Effects of a low-salt diet on flow-mediated dilatation in humans

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ABSTRACT

Background: The effect of salt reduction on vascular function, assessed by brachial artery flow-mediated dilatation (FMD), is unknown.

Objective: Our aim was to compare the effects of a low-salt (LS; 50 mmol Na/d) diet with those of a usual-salt (US; 150 mmol Na/d) diet on FMD.

Design: This was a randomized crossover design in which 29 overweight and obese normotensive men and women followed an LS diet and a US diet for 2 wk. Both diets had similar potassium and saturated fat contents and were designed to ensure weight stability. After each intervention, FMD, pulse wave velocity, augmentation index, and blood pressure were measured.

Results: FMD was significantly greater ($P = 0.001$) with the LS diet (4.89 ± 2.42%) than with the US diet (3.37 ± 2.10%), systolic blood pressure was significantly ($P = 0.02$) lower with the LS diet (112 ± 11 mm Hg) than with the US diet (117 ± 13 mm Hg), and 24-h sodium excretion was significantly lower ($P = 0.0001$) with the LS diet (64.1 ± 41.3 mmol) than with the US diet (156.3 ± 56.7 mmol). There was no correlation between change in FMD and change in 24-h sodium excretion or change in blood pressure. No significant changes in augmentation index or pulse wave velocity were observed.


INTRODUCTION

Endothelial dysfunction, as assessed by flow-mediated dilatation (FMD), precedes the appearance of clinical cardiovascular disease (CVD), may be correlated in its pathogenesis, and is associated with risk factors for CVD including hypertension and obesity (1–3). Coronary endothelial dysfunction, assessed by intracoronary acetyl choline infusion, predicts future CVD events in both diseased and healthy populations. Brachial artery FMD is also a predictor of future cardiovascular events, but is not always independent of risk factors for CVD (4–6). Endothelial function measured by FMD noninvasively in the peripheral circulation correlates with coronary endothelial function (7–9). FMD is primarily mediated by endothelium-derived nitric oxide (NO), which is released in response to shear stress and causes smooth muscle relaxation and arterial dilatation (4).

Although the association between salt and blood pressure (BP) is well known, the effect of salt reduction on FMD has not been well explored in humans. Salt loading has been shown to decrease NO production independently of changes in BP, which suggests a direct effect of sodium (or the hormonal response to sodium) on the endothelium (10). We previously observed, in a post hoc cross-sectional analysis, that FMD was negatively correlated with 24-h sodium excretion ($r = -0.28, P < 0.05$) and was unrelated to BP (11). Hence, a prospective study of the effect of changes in salt intake on FMD was required to show causality.

Increased arterial stiffness predicts the development of CVD and is an independent predictor of mortality in hypertensive patients (12). Augmentation index (AI) measured by applanation tonometry and aortic pulse wave velocity (PWV) are used as indirect measures of arterial stiffness; a faster PWV indicates a stiffer aorta (13). A high salt intake has been shown to be a determinant of arterial stiffness, and a low salt intake has been associated with reduced arterial stiffness in normotensive adults, both epidemiologically and in an intervention (14, 15).

The main aim of this study was to compare the effects of a low-salt (LS) diet with those of a usual-salt (US) diet on FMD in a weight-stable setting. Given our previous finding that FMD was negatively correlated with 24-h sodium excretion and that FMD was not correlated with BP, our primary hypothesis was that the LS diet (50 mmol Na/d) would improve vascular function to a greater extent than would the US diet (150 mmol Na/d)—more so than might be expected by small reductions in BP alone—and that the changes in vascular function would be unrelated to the changes in BP. Our secondary aim was to determine the effect of an LS diet on other measures of vascular function, namely PWV and AI.

SUBJECTS AND METHODS

Subjects

Forty-one men and women were recruited from the Commonwealth Scientific and Industrial Research Organization (CSIRO) volunteer database and were screened at the Clinical Research Unit, CSIRO Human Nutrition, Adelaide, Australia. Subjects were excluded if they had metabolic disease, had CVD, had a systolic BP (SBP) >160 mm Hg at screening, had significant weight loss in the preceding 6 mo (>2 kg), had a body mass index (BMI; in kg/m²) <27 or >40, or were using antihypertensive medication. Previous investigations in overweight/obese subjects have shown an inverse relation between FMD and 24-h sodium excretion, which guided the selection criteria in this investigation (11). Nine volunteers did not meet the selection criteria. Thirty-two volunteers were admitted to the study, one withdrew before the study began for personal reasons, and one withdrew before the study ended for personal reasons unrelated to the dietary intervention. One volunteer withdrew before the study ended because of an inability to comply with the protocol. Twenty-nine subjects (7 men and 22 women) completed the study. The protocol was approved by the CSIRO Human Nutrition Human Research Ethics Committee (HREC06/32), and all subjects gave written informed consent.

Study methods

This study had a randomized crossover design consisting of two 2-wk dietary interventions, with no washout periods between interventions. The participants were not blinded to interventions. The subjects were randomly assigned to either a US diet (150 mmol Na/d) or an LS diet (50 mmol Na/d) by using a numbered random-allocation sequence generated by computer (CLINSTAT software; Martin Bland, York, United Kingdom) by a person blinded to interventions. The participants were not blinded to interventions. One volunteer withdrew before the study ended because of an inability to comply with the protocol. Twenty-nine subjects (7 men and 22 women) completed the study. The protocol was approved by the CSIRO Human Nutrition Human Research Ethics Committee (HREC06/32), and all subjects gave written informed consent.

Diets

The dietary interventions were an LS diet (50 mmol/d) and a US diet (150 mmol/d). Both diets were designed to ensure weight stability and were measured. The background diet was designed to keep potassium intake constant across both diet periods (100 mmol/d) because of the potential BP-lowering effect of potassium (16). Because saturated fat may also affect FMD and BP, our aim was to keep total and saturated fat constant across the diets. No other micro- or macronutrients were targeted. Subjects were advised by a trained dietician on how to achieve either an LS diet (50 mmol/d) or a US diet (150 mmol/d) and to keep their weight stable. The participants were supplied with salt-free bread and butter during the low-salt diet and salted bread and butter during the US diet. No other study foods were provided. The participants prepared all of their meals at home after the advice provided. Participants were given written dietary advice for both diets and education on LS foods and label reading to aid compliance with the diet. Subjects then returned after 2 wk to receive instruction on the next diet. Three-day weighed food records were collected for each week of the study, which included 2 weekdays and 1 weekend day for each record. The food records were collected during the second week of each intervention and were analyzed while the volunteers were present to ensure accuracy by using a computerized database of Australian foods (version 4, 1998, Foodworks Professional Edition; Xyris Software, Highgate Hill, Australia). Scales for weighing food were provided.

Weight and height

Body height was measured at baseline to the nearest 0.1 cm with a stadiometer (SECA, Hamburg, Germany) while the participants were barefoot. Body weight was measured to the nearest 0.05 kg with calibrated electronic digital scales (AMZ 14; Mercury, Tokyo, Japan) while the participants were wearing light clothing and no footwear.

Flow-mediated dilatation

Endothelium-dependent FMD of the right brachial artery was measured, under conditions previously described (17), after each dietary intervention. Subjects lay quietly for 5 min before arterial diameter was measured, in the morning after an overnight fast, with a 7.5-MHz linear array transducer (Accuson Aspen Duplex, Mountain View, CA) before and after forearm ischemia was caused by inflation of a BP cuff applied to the forearm to 200 mm Hg for 5 min. Images were recorded at baseline (before compression), 30 s before cuff release, and then every 15 s after cuff release for 3 min. Arterial diameter was measured offline by a single observer using ultrasonic calipers at end-diastole, incident with the R-wave on the echocardiogram (ECG). The FMD response was calculated as the percentage change from the baseline diameter. The intraobserver CV for FMD was 16% (n = 12), which was calculated in healthy subjects who were scanned on 2 separate occasions after an overnight fast. Endothelium-independent FMD, ie, the response to glyceryltrinitrate (GTN) was not assessed, and flow rates in response to ischemia were not measured. The operator who performed the FMD measurements was unaware of the diet assignment at the time of the test.

Pulse wave velocity

Aortic PWV was measured via Doppler recordings at the carotid and femoral arteries at 2 and 4 wk. Approximately 10 consecutive beats were recorded to cover a complete respiratory cycle. A simultaneous ECG recording was used to calculate the interval between the R-wave and the upstroke of each sound wave. The difference between the average intervals for each artery was calculated. PWV was then determined by dividing the measured surface anatomical distance by this difference.

Augmentation index

Vascular measurements were performed as previously described by using the SphygmoCor BP analysis system (AtCor Medical, Sydney, Australia) at 2 and 4 wk (18). All AI measurements were performed by the same operator, and the intraobserver CV for AI was 12.8% based on data for healthy individuals (n = 12), who were tested on 2 separate occasions and unadjusted for BP differences between visits.
Blood pressure

The subjects rested in a seated position for 5 min before BP was measured with an automated sphygmomanometer (SureSigns V3; Philips, North Ryde, Australia) at baseline and at 2 and 4 wk. Four consecutive BP measurements were taken, each 2 min apart. The first reading was discarded, and the mean of the next 3 consecutive readings with SBP readings within 10 mm Hg and diastolic BP (DBP) readings within 5 mm Hg of each other was used in the study; additional measurement were made if required.

Laboratory analysis

A 24-h urine sample was collected at baseline and at 2 and 4 wk for the measurement of sodium and potassium excretion to assess dietary compliance. Measurements of sodium and potassium were made by the Institute of Medical and Veterinary Sciences (Adelaide, South Australia)—a certified commercial laboratory. Urinary creatinine was used to assess completeness of laboratory analysis used in the study; additional measurement were made if required.

Statistical analysis

For a crossover design, power calculations indicate that to detect a mean difference in FMD of 2.8% absolute, 30 subjects would need to complete the study (z statistic = 0.05; power = 0.8). There was 80% power to detect a 2-m/s difference in PWV and a 5% difference in AI. Analysis of variance with repeated measures was used (with diet as the within-subject factor) with and without covariates, including diet order, BP, and baseline urinary sodium excretion. Pearson correlation analyses were conducted to assess the association of change between variables. Analyses were performed with SPSS 14.0 for Windows (SPSS Inc, Chicago, IL). Significance was set at P < 0.05. Continuous data are presented as means ± SDs.

RESULTS

Subjects

Twenty-nine subjects completed the study. Baseline characteristics are described in Table 1. Weight remained stable across each intervention (LS: 86 ± 9.7 kg; US: 87 ± 9.6 kg; P > 0.05). Baseline weight was correlated with baseline 24-h sodium (r = 0.509, P = 0.005), potassium (r = 0.572, P = 0.001), urea (r = 0.705, P < 0.0001), and creatinine (r = 0.706, P < 0.0001) excretion.

Dietary analysis and compliance

Compliance with the protocol was confirmed by 24-h urinalysis. Twenty-four-hour sodium excretion was significantly lower (2.5-fold; P < 0.001) with the LS diet (64.1 ± 41.3 mmol/L) than with the US diet (156.3 ± 56.7 mmol); 24-h potassium excretion remained constant between treatments (LS: 78.5 ± 29.8 mmol; US: 80.6 ± 25.4 mmol; P = 0.646). Energy, protein, carbohydrate, potassium and total, polyunsaturated, monounsaturated, and saturated fat intakes were not significantly different between diets (Table 2).

Vascular function

Brachial artery FMD

Precompression brachial artery diameter was not significantly different between treatments (LS: 4.14 ± 0.85 mm; US: 4.10 ± 0.78 mm; P = 0.618). Absolute FMD was 1.52% greater (P = 0.001) with the LS diet (4.89 ± 2.42%) than with the US diet (3.37 ± 2.10%)—a relative increase of ≈30%. The data were analyzed to determine whether changes in weight and in BP had an effect on FMD. Changes in FMD remained significant (P = 0.002, P = 0.009, P = 0.007, and P = 0.005, respectively) after weight loss, change in SBP, change in DBP, and saturated fat between treatments were controlled for. Baseline SBP, DBP, and urinary sodium excretion were not correlated with the change in FMD nor with absolute FMD after the LS or US diet.

FMD was not correlated with 24-h sodium excretion after the LS or US diets, and the change in FMD was not correlated with the change in urinary sodium (Figure 1) or with the change in SBP (Figure 2) or DBP. The data were analyzed for carryover effects, with diet order included as a covariate in the analysis of variance; no carryover effect was noted. Age was not related to the change in FMD.

Blood pressure

A small significant reduction in SBP was observed after sodium reduction (LS: 112 ± 11 mm Hg; US: 117 ± 13 mm Hg;
$P = 0.02$), and there were nonsignificant reductions in DBP and mean arterial pressure after sodium reduction (Table 3). There was no correlation between the change in sodium excretion and the change in SBP (Figure 3). Weight and resting BP variables were not correlated at baseline.

**Pulse wave velocity**

There was no change in PWV between treatments (LS: 10.49 ± 4.14 m/s; US: 10.49 ± 3.07 m/s; $P = 0.991$) with or without covariates of weight loss, change in 24-h sodium excretion, change in SBP and DBP, and baseline SBP and DBP.

**Augmentation index**

AI did not change significantly (LS: 27.49 ± 9.02%; US: 28.06 ± 10.19%; $P = 0.581$) with or without covariates of weight loss, change in 24-h sodium excretion, change in SBP and DBP, and baseline SBP and DBP.

### DISCUSSION

The main finding of this study was that a sodium-restricted diet improved endothelial function, assessed by FMD, relative to a US diet in overweight and obese normotensive subjects. Change in FMD was unrelated to changes in BP, which suggests that a mechanism other than change in BP is responsible for the effect of salt on FMD.

In salt-sensitive hypertensive rats, it was shown that vascular relaxation is impaired by a high salt intake, which is thought to be

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**TABLE 3**

<table>
<thead>
<tr>
<th>Measure</th>
<th>Low-salt diet</th>
<th>Usual-salt diet</th>
</tr>
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<tbody>
<tr>
<td>Baseline BA diameter (mm)</td>
<td>4.14 ± 0.85</td>
<td>4.10 ± 0.78</td>
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<tr>
<td>Postrelease BA diameter (mm)</td>
<td>4.33 ± 0.85</td>
<td>4.23 ± 0.77</td>
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<tr>
<td>FMD (%)</td>
<td>4.89 ± 2.42$^2$</td>
<td>3.37 ± 2.10</td>
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<tr>
<td>SBP (mm Hg)</td>
<td>112 ± 11$^a$</td>
<td>117 ± 13</td>
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<tr>
<td>DBP (mm Hg)</td>
<td>72 ± 8</td>
<td>73 ± 7</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>86 ± 9</td>
<td>88 ± 8</td>
</tr>
<tr>
<td>PWV (m/s)</td>
<td>10.49 ± 4.14</td>
<td>10.49 ± 3.07</td>
</tr>
<tr>
<td>AI (%)</td>
<td>27.49 ± 9.02</td>
<td>28.06 ± 10.19</td>
</tr>
<tr>
<td>24-h Sodium excretion (mmol)</td>
<td>64.1 ± 41.3$^d$</td>
<td>156.3 ± 56.7</td>
</tr>
<tr>
<td>24-h Potassium excretion (mmol)</td>
<td>78.5 ± 29.8</td>
<td>80.6 ± 25.4</td>
</tr>
</tbody>
</table>

$^a$ All values are means ± SDs; $n = 29$ (22 women and 7 men). AI, augmentation index; BA, brachial artery; DBP, diastolic blood pressure; FMD, flow-mediated dilatation; MAP, mean arterial pressure; PWV, pulse wave velocity; SBP, systolic blood pressure.

$^2$, $^d$ Significantly different from the usual-salt diet (repeated-measures ANOVA): $^2P < 0.01$, $^dP < 0.05$, $^aP < 0.001$. 

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**FIGURE 1.** Correlation between change in flow-mediated dilatation (FMD) and change in 24-h sodium excretion in 29 overweight and obese men and women after consumption of a usual-salt diet and a low-salt diet for 2 wk each in a crossover design. Pearson correlation analyses were conducted to assess the association of change between variables. There was no significant relation ($r = 0.090$, $P = 0.642$).

**FIGURE 2.** Correlation between change in flow-mediated dilatation (FMD) and change in systolic blood pressure (SBP) in 29 overweight and obese men and women after consumption of a usual-salt diet and a low-salt diet for 2 wk each in a crossover design. Pearson correlation analyses were conducted to assess the association of change between variables. There was no significant relation ($r = -0.082$, $P = 0.671$).

**FIGURE 3.** Correlation between change in systolic blood pressure (SBP) and change in 24-h sodium excretion in 29 overweight and obese men and women after consumption of a usual-salt diet and a low-salt diet for 2 wk each in a crossover design. Pearson correlation analyses were conducted to assess the association of change between variables. There was no significant relation ($r = -0.032$, $P = 0.869$).
due to decreases in endothelial NO synthase activity or an increase in endogenously generated NO synthase inhibitors (19–24). Human studies have shown that NO production during a high-salt diet may be impaired. Bragulat and de la Sierra (25) showed that a high salt intake (250 mmol Na/d) decreased the plasma concentration and urinary excretion of nitrates and the plasma concentration of endothelin, which suggests that this level of salt intake may adversely affect endothelial cell function (25). NO production decreases after salt loading and can be reversed after salt reduction (10, 26). Dishy et al (10) have also shown that the decrease in NO production after salt loading was not related to the changes in BP or in NO-mediated vascular responses.

A suppressant effect of high salt intake on NO production could occur via changes in plasma asymmetric dimethylarginine (ADMA)—a natural NO synthase inhibitor (26, 27). Fujiwara et al (26) showed that plasma ADMA concentration increased after salt loading and decreased after salt reduction, and these changes were inversely correlated with the change in plasma NO ($r = -0.64, P = 0.003$). Fang et al (27) also found significant correlations between plasma ADMA, mean BP, and NO after salt loading in normotensive, salt-sensitive individuals.

It has also been postulated that a high salt intake results in a decreased responsiveness to NO in smooth muscle cells in a rat model (28, 29). In rats, excess dietary salt caused the down-regulation of soluble guanylate cyclase (sGC), the principle target of NO, which produced a decrease in the production of cyclic GMP (cGMP) (30). NO activates sGC to form cGMP, and an elevated cGMP concentration causes vascular smooth muscle relaxation and regulates vascular tone in various vascular beds (31, 32). It was shown that this impairment in sGC results from a direct effect of the salt itself (33). However, we did not measure the response to GTN and accept that smooth muscle relaxation as well as NO production may also have improved with salt reduction. In all of our previous studies of diet, the response to GTN has not changed and we elected to omit this step to reduce the burden on the study participants. Other recent studies of FMD also omitted this step (34). Longer studies that also include this measure of endothelial independent vasodilation are needed.

We found no relation between change in BP and FMD after LS and US salt intakes. Despite this finding, we recognize that it is possible that the effect on FMD occurs via changes in BP. Both measures are quite variable, so a significant relation between the 2 may be hard to observe. More robust measures of these variables, such as 24-h BP monitoring, would be important for future studies to reduce this variability. We also found no relation between the change in sodium and the change in FMD or between the change in sodium and the change in SBP. Urinary sodium excretion varies considerably, and a single 24-h collection may not have been adequate. Liu et al (35) suggests that as many as six 24-h urine collections are needed to establish correlations between urinary sodium and physiologic variables.

Salt reduction reduces BP in normotensive individuals (36). The decreases in BP observed in the present study were consistent with a recent systematic review of BP and salt in normotensive subjects (37). Long-term salt reduction may have additional benefits beyond BP reduction (38, 39). Cook et al (38) reported a 25–30% reduction in risk of a cardiovascular event after long-term (>18 mo) sodium reduction, even though long-term sodium reduction was relatively small (44 and 33 mmol/24 h at 18 and 36–48 mo, respectively). The detrimental effects of a high salt intake beyond BP have been reported previously (40–43). A high salt intake has been shown to cause renal and myocardial fibrosis and left ventricular impairment in rats (41, 42). A high sodium excretion has been associated with ventricular hypertrophy in adults and in children (43–46). Jula and Karanko (39) observed that left ventricular mass decreased by 5.4% after 12 mo of salt reduction.

We speculate that the effect of salt on endothelial function may occur via a neurohumoral response. It has been shown that stress-related endothelial dysfunction can be prevented by blocking cortisol production (47). Salt reduction decreases urinary free cortisol excretion, which might indicate lower cortisol production (48, 49). Cortisol, has been shown to suppress NO release by decreasing endothelial NO synthase protein production and inhibiting intracellular Ca$^{2+}$ mobilization in bovine coronary artery endothelial cells (50). This hypothesis needs to be examined in a prospective study.

PWV, an indirect measure of arterial stiffness and an independent predictor of mortality (51), did not change significantly. The study was powered to detect a 2-m/s difference in PWV, but the intervention may not have been long enough because evidence suggests that long-term sodium reduction (8–60 mo) improves PWV independently of BP (15). AI, another measure of arterial stiffness (52), also did not change significantly.

Limitations of the current study include the short duration and the failure to measure NO metabolites, ADMA, cortisol, or the GTN response. The US diet at baseline was not assessed; therefore, we had no data on differences between intervention and prestudy intakes. The usual diets of the subjects may have changed as a result of the intervention, and other nutrient intakes may also have changed. However, we believe that the randomized design accounted for any effects of the change in diet from baseline. The diets in the 2 intervention phases did not differ significantly, other than in sodium content.

In conclusion, short-term sodium reduction in a weight-stable setting improved endothelial function as assessed by FMD in overweight and obese normotensive subjects. We observed beneficial effects of sodium reduction on BP, but not on other measures of vascular function. These findings further evidence of the benefits of an LS diet on vascular function beyond the reduction in BP.

The authors’ responsibilities were as follows—JBK and PMC: developed the hypotheses tested in the study; KMD: conducted the study, performed the vascular and BP measurements, helped design the diet protocol, performed the statistical analyses, interpreted the results, and drafted the manuscript; JBK: designed the study and diet protocol, interpreted the data, and wrote the manuscript; and PMC: had overall responsibility for the study, performed the statistical analysis, interpreted the results, and wrote the manuscript. None of the authors had any conflicts of interest in relation to this study.

REFERENCES


