Dietary salt loading impairs arterial vascular reactivity[1–3]

Alwyn S Todd, Robert J MacGinley, John BW Schollum, Richard J Johnson, Sheila M Williams, Wayne HF Sutherland, Jim I Mann, and Robert J Walker

ABSTRACT

Background: Studies of sodium have shown improvements in vascular function and blood pressure (BP). The effect of chronic sodium loading from a low-sodium diet to a Western diet on vascular function and BP has been less well studied.

Objective: The objective was to examine the effects of dietary salt intake on vascular function and BP.

Design: Thirty-five hypertensive volunteers met the inclusion criteria. After a 2-wk run-in with a low-sodium diet (60 mmol/d), the participants maintained their diets and were randomly assigned to receive sequentially 1 of 3 interventions for 4 wk, with a 2-wk washout between interventions: sodium-free tomato juice (A), tomato juice containing 90 mmol Na (B), and tomato juice containing 140 mmol Na (C). The outcomes were changes in pulse wave velocity (PWV), systolic BP (SBP), and diastolic BP (DBP).

Results: The difference in PWV between interventions B and A was 0.39 m/s (95% CI: 0.18, 0.60 m/s; $P \leq 0.001$) and between C and A was 0.35 m/s (95% CI: 0.13, 0.57 m/s; $P \leq 0.01$). Differences in SBP and DBP between interventions B and A were 4.4 mm Hg (95% CI: 1.2, 7.8 mm Hg; $P \leq 0.01$) and 2.4 mm Hg (95% CI: 0.8, 4.1 mm Hg; $P \leq 0.001$), respectively, and between interventions C and A were 5.6 mm Hg (95% CI: 2.7, 8.4 mm Hg; $P \leq 0.01$) and 3.3 mm Hg (95% CI: 1.5, 5.0 mm Hg; $P \leq 0.001$), respectively. Changes in PWV correlated with changes in SBP ($r = 0.52$) and DBP ($r = 0.58$).

Conclusions: Dietary salt loading produced significant increases in PWV and BP in hypertensive volunteers. Correlations between BP and PWV suggest that salt loading may have a BP-independent effect on vascular wall function. This further supports the importance of dietary sodium restriction in the management of hypertension. This trial was registered with the Australian and New Zealand Clinical Trials Registry as ACTRN12609000161224. *Am J Clin Nutr* 2010;91:557–64.

INTRODUCTION

Excess dietary salt has long been considered a contributing factor to the development of hypertension, with references relating to the harmful effects of excess salt dating back to >4000 y ago (1). Numerous human studies have shown a causal link between high salt intake, high blood pressure (BP), and cardiovascular disease. Similarly, the reduction of dietary salt intake in clinical and experimental studies has been shown to improve both vascular function and BP (2). The Intersalt study (3) suggested that a decrease in BP of 3.1–6.0 mm Hg can be achieved if salt intake is reduced by 5.9 g/d (100 mmol Na). The Dietary Approaches to Stop Hypertension (DASH) study (4) showed that the DASH diet and a low salt intake resulted in a 7.1-mm Hg reduction in systolic BP (SBP) in normotensive subjects and a 11.5-mm Hg reduction in SBP in hypertensive subjects when compared with the control diet group who had a high salt intake.

He and MacGregor (5), in a meta-analysis of the published studies that achieved a decrease in dietary salt intake of ≥2.4 g/d (40 mmol Na) and had a minimum duration of 4 wk, found a dose-response relation between BP and sodium intake between 3 and 12 g/d (50–200 mmol Na/d) and showed that a reduction in sodium intake of 75 mmol/d can decrease SBP and diastolic BP (DBP) by 5 and 3 mm Hg, respectively, in hypertensive subjects and by 2 and 1 mm Hg, respectively, in normotensive subjects over a period of ≥4 wk.

The few published studies of sodium loading that show differences in BP and vascular function (6–8), tended to have small sample sizes, were of short duration, and often used unphysiologic concentrations of sodium loading and hence have limited applicability to a standard outpatient clinical setting when determining the effects of a longer-term high-salt diet. To convince individuals that dietary salt restriction is beneficial, it is important to show the converse, ie, that dietary salt loading is detrimental.

This study was designed to examine the effect of 4 wk of dietary salt loading on arterial vascular tone and BP in a group of middle-aged hypertensive but otherwise healthy volunteers recruited from the community. We hypothesized that if dietary salt restriction improves arterial vascular tone and BP, then the converse should also occur, ie, increased salt intake would lead to a deterioration in arterial vascular tone. Confirmation of these changes would provide further supporting evidence of a role of dietary sodium restriction in the outpatient management of hypertensive individuals.

[1] From the Departments of Medical & Surgical Sciences (AST, JBWS, WHFS, JIM, and RJW) and Preventive and Social Medicine (SMW), University of Otago, Dunedin, New Zealand; the Deakin University Medical School, Deakin University, Geelong, Australia (RJM); and the Division of Renal Diseases and Hypertension, University of Colorado, Denver, CO (RJJ).

[2] Supported by the National Heart Foundation of New Zealand and the Otago Medical Research Foundation. AST was the recipient of a Neige Todhunter Award from the New Zealand Dietetic Association and a Unilever Research Scholarship.

[3] Address correspondence to RJ Walker, Department of Medical & Surgical Sciences, University of Otago, Great King Street, PO Box 913, Dunedin 9015, New Zealand. E-mail: rob.walker@otago.ac.nz.

Received September 10, 2009. Accepted for publication December 29, 2009. First published online January 27, 2010; doi: 10.3945/ajcn.2009.28645.

This study was designed to examine the effects of dietary salt intake; however, for the purposes of quantification and comparability with other published studies, dietary salt intake is defined in terms of the amount of sodium (mmol).

SUBJECTS AND METHODS

Participants

Volunteers were referred to the study by their general physician. All participants provided written informed consent before participating. Inclusion criteria were prehypertension or hypertension, defined as an SBP >130 mm Hg and a DBP >85 mm Hg or current treatment with antihypertensive therapy; age between 20 and 65 y; nonsmoker; body mass index (in kg/m^2) <30; and no history of cardiovascular disease, diabetes, or renal disease. Demographic characteristics of the population are outlined in **Table 1**.

Study methods

The single-blind randomized crossover design involved 3 intervention phases of 4-wk duration each. There was a 2-wk run in period and a 2-wk washout period between each intervention. During the run-in period, the participants were assigned to a low-sodium diet (60 mmol Na/d). This low-sodium diet was maintained for the duration of the study. After the run-in period, the participants were randomly assigned to the first of 3 intervention phases. The interventions involved the consumption of 500 mL tomato juice/d. The tomato juice contained 0 mmol Na (A), 90 mmol Na (B), or 140 mmol Na (C). For each intervention and between each intervention, the participants remained maintained their low-sodium diet before being assigned to the next phase. The participants were asked to keep exercise levels and alcohol intake constant during the study. Dietary supplements that may have contributed to sodium intake (eg, protein powder and multivitamin supplements) were discontinued for ≥2 wk before prebaseline measurements. All participants maintained their usual antihypertensive medication use because this trial was aimed to reproduce a typical outpatient setting in which dietary advice was complementary to pharmacologic intervention rather than an alternative treatment. Because high-salt interventions can precipitate marked elevations in BP, an elevated BP > 160 (SBP)/100 (DBP) mm Hg was an indication for withdrawal from the study. This study was approved by the Lower South Regional Ethics Committee (Dunedin, New Zealand).

Dietary advice and counseling were given by a registered dietitian. Resources were developed for the study and were designed for 60 mmol Na/d. All resources were provided to the participants at their prebaseline consultation. Dietary diaries were kept by participants for 3 d before the prebaseline measurements and consultation and again during each intervention period. Dietary recalls were used to monitor dietary intake and direct further counseling to improve compliance.

TABLE 1
Demographic and biochemical characteristics of all participants at recruitment (n = 34)[1]

Characteristic	Value
Sex [n (%)]	
Male	13 (38)
Female	21 (62)
Ethnicity [n (%)]	
European	33 (97)
Indian/Asian	1 (3)
No antihypertensive therapy [n (%)]	9 (23)
Antihypertensive therapy [n (%)][2]	25 (77)
≥2 Antihypertensive agents[2]	13 (38)
Age (y)	51.8 ± 7.6[3]
Height (cm)	161 ± 27
BMI (kg/m^2)	25.7 ± 5.2
SBP (mm Hg)	134 ± 15
DBP (mm Hg)	84 ± 9
Pulse wave velocity (m/s)	7.50 ± 0.52
Urinary sodium:creatinine	9.4 ± 6.0

[1] DBP, diastolic blood pressure; SBP, systolic blood pressure.
[2] Angiotensin-converting enzyme inhibitor (n = 15), angiotensin receptor blocker (n = 3), diuretic (n = 11), calcium channel antagonist (n = 4), and β-blocker (n = 7).
[3] Mean ± SD (all such values).

Randomization

Randomization was done by a third party using www.randomisation.com to generate the randomization sequence for the tomato juice interventions. The sequence was given to the Dunedin hospital pharmacy, where a study-dedicated pharmacist added the allocated salt to the tomato juice. The investigators remained blind to the randomization sequence throughout the study. Participants were initially blind to the randomization sequence, but once they began each phase of the intervention they would clearly be aware of the presence or absence of added salt. They were asked not to tell the investigator which tomato juice they had received. Analysis of the data was undertaken with the investigators remaining blinded to treatment allocation until after the initial analyses had been completed.

Blood pressure

BP was measured at the clinic with a calibrated digital BP monitor (model UA-767;AND Instruments Ltd, Tokyo, Japan) at screening and at weeks 0, 1, 2, and 4 of each intervention. The participants were seated for 5 min to rest before ≥4 BP readings were taken over a 10-min period. The first reading was discarded, and the subsequent 3 readings were averaged.

Pulse wave velocity and pulse wave analysis

Pulse wave velocity (PWV) and pulse wave analysis (PWA) were measured by using a SphygmoCor system (AtCor Medical, Sydney, Australia) by a single trained operator. The estimated reliability of measurements was calculated from 3 baseline readings for each patient and found to be 0.89 ($P \leq 0.001$). All measurements were taken at 0, 1, 2, and 4 wk for each intervention. PWA was performed at the radial site, and continuous measurements were taken until a report with a quality index >80 was recorded. Sphygmocor software used the captured peripheral wave form to derive an aortic wave form and to estimate the Aortic Augmentation Index. PWV was measured between the carotid and femoral arteries, with measurements taken from the right carotid to the left femoral arteries and from

the left carotid to the right femoral arteries, and the 2 values were averaged (AtCor Medical Pty Ltd, Sydney, Australia).

Blood and urine samples were collected after an 8-h overnight fast, at entry, and at weeks 0 and 4 of each intervention from all participants. Plasma urea and electrolytes and urinary sodium, potassium, creatinine, and calcium were routinely measured by Southern Community Laboratories (Dunedin, New Zealand) on collection. Additional blood samples were centrifuged, and the plasma was stored (−80 °C) until the conclusion of the study until batch analysis of vasoactive hormones [renin, aldosterone, endothelin-1, insulin, atrial natriuretic peptide (ANP), B-natriuretic peptide (BNP), and *N*-terminal C-natriuretic peptide (CNP); Endolab Canterbury Health Laboratories, Christchurch, New Zealand] and surrogate markers of oxidative stress and inflammation [nitrate and nitrite concentrations, lipid peroxides (9), lipofuscin-like fluorophores (LFs) (10), high-sensitivity C-reactive protein (hs-CRP)] were analyzed in the Hunter Nye Laboratory (Department of Medicine, University of Otago, Dunedin, New Zealand). Plasma LFs, which are also a marker of dietary lycopene intake as well as oxidation status, were measured at 350 nm excitation and 460 nm emission in the ethanol/diethyl ether (3/1; vol:vol) extract of plasma. Endogenous plasma MMP-9 was assessed in heparinized plasma samples by using the Biotrak Activity Assay System (Amersham Biosciences, Buckinghamshire, United Kingdom). This system measures the endogenous activity of the specific matrix metalloproteinase (MMP) by measuring substrate cleavage of antibody captured MMP isoforms. The CV for MMP-9 assays ranged from 3.7% to 4.4% (average: 4.1%) (11). Compliance with the interventions was continuously assessed throughout the study and consisted of 3 different but complementary components: dietary diaries, urinary electrolytes, and plasma LFs.

Statistical analysis

The primary outcome was the change in PWV. The power calculation for the study was calculated on the basis of an estimated 10% change in PWV after salt loading. The SD of 2 m/s was derived from the study by Liang et al (12). Because hypertensive patients had not previously been studied, we increased the SD to 2.4 m/s. Thus, the study was powered with a sample size of 31 at 90% to detect a significant difference of 0.12 m/s (10%) in PWV by using the 5% level of significance.

The data were analyzed in accordance with an intention-to-treat analysis. A mixed model, with a random effect for participants, using likelihood methods was used to analyze the data. The model included terms for order, treatment, and week-0 (baseline) observations for each dependent variable. All values were adjusted for time and order, and an effect for time was also included when observations were made at weeks 1, 2, and 4. Because the interaction effects for time and treatment were not statistically significant, they were not included in the model. The results are presented as differences (95% CIs) between the treatments averaged over weeks 1, 2, and 4. Stata 10 was used to analyze the data (Stata Statistical Software version 10, 2007; StataCorp LP, College Station, TX).

A sensitivity analysis was carried out to consider the effects of the missing values on the differences between treatments. Because some of the participants were withdrawn from treatments B and C because of an excessively high BP or because they failed to complete treatment C when it was the last treatment, the missing values were unlikely to be missing at random. Therefore, an estimate for the expected differences between treatments was proposed for PWV and SBP. Differences (mean ± SD) of 1.5 ± 0.5 m/s for PWV and of 9 ± 0.5 mm Hg for SBP were used in a pattern mixture model after chained equations were used to impute the missing values.

RESULTS

Participants

A total of 35 participants were assigned to the low-sodium diet; however, one person withdrew during the first week. Thirty-four participants were randomly assigned to the first intervention phase (**Figure 1**). The baseline characteristics of the participants are presented in Table 1. Thirty-three participants successfully completed intervention A, 32 participants completed the 4 wk of intervention B, and 24 participants completed the 4 wk of intervention C (Figure 1). One participant was withdrawn from intervention B because of an elevated BP (>160/100 mm Hg) and peripheral fluid retention, 7 participants were withdrawn from intervention C because of an elevated BP (>160/100 mm Hg) and symptoms of headaches, nausea, vomiting, frequent bowel motions, fluid retention, or general ill feelings. One participant withdrew from the study because of a broken collar bone, and 2 participants refused intervention C. Recruitment characteristics are shown in Table 1. There were no significant differences observed between values measured at the end of each washout period and those at baseline.

Pulse wave velocity

Mean (± SD) values for carotid to femoral PWV are shown in **Table 2**. The difference between intervention B (dietary sodium: 150 mmol/d) and intervention A (dietary sodium: 60 mmol/d) for PWV was 0.39 m/s (95% CI: 0.18, 0.60; $P \leq 0.001$). The difference between intervention C (dietary sodium: 200 mmol/d)

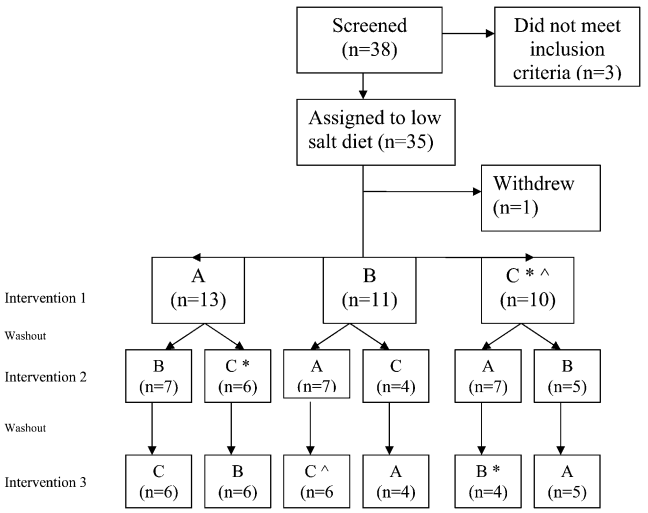

FIGURE 1. Recruitment and treatment allocation of participants. A, 60 mmol Na/d; B, 150 mmol Na/d; C, 200 mmol Na/d. *n = 1 withdrew after 1 wk; ^n = 1 withdrew after 2 wk.

TABLE 2
Pulse wave velocity (PWV), arterial pressure, augmentation index (AIx), and differences between interventions A, B, and C at weeks 1, 2, and 4[1]

	Mean ± SD			Difference (95% CI)		
	A	B	C	B−A	C−A	C−B
PWV (m/s)						
Week 0	7.50 ± 1.24	7.48 ± 0.93	7.55 ± 1.21			
Week 1	7.35 ± 1.04	7.77 ± 1.24	7.76 ± 1.47			
Week 2	7.29 ± 0.83	7.64 ± 1.10	7.57 ± 1.08			
Week 4	7.34 ± 1.12	7.63 ± 0.95	7.84 ± 1.20	0.39 (0.18, 0.60)[2]	0.35 (0.13, 0.57)[3]	−0.04 (−0.26, 0.18)
SBP (mm Hg)						
Week 0	126 ± 11	128 ± 14	130 ± 14			
Week 1	126 ± 13	132 ± 14	135 ± 14			
Week 2	129 ± 13	133 ± 14	133 ± 13			
Week 4	127 ± 11	133 ± 17	137 ± 15	4.4 (1.7, 7.1)[3]	5.8 (2.9, 8.6)[2]	1.4 (−1.5, 4.2)
DBP (mm Hg)						
Week 0	81 ± 7	81 ± 8	83 ± 8			
Week 1	80 ± 8	82 ± 8	84 ± 6			
Week 2	80 ± 8	82 ± 9	84 ± 7			
Week 4	79 ± 7	84 ± 7	85 ± 7	2.5 (0.8, 4.2)[3]	3.4 (1.7, 5.2)[2]	0.9 (−0.8, 2.7)
AIx						
Week 0	92 ± 20	91 ± 21	94 ± 21			
Week 1	86 ± 19	91 ± 22	92 ± 17			
Week 2	88 ± 23	90 ± 22	92 ± 18			
Week 4	91 ± 21	96 ± 28	99 ± 31	5.5 (1.4, 9.6)[3]	4.4 (0.1, 8.6)[4]	1.1 (−5.4, 3.2)
Aortic AIx						
Week 0	27 ± 11	28 ± 10	30 ± 10			
Week 1	24 ± 12	29 ± 11	29 ± 11			
Week 2	26 ± 14	29 ± 12	30 ± 11			
Week 4	30 ± 11	29 ± 10	28 ± 10	2.1 (0.2, 4.1)[4]	0.3 (−1.8, 2.3)	−1.9 (−4.0, 0.2)

[1] A, 60 mmol Na/d; B, 150 mmol Na/d; C, 200 mmol Na/d. DBP, diastolic blood pressure; SBP, systolic blood pressure. The analysis was adjusted for week 0, order, and time.
[2] $P \leq 0.001$.
[3] $P \leq 0.01$.
[4] $P \leq 0.05$.

and intervention A was 0.35 m/s (95% CI: 0.13, 0.57; $P \leq 0.01$). The difference between interventions B and C, −0.04 m/s (95% CI: −0.26, 0.18), was not statistically significant.

Blood pressure

The difference in SBP between interventions B and A was 4.4 mm Hg (95% CI: 1.7, 7.1), between C and A was 5.8 mm Hg (95% CI: 2.8, 8.6), and between C and B was 1.4 mm Hg (95% CI: −1.5, 4.2). The respective differences for DBP were 2.5 mm Hg (95% CI: 0.8, 4.2), 3.4 mm Hg (95% CI: 1.7, 5.2), and 0.9 mm Hg (95% CI: 0.8, 2.7).

Pulse wave analysis

The peripheral and aortic augmentation indexes were significantly higher in intervention B than in intervention A: 5.5 (95% CI: 1.4, 9.6) and 2.1 (95% CI: 0.2, 4.1), respectively (Table 2). There was no significant difference between intervention C and interventions A or B. Although increased sodium intake increased both BP and PWV, the correlation between changes in BP and PWV were less marked: SBP and PWV ($r = 0.52$) and DBP and PWV ($r = 0.58$) (**Figure 2**).

Sensitivity analysis for the 23 missing outcome values suggested that the difference in PWV between groups B and A was 0.41 m/s (95% CI: 0.04, 0.84) and that between groups C and A was 0.47 m/s (95% CI: −0.30, 1.23). The differences for SBP were 4.8 mm Hg (95% CI: 2.0, 7.7) and 6.9 mm Hg (95% CI: 3.8, 10.0) for the same comparisons.

Plasma electrolytes and vasoactive hormones are shown in **Table 3**. Plasma sodium remained constant across all 3 interventions; however, small significant decreases were observed in plasma creatinine and plasma potassium in the highest sodium-supplemented group. There was a small but significant difference in plasma uric acid with sodium loading, which resulted in a decrease in plasma uric acid concentrations of 0.02 mmol/L (95% CI: −0.04, 0.01; $P \leq 0.01$) in intervention B and of 0.03 mmol/L (95% CI: −0.04, 0.01; $P \leq 0.01$) in intervention C.

Sodium loading produced the appropriate lowering of plasma renin activity and aldosterone after adjustment for baseline, order, and time (Table 3). There were no changes in the concentrations of endothelin-1 or the natriuretic peptides (ANP and BNP) across all groups (**Table 4**). Markers of oxidative stress, lipid peroxides, total nitrate production, or hs-CRP did not change across any study group (data not shown). Because hypertension can be associated with an insulin-resistant state, insulin sensitivity was calculated (13) but failed to show any difference across study groups (data not shown).

MMP-9 was measured as a surrogate marker for increased matrix turnover in the arterial vessel wall. In response to the sodium loading (intervention C), there was a reduction in MMP-9 activity that appeared to be inversely related to PWV, which was

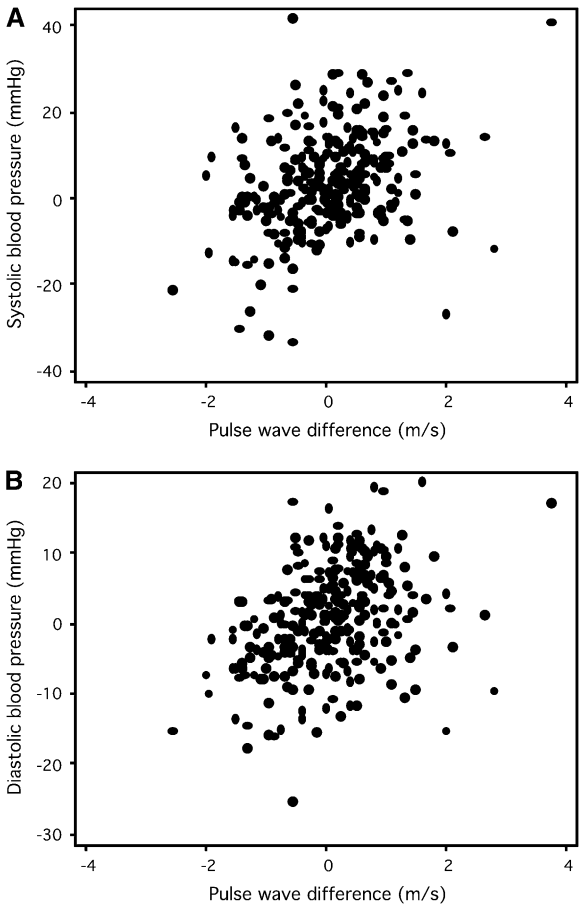

FIGURE 2. Correlations between differences in systolic blood pressure and differences in pulse wave velocity (A) and differences in diastolic blood pressure and differences in pulse wave velocity (B). Correlations were determined by random-effects generalized least squares regression: systolic blood pressure and pulse wave velocity ($r = 0.52$, $P < 0.05$) and diastolic blood pressure and pulse wave velocity ($r = 0.58$, $P < 0.05$).

not significant (Table 4). *N*-terminal C-natriuretic peptide (CNP), which is also synthesized in the vessel wall, decreased significantly by 1.01 pmol/L (95% CI: −1.75, 0.27; $P < 0.01$ after adjustment for order and time) in intervention C compared with intervention A (Table 4).

Antihypertensive therapy

All participants initially maintained their antihypertensive therapy at the commencement of the study. Six of 24 patients receiving antihypertensive therapy had a reduction in antihypertensive therapy during the study because of the development of symptomatic hypotension with the low-sodium diet. Conversely, 5 subjects were withdrawn early from the sodium-loading interventions because their BP rose rapidly to exceed the preset safety targets for BP.

Analysis of 4-d dietary diaries showed that macro- and micronutrient intakes were similar across all 3 interventions (**Table 5**). Dietary sodium and potassium intakes from food remained constant across all 3 interventions. Total sodium intake from food and juice was 55 mmol/d in intervention A, 149 mmol/d in intervention B, and 197 mmol/d in intervention C.

Urinary analysis at week 4 for sodium:creatinine excretion after adjustment for baseline, order, and time showed a difference in early morning sodium excretion between interventions A and B of 4.5 (95% CI: 1.2, 7.8; $P \leq 0.01$), between interventions A and C of 8.2 (95% CI: 4.8, 11.6; $P \leq 0.001$), and between interventions B and C of 3.7 (95% CI: −0.3, 7.0; $P \leq 0.05$), consistent with the participants' consumption of the corresponding juice (**Table 6**). There was no difference in the urinary potassium:creatinine ratio between interventions A and C. Urinary sodium:creatinine excretion correlated with urinary calcium:creatinine excretion ($r = 0.70$).

Plasma LF concentrations increased significantly during all 3 interventions ($P < 0.001$); however, the magnitudes of these increases were not significantly different: 3.69 U/mL (intervention A), 3.93U/mL (intervention B), and 3.66 U/mL (intervention C) between interventions consistent with participant compliance with tomato juice intake (Table 6).

DISCUSSION

The major finding was that increased sodium (salt) intake to levels currently consumed in the Western diet in subjects consuming a low-salt diet caused an increase in PWV and BP. A sodium load of 150 and 200 mmol/d produced significant increases in PWV ($P \leq 0.001$) compared with the low-sodium diet. A sensitivity analysis of the missing data due to withdrawal

TABLE 3
Plasma sodium, electrolytes, and hormones at final observation for each treatment[1]

	Mean ± SD			Difference (95% CI)		
	A	B	C	B−A	C−A	C−B
Sodium (mmol/L)	140 ± 2	139 ± 2	139 ± 2	−0.8 (−8.1, 6.5)	−5.7 (−13.1, 1.7)	−4.9 (−2.3, 12.2)
Potassium (mmol/L)	4.1 ± 0.3	4.2 ± 0.3	4.0 ± 0.3	0.01 (−0.11, 0.13)	−0.11 (−0.24, 0.00)[2]	−0.12 (−0.24, −0.01)
Urea (mmol/L)	5.7 ± 1.4	5.8 ± 1.2	5.4 ± 1.2	0.08 (−0.46, 0.63)	−0.23 (−0.77, 0.32)	−0.31 (−0.23, 0.85)
Creatinine (mmol/L)	76 ± 10	76 ± 11	74 ± 12	0.0 (−2.9, 3.0)	−3.0 (−6.0, 0.4)[2]	−3.1 (−6.0, −0.1)[2]
Uric acid (mmol/L)	0.33 ± 0.08	0.31 ± 0.06	0.29 ± 0.07	−0.02 (−0.04,0.01)[3]	−0.03 (−0.04, −0.01)[3]	0.00 (−0.02, 0.02)
Renin activity ($nmol \cdot L^{-1} \cdot s^{-1}$)	3.2 ± 4.9	1.7 ± 1.6	1.4 ± 1.4	−1.05 (−1.99, −0.12)[2]	−1.07 (−2.04, −0.11)[2]	−0.02 (−0.94, 0.98)
Aldosterone (pmol/L)	320 ± 130	232 ± 86	201 ± 76	−84 (−124, −44)[4]	−122 (−163, −81)[4]	−38 (−79, 3)
Endothelin-1 (pmol/L)	1.90 ± 0.68	2.07 ± 0.63	2.09 ± 0.59	0.16 (−0.15, 0.47)	0.21 (−0.11, 0.53)	0.05 (−0.27, 0.37)

[1] A, 60 mmol Na/d; B, 150 mmol Na/d; C, 200 mmol Na/d. The analysis was adjusted for week 0, order, and time.
[2] $P \leq 0.05$.
[3] $P \leq 0.01$.
[4] $P \leq 0.001$.

TABLE 4
Differences in surrogate plasma markers of endothelial function between interventions A and C at week 4[1]

	Mean ± SD		Difference (95% CI)	
	A	C	C−A	*P*
ANP (pmol/L)	21.1 ± 10.0	22.5 ± 9.7	1.6 (−3.5, 6.8)	0.535
BNP (pmol/L)	4.7 ± 2.2	5.4 ± 3.1	0.7 (−0.7, 2.1)	0.325
N-terminal CNP (pmol/L)	16.3 ± 3.5	15.2 ± 2.7	−1.0 (−1.8, −0.3)	0.008
Active MMP-9 (ng/mL)	0.746 ± 0.476	0.651 ± 0.443	−0.122 (−0.249, 0.005)	0.060

[1] A, 60 mmol Na/d; B, 150 mmol Na/d; C, 200 mmol Na/d. ANP, atrial natriuretic peptide; BNP, B-natriuretic peptide; CNP, C-natriuretic peptide; MMP-9, matrix metalloproteinase-9. The analysis was adjusted for week 0, order, and time.

from the high sodium intervention increased the effect on PWV. Conversely, a dietary sodium reduction of a similar magnitude (100 mmol/d) in a group of 35 nonmedicated healthy postmenopausal women, whose SBP ranged from 130 to 159 mm Hg, was associated with a significant improvement in PWV in the order of 0.125 ± 0.1 m/s ($P < 0.01$) (14). Seals et al (14) suggested that the effect of dietary sodium restriction was due to a decrease in the stiffness of the large elastic arteries. Gates et al (15) showed that changes in central arterial compliance correlated with sodium intake in a small study of older men and women with stage 1 hypertension (15). The improvement in carotid artery compliance was observed within 1 to 2 wk and was sustained at 4 wk after volunteers restricted their sodium intake to 54 mmol/d compared with a baseline intake of 135 mmol/d (15). Our study paralleled these observations.

In the present study, the high dietary sodium intake (150–200 mmol/d) produced a 4.4–5.6-mm Hg increase in SBP and 2.4–3.3-mm Hg increase in DBP. It is likely that the higher sodium intake would have resulted in higher mean BPs, because 5 subjects had to be withdrawn because of a rise in BP above the preset safety levels (BP > 160/100). If we allow for the missing data, the differences for SBP between interventions C and A would be 6.9 mm Hg (95% CI: 3.8, 10.0). Indeed, this is likely an important reason why there was no pronounced difference between the modest and high sodium intake groups evident after the intention-to-treat analysis.

Changes in BP in this study were similar to findings from previous intervention studies of dietary sodium reduction, with the highest level of sodium intake producing the highest BP and the lowest level of sodium intake producing the lowest level of BP. In the meta-analysis undertaken by He and MacGregor (5), a reduction in sodium intake of ≈75 mmol/d can decrease BP by 5 ± 3 mm Hg in hypertensive subjects and of 2 ± 1 mm Hg in normotensive subjects over a period of ≥4 wk.

Interestingly, whereas an increased sodium intake increased both BP and PWV, the correlation between changes in BP and PWV were less marked: SBP and PWV ($r = 0.52$) and DBP and PWV ($r = 0.58$). This indicates that the increased dietary sodium intake may have BP-independent effects on arterial vascular function. Because the volunteers in this study had very well-controlled BP (74% were receiving antihypertensive therapy and had an average baseline BP of 134/84 mm Hg), the effects of good BP control and the antihypertensive agents used may have modified the relation between dietary sodium, BP, and arterial wall function (16).

We examined many potential mechanisms by which the increased sodium intake could have mediated the changes in arterial wall function. Oxidative stress and inflammation are mediators of endothelial dysfunction (17). However the markers measured in this study (hs-CRP, nitrate-nitrite concentrations, lipid peroxides, and insulin resistance) did not show any significant correlation. CNP, a novel vasoactive peptide thought to be derived from the vessel wall, may play a role in mediating the changes in vascular tone related to sodium intake (18). There was a significant downward trend in CNP values at the completion of the high-sodium intervention (200 mmol/d) compared with the low-sodium intervention (60 mmol/d). Vascular remodeling in the setting of hypertension involves the interaction of the MMPs

TABLE 5
Dietary intake data during each intervention[1]

Nutrient	Usual diet (n = 34)	A (n = 19)	B (n = 18)	C (n = 17)
Energy (kJ)	9364 ± 4219	7242 ± 2559	7614 ± 2183	8032 ± 2822
Protein (g)	88 ± 24	79 ± 29	84 ± 24	81 ± 28
Fat, total (g)	74 ± 33	57 ± 24	56 ± 20	65 ± 25
Saturated fat (g)	27 ± 13	19 ± 8	19 ± 7	22 ± 7
Carbohydrate (g)	287 ± 200	210 ± 76	228 ± 78	235 ± 101
Alcohol (g)	13 ± 14	11 ± 16	11 ± 14	11 ± 16
Sodium				
(mg)	2607 ± 1289	1254 ± 397	1357 ± 486	1306 ± 335
(mmol)	113 ± 55	78 ± 17	83 ± 21	75 ± 14
Potassium (mg)	4336 ± 2176	3634 ± 1086	3835 ± 1046	3605 ± 1636
Calcium (mg)	1019 ± 794	739 ± 323	759 ± 382	724 ± 290

[1] All values are means ± SDs. The data exclude energy and nutrients provided by a tomato juice supplement that was consumed during interventions A, B, and C. A, 60 mmol Na/d; B, 150 mmol Na/d; C, 200 mmol Na/d.

TABLE 6
Differences in markers of compliance at the final observation for each treatment[1]

	Mean ± SD			Difference (95% CI)		
	A	B	C	B−A	C−A	C−B
Urinary sodium:creatinine						
Week 0	6.7 ± 5.4	6.5 ± 4.8	6.8 ± 4.6			
Week 4	7.6 ± 8.6	11.8 ± 6.0	15.9 ± 8.5	4.5 (1.2, 7.8)[2]	8.2 (4.8, 11.6)[3]	3.7 (−0.3, 7.0)[4]
Plasma lipofuscin-like fluorophores (U/mL)						
Week 0	5.35 ± 1.92	5.44 ± 2.10	5.45 ± 1.83			
Week 4	9.04 ± 3.10	9.37 ± 3.03	9.11 ± 3.05	0.38 (−0.53, 1.30)	0.07 (−0.87, 1.01)	−0.31 (−1.25, 0.63)

[1] A, 60 mmol Na/d; B, 150 mmol Na/d; C, 200 mmol Na/d. The analysis was adjusted for week 0, order, and time.
[2] $P \leq 0.01$.
[3] $P \leq 0.001$.
[4] $P \leq 0.05$.

(MMP-2 and MMP-9) in the arterial wall (19). Comparison between the high sodium intake and low sodium intake at the end of 4 wk showed a decrease in MMP-9 (Table 4) that was not significant, probably because of the relatively small numbers of participants who completed the full 4 wk of the high-sodium arm. When considered in conjunction with the increased PWV evident after the high sodium intake, this finding supports the concept that high sodium intakes reduce extracellular matrix turnover and contribute to vascular wall stiffness (19, 20). However, larger studies are required to test this possibility. In addition, only 7 of the 34 participants were not taking antihypertensive drugs. The most common agent was an angiotensin-converting enzyme inhibitor, and these agents have been clearly shown to have protective actions on vascular function (21). Other agents, including thiazides and the dihydropyridines, also have an effect on vascular tone and will have minimized the ability of this study to identify the mechanistic pathways for the observed changes.

An interesting observation in this study was the reduction in plasma uric acid concentrations in the high-sodium group. This cannot be explained by changes in plasma volume because plasma creatinine was only decreased by ≈5%, and plasma uric acid decreased by 12% in intervention C. Urinary excretion of uric acid remained constant and nitrate production was not enhanced, which excludes other possible explanations and was not modified by the presence of a thiazide diuretic. Increased plasma uric acid concentrations have been correlated with an increased risk of hypertension and cardiovascular disease (22). Watanabe et al (23) have postulated that hyperuricemia provided a survival advantage because it enhanced sodium sensitivity and BP maintenance under low-dietary-sodium conditions. Although this may have been advantageous prehistorically, now, under conditions of constant sodium loading observed in most of the industrialized world, hyperuricemia may contribute to the maintenance of hypertension (23). This study suggests that this warrants further investigation.

The anthropometric status of the participants remained relatively constant throughout the study. There was a small but statistically nonsignificant increase in weight in the high-sodium group. The diet was not designed to alter energy or fat intakes; therefore, weight and body mass index were expected to remain constant. The changes observed in BP during this study are comparable with the effect of a single antihypertensive agent (21). Moreover, ≈25% of patients in this study had their antihypertensive therapy reduced because of symptomatic hypotension during the low-salt phases. Dietary changes associated with a low-salt diet were achieved and maintained throughout by ongoing dietary counseling. Compliance with the study diet was relatively high, as evidenced by measurements of urinary sodium excretion, with baseline values (low salt) for all 3 interventions comparable and the expected increase in urinary sodium excretion after interventions with the added salt to the tomato juice. Compliance with the tomato juice vehicle appeared to be similar within each of the interventions, as judged by similar increases in plasma LF concentrations.

Limitations of this study included the relatively small numbers, although the study was powered appropriately to detect a 10% change in PWV with sodium loading. This study was deliberately designed to recruit participants from the community and to place the interventions within a community setting rather than a highly contrived metabolic laboratory. Measurement of 24-h ambulatory BPs along with 24-h urinary sodium and potassium excretion would have enhanced the validity of the results; however, given the community setting for this study, these measurements were too difficult to implement and maintain participant involvement. The high compliance rates for the study would support this compromise in measurements versus outcomes. It could be argued that a greater effect would have been seen if the participants were weaned off their antihypertensive therapy. Implementation of the baseline low-sodium diet lowered medication requirements in some participants. Conversely, despite the use of concurrent medications, some participants had to be withdrawn from the high dietary sodium arms because of a rapid rise in BP, which exceeded our safety monitoring criteria. It would have been interesting to observe at what point their BP would have reset, as would be expected according to Guyton's pressure natriuresis hypothesis (24). The results of this relatively short-term intervention needs to be linked to longer-term studies of different dietary sodium intakes to allow for the effect of different regulatory mechanisms linking BP and vascular reactivity to be fully expressed.

In summary, dietary sodium loading rapidly and significantly increased PWV in hypertensive participants via both BP-dependent and BP-independent effects. These findings confirm the potentially detrimental effects of a high dietary salt intake, with increases in BP and PWV evident within a short time frame (4 wk). Further research is needed to establish the effects of

dietary sodium intake on arterial wall function in other populations, especially in high-risk groups such as those with obesity, diabetes, cardiovascular disease, and kidney disease.

The authors' responsibilities were as follows—AST: undertook all of the clinical studies, devised and supervised the diet along with the necessary support program to ensure participant compliance, collected the data, analyzed the results, and wrote the manuscript; RJM and RJW (Principal Investigators): responsible for the original grant funding, design of the study, recruitment of participants, analyses of the results, and writing the manuscript; WHFS: performed the laboratory studies and contributed to the analyses of the data and manuscript; RJJ: involved in the design of the study, analyses of results, and manuscript writing; JBWS: provided clinical supervision of the participants and contributed to the analyses of the results and writing of the manuscript; and SMW (biostatistician): responsible for the statistical analyses of the data and writing of the manuscript. None of the authors declared a conflict of interest.

REFERENCES

1. Veith I. Yellow Emperor's classic of internal medicine. Berkeley, CA: University of California Press, 2002.
2. Meneton P, Jeunemaitre X, De Wardener HE, MacGregor GA. Links between dietary salt intake, renal salt handling, blood pressure, and cardiovascular diseases. Physiol Rev 2005;85:679–715.
3. Intersalt Collaborative Research Group. Intersalt: an international study of electrolyte excretion and blood pressure. Results for 24h urinary sodium and potassium excretion. BMJ 1988;297:319–28.
4. Sacks FM, Svetkey LP, Vollmer WM, et al, The DASH-Sodium Collaborative Research Group. Effects on blood pressure of reduced dietary sodium and the Dietary Approaches to Stop Hypertension (DASH) Diet. N Engl J Med 2001;344:3–10.
5. He FJ, MacGregor GA. Effect of longer-term modest salt reduction on blood pressure. Cochrane Database Systc Rev 2004;3:CD004937.
6. Weinberger MH. Cardiovascular and hormonal response to extremes of sodium intake in normal black and white men. Circulation 1979;60: 697–706.
7. Hurwitz S, Fisher NL, Ferri C, Hopkins PN, Williams GH, Hollenberg NK. Controlled analysis of blood pressure sensitivity to sodium intake: interactions with hypertension type. J Hypertens 2003;21:951–59.
8. Roos JC, Koomans HA, Dorhout Mees EJ, Delawi IM. Renal sodium handling in normal humans subjected to low, normal, and extremely high sodium supplies. Am J Physiol 1985;249:F941–7.
9. Tatzber F, Griebenow S, Wonisch W, Winkler R. Dual method for the determination of peroxidase activity and total peroxides-iodine leads to a significant increase of peroxidase in human sera. Anal Biochem 2003; 316:147–53.
10. Tsuchida M, Miura T, Mizutani K, Aibara K. Fluorescent substances in mouse and human sera as a parameter of in vivo lipid peroxidation. Biochim Biophys Acta 1985;834:196–204.
11. Jones GT, Kay IP, Chu JW, et al. Elevated plasma active matrix metalloproteinase-9 level is associated with coronary artery in-stent restenosis. Arterioscler Thromb Vasc Biol 2006;26:e121–5.
12. Liang YL, Teede H, Kotsopoulos D, et al. Non-invasive measurements of arterial structure and function: repeatability, interrelationships and trial sample size. Clin Sci (Lond) 1998;95:669–79.
13. McAuley KA, Williams SM, Mann JI, et al. Diagnosing insulin resistance in the general population. Diabetes Care 2001;24:460–4.
14. Seals DR, Tanaka H, Clevenger CM, et al. Blood pressure reductions with exercise and sodium restriction in postmenopausal women with elevated systolic pressure: role of arterial stiffness. J Am Coll Cardiol 2001;38:506–13.
15. Gates PE, Tanaka H, Hiatt WR, Seals DR. Dietary sodium restriction rapidly improves large elastic artery compliance in older adults with systolic hypertension. Hypertension 2004;44:35–41.
16. Pannier BM, Guerin AP, Marchais SJ, London GM. Different aortic reflection wave responses following long-term angiotensin-converting enzyme inhibition and beta-blocker in essential hypertension. Clin Exp Pharmacol Physiol 2001;28:1074–7.
17. Forstermann U. Oxidative stress in vascular disease: causes, defence mechanisms and potential therapies. Nat Clin Pract Cardiovasc Med 2008;5:338–49.
18. Scotland RS, Ahluwalia A, Hobbs AJ. C-type natriuretic peptide in vascular physiology and disease. Pharmacol Ther 2005;105:85–93.
19. Dhingra R, Pencina MJ, Schrader P, et al. Relations of matrix remodeling biomarkers to blood pressure progression and incidence of hypertension in the community. Circulation 2009;119:1101–7.
20. Yasmin, McEniery CM, O'Shaughnessy KM, et al. Variation in the human matrix metalloproteinase-9 gene is associated with arterial stiffness in healthy individuals. Arterioscler Thromb Vasc Biol 2006;26: 1799–805.
21. Asmar RG, London GM, O'Rourke ME, Safar ME. Improvement in blood pressure, arterial stiffness and wave reflections with a very-low-dose perindopril/indapamide combination in hypertensive patient: a comparison with atenolol. Hypertension 2001;38:922–6.
22. Verdecchia P, Schillaci G, Reboldi G, Santeusanio F, Porcellati C, Brunetti P. Relation between serum uric acid and risk of cardiovascular disease in essential hypertension: the PIUMA Study. Hypertension 2000; 36:1072–8.
23. Watanabe S, Kang D, Feng L, et al. Uric acid, hominoid evolution, and the pathogenesis of salt-sensitivity. Hypertension 2002;40:355–60.
24. Guyton A. The surprising kidney-fluid mechanism for pressure control—its infinite gain! Hypertension 1990;16:725–30.