soluble E-selectin, soluble thrombomodulin, soluble vascular cell adhesion molecule-1 (sVCAM-1), soluble intercellular adhesion molecule-1 (sICAM-1), CRP, serum amyloid A, TNF- α , monocyte chemoattractant protein-1, IL-1 β , IL-6 and IL-8 were measured in EDTA plasma using a multi-array detection system (SECTOR Imager 2400; Meso Scale Discovery) in the laboratory of the Maastricht University Medical Center, as previously described⁽¹⁹⁾.

Endothelin-1 and von Willebrand factor (vWf) were determined by ELISA in the laboratory of the Maastricht University Medical Center from EDTA plasma and citrate plasma, respectively. Levels of vWf were expressed as a percentage of vWf detected in pooled citrated plasma of healthy volunteers⁽¹⁹⁾. Heparin plasma levels of nitric oxide (NO) were determined at the RIKILT Institute of Food Safety by estimating NO by the chemiluminescence formed after the release of NO from NO₂, NO₂⁻ and nitrosated and nitrosylated species, as described previously⁽²⁰⁾. Intra-assay CV were <10% for all biomarker measurements, except for IL-1 β (10.4%). Inter-assay CV were <10% for all biomarker measurements, except for sVCAM-1 (12.8%), IL-1 β (12.4%) and NO (18.6%).

Na and K levels were determined in 24 h urine samples in a certified laboratory using ion-selective electrodes module on the Modular P of Roche.

Flow-mediated dilation and blood pressure. FMD was measured by a trained staff member according to established guidelines⁽²¹⁾. After rest of at least 20 min in the supine position, longitudinal ultrasonographic images of the right brachial artery were continuously recorded. Baseline arterial diameter was recorded for 3 min, after which a pressure cuff on the forearm was inflated to 200 mmHg for 5 min to induce reactive hyperaemia. After cuff release, images were recorded for 5 min for the determination of the maximum arterial diameter. Images were processed automatically using custom-written software (DuplexFMD, Department of Biomedical Engineering, Maastricht University). FMD was calculated as the percentage change in arterial diameter from baseline to the maximum value after cuff release. Endothelium-independent dilation (EID) was assessed as the maximum change in arterial diameter over a 5 min

period following sublingual administration of nitroglycerin (400 µg). Before data analysis, recordings of insufficient quality due to movements of subjects or unclear images of the arterial wall were excluded, leaving twenty-two subjects for the analysis of FMD for Na *v.* placebo supplementation and twenty-four subjects for K *v.* placebo supplementation.

Office brachial BP was measured in the supine position after at least 10 min of rest using an automated oscillometric device (Dinamap Pro 100). Ambulatory BP monitoring was performed for 24 h using Spacelabs 90217 devices (Spacelabs Medical Inc.), as described previously⁽³⁾.

Microvascular vasomotion. Skin blood flow was measured using an LDF system (PeriFlux 5000; Perimed) with a laser Doppler probe (PF 457; Perimed) at approximately 2 cm distal to the wrist on the back of the left hand. Subjects were in the supine position, and after a rest of at least 20 min skin temperature was set at 30°C and skin blood flow was recorded. Spectral analysis was performed using Fast-Fourier transform analysis of skin LDF (Perisoft for Windows version 2.5; Perimed) to determine the power spectral density of the LDF signal. The power density was calculated in the total frequency spectrum of 0.01–1.60 Hz and in five frequency sub-intervals to determine the contribution of oscillations of endothelial (0.01–0.02 Hz), neurogenic (0.02–0.06 Hz), myogenic (0.06–0.15 Hz), respiratory (0.15–0.40 Hz) and heart beat origin (0.40–1.60 Hz) to microvascular vasomotion⁽²²⁾. Data were expressed as arbitrary perfusion units. Recordings of insufficient quality due to movements of subjects were excluded before data analysis, leaving twenty-three subjects for the analysis of Na *v.* placebo supplementation and twenty-three subjects for K *v.* placebo supplementation.

Statistical analysis

Data were analysed according to the intention-to-treat principle, using a predefined statistical analysis plan. FMD was defined as the primary outcome. The present study had 80 % power to detect a FMD difference of 1.0 % for a SD of 1.7 % and a two-sided α of 0.05. Circulating biomarkers with a skewed distribution were transformed taking the natural logarithm. After analysing

biomarkers individually, overall Z scores were created for a set of biomarkers of endothelial function and of low-grade inflammation (online Supplementary Methods)⁽¹⁹⁾. For each outcome measure, a mixed-effects model with covariance structure compound symmetry was used to estimate the effect of the active treatment compared with placebo. ‘Treatment’ and ‘period’ were included as fixed effects and ‘subject’ as the random effect. In the sensitivity analysis, analyses were repeated after the exclusion of intervention periods in which subjects were non-compliant. Values reported in text and tables are expressed as mean with standard deviation, median with interquartile range for skewed variables or treatment effect with 95 % CI. Two-sided *P* values <0.05 were considered statistically significant. Analyses were performed using SAS software version 9.2 (SAS Institute).

Results

Subjects and compliance

Baseline characteristics of the twenty-four men and twelve women who completed the study are reported in Table 1. Subjects were on average 65.8 years old and their BMI was 27.2 kg/m². During screening, their 24 h urinary excretion was 153.7 (SD 63.6) mmol for Na and 81.8 (SD 25.6) mmol for K. This decreased to 90.8 (SD 26.6) mmol and 49.0 (SD 13.4) mmol, respectively, after 1-week run-in. Subjects had a mean office SBP/diastolic BP of 145.3/80.6 mmHg, and 69 % of them (25/36) had an SBP ≥ 140 mmHg during screening. BP was 133.4/75.7 mmHg after the 1-week run-in period on the low-Na, low-K diet. Baseline characteristics of the subjects with FMD or vasomotion data did not essentially differ from the overall study population (online Supplementary Tables S1 and S2, respectively). Based on returned capsules and diary entries, 86 % (31/36) of the subjects were compliant, ingesting over 80 % of the capsules during each intervention period.

Sodium supplementation

Na supplementation increased urinary Na excretion by 97.6 mmol/24 h (95 % CI 81.0, 114.1; *P* < 0.001) and 24 h SBP

Table 1. Baseline characteristics of the thirty-six subjects who completed the study (Mean values and standard deviations)

	Total (n 36)		Men (n 24)		Women (n 12)	
	Mean	SD	Mean	SD	Mean	SD
Age (years)	65.8	8.8	66.0	9.3	65.4	8.2
Height (cm)	175.5	9.3	178.8	8.9	168.8	5.8
Weight (kg)	84.3	18.5	87.9	19.0	77.2	16.0
BMI (kg/m ²)	27.2	4.7	27.3	4.8	27.0	4.6
Waist circumference (cm)	99.9	14.8	103.0	15.0	93.8	12.9
Pre-run-in Na excretion (mmol/24 h)	153.7	63.6	160.2	71.4	140.8	44.1
Pre-run-in K excretion (mmol/24 h)	81.8	25.6	82.2	28.7	81.0	19.3
Post-run-in Na excretion (mmol/24 h)	90.8	26.6	98.3	26.3	75.9	20.8
Post-run-in K excretion (mmol/24 h)	49.0	13.4	50.5	13.6	46.0	13.0
Pre-run-in office SBP (mmHg)	145.3	11.2	147.7	10.3	140.4	11.7
Pre-run-in office DBP (mmHg)	80.6	8.0	82.8	7.7	76.2	7.0
Post-run-in office SBP (mmHg)	133.4	14.7	136.4	14.7	127.5	13.3
Post-run-in office DBP (mmHg)	75.7	8.3	77.8	8.0	71.4	7.6

SBP, systolic blood pressure; DBP, diastolic blood pressure

Table 2. Effects of 4-week supplementation with sodium (3g/d) or placebo on urinary, clinical and blood parameters, flow-mediated dilation and microvascular vasomotion in untreated pre-hypertensive and hypertensive adults (Unadjusted mean values and standard deviations; medians and interquartile ranges; mean differences and 95 % confidence intervals)

	Values after 4 weeks of intervention				Treatment effect*		
	Na		Placebo		Na v. placebo		
	Mean	SD	Mean	SD	Mean difference	95 % CI	P
Urinary parameters							
Na (mmol/24 h)	202.9	54.8	105.1	39.7	97.6	81.0, 114.1	<0.001
K (mmol/24 h)	53.2	16.6	55.3	16.7	-2.2	-10.2, 5.7	0.58
Weight (kg)	82.5	18.3	82.5	18.3	-0.1	-0.7, 0.5	0.71
Ambulatory BP							
24-h systolic BP (mmHg)	136.8	14.4	129.4	14.1	7.5	4.4, 10.5	<0.001
24-h diastolic BP (mmHg)	79.2	8.9	76.5	8.3	2.7	1.1, 4.2	<0.001
Blood parameters†							
Nitric oxide (nmol/l)							
Median	62.4		59.9				
Interquartile range	54.0–83.6		48.1–86.0				
Ln nitric oxide (nmol/l)	4.20	0.38	4.17	0.49	0.03	-0.09, 0.15	0.62
Endothelin-1 (pg/ml)	2.47	0.73	2.22	0.63	0.24	0.03, 0.45	0.023
Soluble E-selectin (ng/ml)	10.6	4.9	10.6	4.5	0.05	-0.77, 0.87	0.90
Soluble thrombomodulin (ng/ml)	3.89	0.71	3.88	0.77	0.01	-0.08, 0.10	0.85
von Willebrand factor (%‡)	136.0	54.3	129.8	34.6	5.7	-5.8, 17.3	0.33
Soluble vascular cellular adhesion molecule-1 (ng/ml)	367.8	78.5	366.2	73.7	1.5	-11.4, 14.4	0.82
Soluble intercellular adhesion molecule-1 (ng/ml)	227.2	45.9	232.7	45.0	-5.2	-13.3, 3.0	0.21
TNF- α (pg/ml)	9.22	2.61	9.09	2.10	0.15	-0.27, 0.58	0.48
C-reactive protein (μg/ml)							
Median	1.29		1.43				
Interquartile range	0.61–2.34		0.76–2.88				
Ln C-reactive protein (μ g/ml)	0.23	1.04	0.33	1.01	-0.10	-0.33, 0.14	0.43
Serum amyloid A (μg/ml)							
Median	1.46		2.15				
Interquartile range	0.94–2.76		1.00–3.70				
Ln serum amyloid A (μ g/ml)	0.44	0.80	0.61	0.81	-0.17	-0.35, 0.01	0.064
Monocyte chemoattractant protein-1 (pg/ml)							
Median	207.0		219.3				
Interquartile range	192.0–236.3		195.8–243.2				
Ln monocyte chemoattractant protein-1 (pg/ml)	5.39	0.21	5.42	0.23	-0.03	-0.07, 0.01	0.099
IL-1β (pg/ml)							
Median	0.41		0.45				
Interquartile range	0.18–0.55		0.20–0.62				
Ln IL-1 β (pg/ml)	-1.18	1.01	-1.15	1.01	-0.04	-0.13, 0.05	0.41
IL-6 (pg/ml)							
Median	1.42		1.31				
Interquartile range	1.09–2.00		1.07–1.94				
Ln IL-6 (pg/ml)	0.46	0.72	0.45	0.65	0.02	-0.16, 0.20	0.81
IL-8 (pg/ml)§	3.30	0.99	3.55	1.14	-0.25	-0.50, 0.00	0.052
Z score endothelial function	0.083	0.692	0.000	0.640	0.080	-0.029, 0.189	0.15
Z score low-grade inflammation¶	-0.051	0.563	0.000	0.546	-0.047	-0.189, 0.094	0.51
Flow-mediated dilation**							
Baseline brachial artery diameter (mm)	4.59	0.90	4.56	0.78	0.03	-0.11, 0.17	0.64
Post-release brachial artery diameter (mm)	4.71	0.90	4.69	0.78	0.04	-0.11, 0.18	0.61
Flow-mediated dilation (mm)	0.13	0.08	0.13	0.09	0.00	-0.03, 0.04	0.80
Flow-mediated dilation (%)	2.94	1.88	3.01	2.15	0.06	-0.75, 0.86	0.89
Vasomotion – power density (AU)††							
Endothelial frequency interval	2.22	1.25	2.46	1.09	-0.21	-0.83, 0.40	0.49
Neurogenic frequency interval	3.76	1.96	3.83	1.63	-0.05	-1.03, 0.93	0.92
Myogenic frequency interval	4.10	2.42	3.62	2.41	0.57	-0.65, 1.78	0.35
Respiratory frequency interval‡‡	6.04	3.34	6.55	6.04	-0.26	-2.59, 2.07	0.82
Heart beat frequency interval	23.5	14.9	23.3	13.8	0.09	-6.94, 7.13	0.98
Total	39.6	20.9	39.7	21.4	0.06	-10.77, 10.88	0.99

BP, blood pressure; AU, arbitrary units.

* Data are obtained from linear mixed models for repeated measurements using the compound symmetry covariance structure.

† Based on thirty-six subjects, for difference in urinary Na excretion of 97.6 mmol/24 h (95 % CI 81.0, 114.1; $P < 0.001$).

‡ Treatment effect after excluding one outlying value of 366 % during Na supplementation was -1.1 % (95 % CI -6.8, 4.5; $P = 0.70$).

§ Treatment effect after excluding one outlying value of 8.25 pg/ml during placebo supplementation was -0.13 pg/ml (95 % CI -0.33, 0.07; $P = 0.19$).

|| Included were endothelin-1, soluble E-selectin, soluble thrombomodulin, von Willebrand factor, soluble vascular cellular adhesion molecule-1 and soluble intercellular adhesion molecule-1.

¶ Included were soluble intercellular adhesion molecule-1, C-reactive protein, serum amyloid A, TNF- α , monocyte chemoattractant protein-1, IL-1 β , IL-6 and IL-8.

** Based on twenty-two subjects, for difference in urinary Na excretion of 99.6 mmol/24 h (95 % CI 78.6, 120.6; $P < 0.001$).

†† Based on twenty-three subjects, for difference in urinary Na excretion of 102.7 mmol/24 h (95 % CI 81.4, 124.0; $P < 0.001$).

‡‡ Treatment effect after excluding one outlying value of 30.48 AU during placebo supplementation was 0.67 AU (95 % CI -1.25, 2.59; $P = 0.49$).

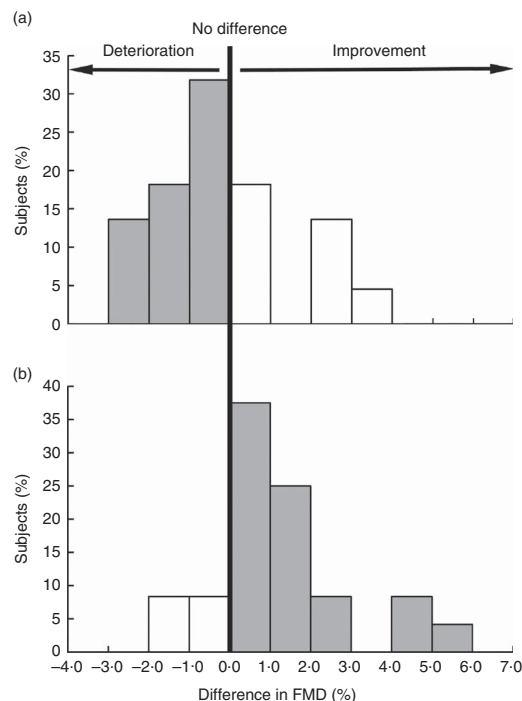


Fig. 1. Effects of 4-week sodium (a) and potassium (b) supplementation on flow-mediated dilation (FMD) in untreated pre-hypertensive and hypertensive adults, compared with placebo.

by 7.5 mmHg (95 % CI 4.4, 10.5; $P < 0.001$) compared with placebo (Table 2). After Na supplementation, endothelin-1 levels were 0.24 pg/ml (95 % CI 0.03, 0.45; $P = 0.023$) higher compared with placebo. Other individual biomarkers of endothelial function and the aggregate Z score of endothelial function (0.080; 95 % CI -0.029, 0.189; $P = 0.15$) did not change. In addition, markers of low-grade inflammation and the aggregate Z score (-0.047; 95 % CI -0.189, 0.094; $P = 0.51$) were unaffected. Differences in FMD are depicted in Fig. 1, with, on average, no effect of Na supplementation on FMD (0.06%; 95 % CI -0.75, 0.86; $P = 0.89$). Moreover, EID (-0.01%; 95 % CI -1.77, 1.75; $P = 0.99$) was unaffected. No effects of Na supplementation were seen on the power densities of the different frequency intervals determining microvascular vasomotion (Table 2).

Potassium supplementation

K supplementation increased urinary K excretion by 62.9 mmol/24 h (95 % CI 54.9, 70.8; $P < 0.001$) and decreased 24 h SBP by 3.9 mmHg (95 % CI 0.9, 6.9; $P = 0.013$), compared with placebo (Table 3). K supplementation resulted in a lower IL-8 of 0.28 pg/ml (95 % CI 0.03, 0.53; $P = 0.031$). In the sensitivity analysis, after the exclusion of intervention periods in which subjects were non-compliant, the effect on IL-8 was no longer significant (-0.24 pg/ml; 95 % CI -0.50, 0.03, $P = 0.080$). K supplementation had no effect on other individual biomarkers, on the Z score of endothelial function (0.007; 95 % CI -0.102, 0.115; $P = 0.90$) or low-grade inflammation (-0.032; 95 % CI -0.173, 0.109; $P = 0.66$). However, in the sensitivity analysis, K supplementation increased vWf by 12.5 % (95 % CI 1.0, 23.9, $P = 0.033$) compared with placebo. FMD was 1.16 %

(95 % CI 0.37, 1.96; $P = 0.005$) higher after K supplementation compared with placebo, with FMD being improved in 83 % (20/24) of the subjects after K supplementation compared with placebo (Fig. 1). K supplementation had no effect on EID (0.51%; 95 % CI -1.17, 2.19; $P = 0.54$). The power densities of the frequency intervals related to activities determining vasomotion were unaffected (Table 3).

Discussion

In untreated pre-hypertensive and hypertensive adults on a fully controlled diet that was relatively low in Na and K, 4 weeks of Na supplementation had no effect on endothelial function or low-grade inflammation, except for an increase in endothelin-1. K supplementation lowered the inflammatory marker IL-8 and increased the functional biomarker FMD.

A major strength of the present study is the comprehensive assessment of endothelial function and low-grade inflammation by an extensive set of circulating biomarkers, the functional biomarker FMD and microvascular vasomotion. All measurements were performed in a fasting state at fixed times of the day using a strict protocol. We used high-sensitivity assay techniques, and eleven circulating biomarkers were assessed simultaneously to minimise between-assay variation. Our study had limited variability in diet and lifestyle behaviours due to the provision of a fully controlled diet and the instruction to keep other lifestyle factors such as physical activity constant. In addition, a large contrast in Na and K intake was achieved, the compliance was high and the drop-out rate was low. Moreover, although the number of subjects in this study was limited, the study had ample power (80 %) to show a clinically relevant effect on FMD of 1.0%^(23,24).

Our study showed that for an increase in Na intake of 2.2 g/d (based on urinary excretions), the potent vasoconstrictor and pro-inflammatory peptide endothelin-1 increased by 0.24 pg/ml. Other circulating biomarkers of endothelial function and low-grade inflammation were unaffected. These findings are in line with the results of a randomized controlled trial, in which lowering Na intake moderately for 6 weeks resulted in a decrease in endothelin-1 and no changes in ICAM-1, VCAM-1 and E-selectin⁽⁶⁾. Other randomized controlled trials also indicated lower levels of endothelin-1 at lower Na intakes, but findings were not significant^(7,9). In contrast, in seventeen adults with moderately elevated BP, endothelin-1 levels were higher after 4 weeks of Na restriction than after normal Na intake (6.3 v. 5.9 pg/ml); however, this was not statistically significant⁽⁸⁾. Although not as comprehensively assessed as in our study, other randomized controlled trials with a 4-week intervention also observed no significant effects of Na intake on inflammatory markers⁽⁸⁻¹⁰⁾.

We found no effect of Na supplementation on FMD. Other cross-over studies demonstrated improvements in FMD of 1.5–2.4 % after Na reductions for 2–6 weeks^(6,8,16). In these studies, in contrast to our study, K intake was not reduced. Moreover, our subjects had, on average, a high baseline brachial artery diameter and a low FMD compared with these studies. We excluded FMD recordings of low quality, but baseline characteristics of the subjects included in the FMD analysis were similar to all thirty-six subjects and randomisation was maintained.

Table 3. Effects of 4-week supplementation with potassium (3 g/d) or placebo on urinary, clinical and blood parameters, flow-mediated dilation and microvascular vasomotion in untreated pre-hypertensive and hypertensive adults (Unadjusted mean values and standard deviations; medians and interquartile ranges; mean differences and 95 % confidence intervals)

	Values after 4 weeks of intervention				Treatment effect*		
	K		Placebo		K v. placebo		P
	Mean	SD	Mean	SD	Mean difference	95 % CI	
Urinary parameters							
Na (mmol/24 h)	96.5	39.0	105.1	39.7	-8.9	-25.4, 7.6	0.29
K (mmol/24 h)	118.1	32.2	55.3	16.7	62.9	54.9, 70.8	<0.001
Weight (kg)	82.3	18.2	82.5	18.3	-0.3	-0.9, 0.2	0.28
Ambulatory BP							
24-h systolic BP (mmHg)	125.6	13.3	129.4	14.1	-3.9	-6.9, -0.9	0.013
24-h diastolic BP (mmHg)	74.9	7.8	76.5	8.3	-1.6	-3.2, -0.1	0.039
Blood parameters†							
Nitric oxide (nmol/l)							
Median	59.7		59.9				
Interquartile range	50.8-81.2		48.1-86.0				
Ln nitric oxide (nmol/l)	4.15	0.37	4.17	0.49	-0.02	-0.14, 0.10	0.70
Endothelin-1 (pg/ml)	2.14	0.61	2.22	0.63	-0.09	-0.29, 0.12	0.42
Soluble E-selectin (ng/ml)	10.5	4.3	10.6	4.5	-0.13	-0.94, 0.69	0.76
Soluble thrombomodulin (ng/ml)	3.92	0.77	3.88	0.77	0.03	-0.06, 0.12	0.47
von Willebrand factor (%)‡	138.0	44.1	129.8	34.6	8.1	-3.4, 19.6	0.17
Soluble vascular cellular adhesion molecule-1 (ng/ml)	363.0	73.6	366.2	73.7	-2.9	-15.8, 10.0	0.66
Soluble intercellular adhesion molecule-1 (ng/ml)	231.0	47.9	232.7	45.0	-1.6	-9.7, 6.6	0.71
TNF- α (pg/ml)	9.40	2.84	9.09	2.10	0.31	-0.11, 0.74	0.15
C-reactive protein (μg/ml)							
Median	1.73		1.43				
Interquartile range	0.53-2.58		0.76-2.88				
Ln C-reactive protein (μ g/ml)	0.33	1.09	0.33	1.01	0.00	-0.24, 0.24	0.99
Serum amyloid A (μg/ml)							
Median	1.92		2.15				
Interquartile range	1.09-2.73		1.00-3.70				
Ln serum amyloid A (μ g/ml)	0.61	0.75	0.61	0.81	0.01	-0.17, 0.19	0.94
Monocyte chemoattractant protein-1 (pg/ml)							
Median	211.4		219.3				
Interquartile range	192.9-233.1		195.8-243.2				
Ln monocyte chemoattractant protein-1 (pg/ml)	5.39	0.22	5.42	0.23	-0.02	-0.06, 0.01	0.21
IL-1β (pg/ml)							
Median	0.40		0.45				
Interquartile range	0.23-0.62		0.20-0.62				
Ln IL-1 β (pg/ml)	-1.13	1.01	-1.15	1.01	0.01	-0.08, 0.11	0.80
IL-6 (pg/ml)							
Median	1.40		1.31				
Interquartile range	1.02-2.02		1.07-1.94				
Ln IL-6 (pg/ml)	0.42	0.54	0.45	0.65	-0.03	-0.21, 0.15	0.74
IL-8 (pg/ml)§	3.27	0.78	3.55	1.14	-0.28	-0.53, -0.03	0.031
Z score endothelial function	0.008	0.669	0.000	0.640	0.007	-0.102, 0.115	0.90
Z score low-grade inflammation¶	-0.033	0.524	0.000	0.546	-0.032	-0.173, 0.109	0.66
Flow-mediated dilation**							
Baseline brachial artery diameter (mm)	4.54	0.76	4.60	0.80	-0.06	-0.19, 0.08	0.41
Post-release brachial artery diameter (mm)	4.72	0.78	4.73	0.79	0.00	-0.15, 0.14	0.98
Flow-mediated dilation (mm)	0.18	0.09	0.12	0.09	0.05	0.02, 0.09	0.006
Flow-mediated dilation (%)	4.04	1.82	2.85	2.13	1.16	0.37, 1.96	0.005
Vasomotion – power density (AU)††							
Endothelial frequency interval	2.28	0.95	2.39	1.04	-0.18	-0.80, 0.43	0.55
Neurogenic frequency interval	3.80	1.58	3.83	1.63	-0.06	-1.04, 0.91	0.90
Myogenic frequency interval	3.52	1.86	3.64	2.40	-0.07	-1.28, 1.14	0.90
Respiratory frequency interval‡‡	5.92	3.50	6.38	6.03	-0.59	-2.91, 1.74	0.62
Heart beat frequency interval	22.1	9.8	23.6	14.0	-1.60	-8.62, 5.43	0.65
Total	37.6	13.3	39.8	21.4	-2.78	-13.58, 8.03	0.61

BP, blood pressure; AU, arbitrary units.

* Data are obtained from linear mixed models for repeated measurements using the compound symmetry covariance structure.

† Based on thirty-six subjects, for difference in urinary K excretion of 62.9 mmol/24 h (95 % CI 54.9, 70.8; $P < 0.001$).‡ Treatment effect after excluding one outlying value of 366 % during Na supplementation was 8.3 % (95 % CI 2.7, 13.9; $P = 0.004$).§ Treatment effect after excluding one outlying value of 8.25 pg/ml during placebo supplementation was -0.17 pg/ml (95 % CI -0.37, 0.03; $P = 0.10$).

|| Included were endothelin-1, soluble E-selectin, soluble thrombomodulin, von Willebrand factor, soluble vascular cellular adhesion molecule-1 and soluble intercellular adhesion molecule-1.

¶ Included were soluble intercellular adhesion molecule-1, C-reactive protein, serum amyloid A, TNF- α , monocyte chemoattractant protein-1, IL-1 β , IL-6 and IL-8.** Based on twenty-four subjects, for difference in urinary K excretion of 67.7 mmol/24 h (95 % CI 57.5, 78.0; $P < 0.001$).†† Based on twenty-three subjects, for difference in urinary K excretion of 65.0 mmol/24 h (95 % CI 54.4, 75.6; $P < 0.001$).‡‡ Treatment effect after excluding one outlying value of 30.48 AU during placebo supplementation was 0.43 AU (95 % CI -1.49, 2.35; $P = 0.66$).

In our study, doubling the intake of K from 2.2 to 4.6 g/d had no effect on circulating biomarkers of endothelial function, whereas the inflammatory marker IL-8 was reduced. Other randomized controlled trials with durations of 4 weeks or more also observed no effect of increased K intake on endothelial biomarkers^(11–13). To our knowledge, in randomized controlled trials, the effects of supplemental K on inflammatory biomarkers have only been investigated by measuring high-sensitivity CRP, which, in line with our study, was not affected^(12,13). As other cytokines did not change, we cannot exclude the possibility that our findings for IL-8 were per chance because of the large number of outcomes that we examined. Moreover, in the sensitivity analysis, the effect on IL-8 was no longer significant after the exclusion of intervention periods in which subjects were non-compliant.

The functional biomarker FMD was improved by 1.16% following K supplementation, which may contribute to cardiovascular risk reduction. In the meta-analyses, each 1% increase in FMD was associated with a 8–13% lower risk of cardiovascular events^(23,24). A randomized controlled trial investigating the effects of increased K intake using potassium chloride supplements revealed in forty-two untreated hypertensives a significant increase of 2.7% in FMD for an increased urinary K excretion of 45 mmol/24 h⁽¹⁷⁾. This study also showed a 1.5% increase in FMD after 4-week supplemental potassium bicarbonate. In contrast, increasing K intake for 6 weeks through potassium citrate supplements and fruit and vegetables with a maximum increase in urinary K of 27 mmol/24 h resulted in no effect on FMD in forty-eight early hypertensives⁽¹²⁾. Possibly, a minimum dose of K is required to improve FMD. Skin microvascular vasomotion, which is thought to be partly dependent on endothelial function, was unaffected by K supplementation. It is uncertain to what extent endothelial function of the microvessel is similar to that of macrovessels (e.g. brachial artery). Moreover, our data on microvascular vasomotion should be considered exploratory, and need confirmation by others using similar methods.

In the present cross-over study, we have previously shown that 24 h BP was 7.5/2.7 mmHg higher after Na supplementation and 3.9/1.6 mmHg lower after K supplementation⁽³⁾. The results of the present investigation suggest that supplemental Na for 4 weeks can increase BP without altering endothelial function. The endothelium may play a role in the BP effects of K. However, we saw no effect of K intake on the circulating biomarkers of endothelial function, while FMD was affected. Endothelial function is a complex process involving a number of factors and it needs to be determined which factors are directly related to K intake-induced changes in FMD. Furthermore, we cannot conclude whether the improvement in endothelial function preceded or followed BP reduction, or whether these changes are independent.

In conclusion, a 4-week increase in Na intake had, besides an increase in endothelin-1, no effect on endothelial function and low-grade inflammation in subjects with untreated elevated BP. Increasing K intake improved endothelial function as assessed by FMD, but did not affect other indicators of endothelial function or low-grade inflammation. This suggests that K intake may have protective effects on endothelial function. Other studies replicating these findings and further studies about the

mechanisms underlying the effect of K intake on endothelial function are warranted.

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L. G. and J. M. G. designed the study; L. G. and J. I. D. carried out the study; L. G., Y. H. A. M. K. and J. M. G. analysed the data; L. G., J. I. D., C. G. S., Y. H. A. M. K., S. J. L. B., P. C. H. H. and J. M. G. wrote the manuscript. All the authors read and approved the final version of the manuscript.

There are no conflicts of interest to declare.

Supplementary material

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