Adverse Cardiovascular Effects of Acute Salt Loading in Young Normotensive Individuals

Nikolaos Tzemos, Pitt O. Lim, Suzanne Wong, Allan D. Struthers, Thomas M. MacDonald

Abstract—We sought to explore the effects of salt loading in young normotensives on vascular endothelial function, echocardiographic left ventricular diastolic function, and electrocardiographic QT dispersion. Sixteen healthy normotensive male volunteers were randomized in a double-blind crossover fashion to 5-day treatment periods with either placebo or salt tablets (200 mmol/d of sodium) separated by a 2-week washout period. Throughout the study the volunteers were asked to maintain a low-salt diet. Forearm venous occlusion plethysmography and intraarterial infusions of acetylcholine (ACh), sodium nitroprusside (SNP), and N\textsuperscript{G}-monomethyl-l-arginine (L-NMMA) were used to assess vascular reactivity. Baseline and postsalt loading 12-lead ECGs and echocardiograms were also obtained. Twenty-four-hour ambulatory systolic blood pressure rose (117±11 to 121±8 mm Hg) significantly with salt loading. The endothelium-dependent responses to ACh were significantly blunted with salt compared to placebo (ΔFBF% 403 [50] versus 296 [31]; P<0.05) and L-NMMA (ΔFBF% −47.2 [4] versus −31 [3]; P<0.01). In contrast, the endothelium-independent response to SNP was not different between treatments. Color M-mode flow propagation velocity (CMMFPV), a preload index of left ventricular diastolic function, was significantly reduced with salt (64 [6] versus 59 [16] cm/s; P<0.05) suggesting increased ventricular stiffness. QT dispersion was also significantly increased with salt (58 [16] versus 48 [17] ms; P=0.02). Salt loading impaired vascular endothelial function, left ventricular mechanical relaxation, and electric repolarization in young healthy normotensives. (Hypertension. 2008;51:1525-1530.)

Key Words: salt loading ■ endothelium ■ diastolic dysfunction ■ QT dispersion ■ blood pressure

Any risk factors associated with atherosclerotic diseases have been identified over the last 5 decades. However, epidemiological data show that these conventional risk factors account for most but not all coronary artery disease burden.1–3 This suggests that other factors must be causal1 and excess salt intake is a possible candidate.4 There is evidence in animals that salt produces hypertension and direct target-organ damage such as myocardial hypertrophy, vascular hypertrophy, and fibrosis.5,9 However, in man, the role of salt in cardiovascular disease continues to be contentious,6 with most studies in man concentrating on the relationship between salt intake and blood pressure.5,9

Salt may have blood pressure–independent adverse effect on target organs3,9 with increasing coronary events, independently of blood pressure,4 and left ventricular diastolic dysfunction.10 We have previously reported that in hypertensive subjects with increased aldosterone activity, salt loading not only impairs left ventricular diastolic function, but also increases QT dispersion, a measure of cardiac electric depolarization.11 One study has previously examined the effect of sodium loading on vascular endothelium in 7 normal subjects and found no significant effect on endothelial-dependent vasodilation.12 However, our prior experience prompted us to repeat this with a larger study group.11

We therefore explored whether salt loading has adverse effects on cardiovascular system in healthy volunteers. We studied the effects of salt on vascular endothelial function and on NO bioactivity, as it is accepted that endothelial dysfunction precedes frank atherosclerotic disease.13 Secondly, we studied whether excess salt would adversely affect the left ventricular diastolic function and QT dispersion in normal individuals. These 2 effects may be interrelated because left ventricular systolic and diastolic functions are influenced in turn by the coronary endothelial function.14 Left ventricular diastolic dysfunction in turn is also recognized to be an early sign of cardiac target-organ damage.15

Methods

Study Population

Sixteen healthy white male subjects were recruited by advertisement in a local newspaper and from within the University. Exclusion criteria included a history of hypertension, coronary artery disease, diabetes, hyperlipidemia (fasting cholesterol >5.0 mmol/L), renal impairment, and cerebrovascular and peripheral vascular diseases. We also excluded subjects with a known first-degree family history of any of the above-described diseases. None of the subjects were taking before or during the study any drugs which could have affected the endothelium. Written informed consent was obtained.

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from each study subject, and this study had the approval of the Tayside committee on medical research ethics.

**Study Protocol**

This was a randomized, double-blind, and placebo-controlled cross-over study. Subjects were randomized to receive either salt supplementation or matching placebo in a double-blind crossover manner, each for 5 days, with a 2-week washout period between treatments. During the study subjects were instructed to maintain a low sodium diet (<40 mmol/d). Salt supplement was provided as sodium chloride tablets (each with 10 mmol of sodium), and the total amount given was 200 mmol/d of sodium in 2 divided doses as previously described.16 Twenty-four-hour ambulatory blood pressure monitoring (ABPM) and 24-hour urinary collection for sodium excretion were undertaken at the end of each treatment period (day 5).

Blood pressure was measured (mean of 3 measurements) at the beginning of each visit (day 6) after 10 minutes rest in seated position using a semiautomatic oscillometric monitor (OMRON 705CP). ABPM was recorded using SpaceLabs model 90207 recorders (Redmond). Recordings were taken every 15 minutes during the daytime (8 AM to 10 PM) and every 30 minutes at nighttime (10 PM to 8 AM).

**Vascular Studies**

Each subject underwent 2 endothelial function assessments undertaken in the morning of day 6 of each treatment. All vascular studies were conducted by the same operator (N.T.) after an overnight fast and in a quiet temperature-controlled laboratory (24°C ±0.5°C) with dimmed lights. Alcohol- and caffeine-containing beverages were avoided for at least 24 hours before the study day. After a supine rest of 30 minutes the nondominant brachial artery was cannulated with a 27-gauge steel needle mounted onto a 16-gauge polyethylene epidural catheter under local anesthesia with 1% lidocaine. Forearm blood flow (FBF) was measured simultaneously in both arms by strain-gauge venous occlusion plethysmography as previously described.17 Blood pressure and heart rate were noninvasively (OMRON, HEM-705CP) recorded in the noninfused (control) arm before each infusion.

**Hemodynamic Measurements and Drugs Infusions**

FBF was measured during the last 2 minutes after each infusion period and was expressed as mL·min⁻¹·100·mL⁻¹ forearm volume according to the Whitney method.18 Resting baseline FBF were obtained at least 30 minutes after needle placement to ensure that the blood flow in the cannulated arm had stabilized. After resting baseline FBF measurements, each study subject received intraarterial infusions of incremental doses of acetylcholine (Miochol, CIBAVision), sodium nitroprusside (David Bull Laboratories), and Nω-monomethyl-l-arginine (Clinalfa). The muscarinic agonist acetylcholine (ACh) was used to assess endothelium-dependent vasodilatation (stimulated NO release) whereas sodium nitroprusside (SNP), an exogenous source of NO, was used to assess endothelium-independent vasodilatation. Cumulative dose response curves were constructed after infusions of ACh 25, 50, and 100 nmol/min and SNP 4.2, 12.6, and 37.8 nmol/min, each incremental dose for 5 minutes. The endothelial-dependent vasoconstriction was assessed by using the competitive NO synthase antagonist Nω-monomethyl-L-arginine (L-NMMA) infused at 1.2, and 4 µmol/min, each for 5 minutes. After each agent, care was taken for FBF to reach the baseline values, generally at least after 30 minutes. The order of the vasoactive drugs infused was identical in all the study visits. Drugs, saline, and 5% dextrose were infused at flow rates of 1 mL/min by means of a constant-rate infusion pump (Braun). All studies were performed by the same operator (N.T.), blinded to the other measurements. PRA and plasma aldosterone were measured using standard radioimmunoassay techniques.22 Twenty-four-hour urinary collection for sodium excretion was measured using flame photometry.

**Statistical Analysis**

Five recordings at each infusion step were measured for both infused and control arms. Because blood pressure and baseline forearm flows did not vary significantly during visits the FBF ratio between infused and control arms in response to drugs was expressed as percentage of the ratio measured during the placebo period (ΔFBF% [mean, SD]), Blood pressure and heart rate were noninvasively (OMRON, HEM-705CP) recorded in the noninfused (control) arm before each infusion.

**Results**

**Baseline and Hemodynamic Characteristics**

Sixteen male subjects with a mean age of 27 (SD 8) years and body mass index (BMI) of 25 (SD 2) kgm⁻² were studied. 24-hour ABP at the end of the 5-day placebo period confirmed normotension (Table 1). With salt supplementation, daytime and 24-hour ambulatory systolic BP rose significantly (Table 1). Similarly, urinary sodium excretion rose significantly with salt supplementation (Table 2). There were no significant differences in baseline plasma electrolytes or cholesterol levels and weight between the 2 study periods (Table 2). However, a small but significant fall in serum potassium was noted with salt supplementation (Table 2). As expected, plasma aldosterone fell with salt supplementation (Table 2).

**Echocardiographic Examination**

Echocardiographic examinations were conducted by a single operator (N.T.), blinded to the rest of the measurements, using a Hewlett Packard Sonos 2500. All echocardiographic parameters were measured in triplicate and averaged over 3 cardiac cycles. The following indexes of LV dimensions were recorded/derived from M-mode measurements obtained at the level of the papillary muscles in the short-axis view: LV internal diameter in diastole (LVIDD), LV internal diameter in systole (LVIDS), fractional shortening (FS), and LV mass index (LVMi). The left atrial size was measured in the parasternal long-axis view. To assess diastolic function, transmitral Doppler indexes (peak velocities of the early E and late A waves and E/A ratio) and E deceleration time (E DT) were obtained as previously described.20 Additionally, color M-mode flow propagation velocity (CMMPFV) was measured according to the method described by Brun and colleagues.21
Tzemos et al Endothelial and Myocardial Effects of Salt J527

Table 1. Hemodynamic Data of the Study Population

<table>
<thead>
<tr>
<th>Variable</th>
<th>High Salt</th>
<th>Placebo</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daytime SBP, mm Hg</td>
<td>128 (9)</td>
<td>122 (9)*</td>
<td>0.02</td>
</tr>
<tr>
<td>Daytime DBP, mm Hg</td>
<td>76 (9)</td>
<td>76 (18)</td>
<td>0.5</td>
</tr>
<tr>
<td>Night-time SBP, mm Hg</td>
<td>116 (4)</td>
<td>114 (4)</td>
<td>0.2</td>
</tr>
<tr>
<td>Night-time DBP, mm Hg</td>
<td>68 (3)</td>
<td>67 (4)</td>
<td>0.5</td>
</tr>
<tr>
<td>24-hour SBP, mm Hg</td>
<td>121 (8)</td>
<td>117 (12)†</td>
<td>0.005</td>
</tr>
<tr>
<td>24-hour DBP, mm Hg</td>
<td>71 (2)</td>
<td>70 (3)</td>
<td>0.5</td>
</tr>
<tr>
<td>24-hour Heart rate, beats/min</td>
<td>67 (11)</td>
<td>68 (8)</td>
<td>0.3</td>
</tr>
<tr>
<td>Absolute basal FBF</td>
<td>3.0 (4)</td>
<td>3.2 (0.3)</td>
<td>0.5</td>
</tr>
</tbody>
</table>

ABP indicates ambulatory blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBF, forearm blood flow (ml^{-1} - 100 mL^{-1} - forearm volume).

Results are expressed as mean (SD).

*P<0.05, †P<0.001.

Vascular Studies

ACh produced a marked dose-dependent increase in FBF in both treatment groups (Figure). However, salt loading was associated with significantly less endothelium-dependent vasodilation (\( \Delta \text{FBF}_\% = 141 [15], 195 [21], 296 [31] \), with respect to the baseline value) in response to ACh (25, 50, 100 nmol/min, respectively) compared to placebo treatment (196 [41], 310 [46], 403 [50], mean [SEM], P<0.05 for the whole dose range). In a multivariate analysis model only urinary sodium excretion was a significant predictor of the maximal ACh% response (P<0.001).

Similarly, the vascular vasoconstrictive response to L-NMMA was significantly blunted with salt loading as compared to placebo (max\( \Delta \text{FBF}_\% = -31 [3] \) versus -47.2 [4], P<0.01 for the whole dose range). The response to SNP (NO-independent response) was not different between the 2 treatments (Figure).

Echocardiographic and Ectolectrocardiographic Parameters

None of the subjects had echocardiographic or electrocardiographic left ventricular hypertrophy. Structural echocardiographic indexes were not different between treatment periods (Table 3). The LV diastolic filling indexes were not significantly altered with salt loading apart from the color M-mode flow propagation velocity index (CMMFVP) which deteriorated suggesting increased ventricular stiffness. QT dispersion was significantly increased with salt loading compared to placebo.

Discussion

The major findings of this study, which is the biggest of its kind, were that oral salt loading in young normotensive individuals was associated with reduced vascular NO bioactivity, impaired left ventricular myocardial relaxation, and increased electrocardiographic QT dispersion.

Salt Loading and Habitual Alimentary Salt Intake

In the Health Survey for England\textsuperscript{23} the mean urinary sodium excretion of 16- to 24-year-old subjects was 148.5 mmol/L with a wide range (5th percentile 28.2, 95th percentile 260.4 mmol/L). In our study, the volunteers were asked to maintain a low sodium diet of <40 mmol/day for 5 days which in fact achieved a mean 24-hour urinary sodium excretion of 76 mmol/day. Conversely, during sodium loading of 200 mmol/day the urinary sodium excretion achieved was 225 mmol/day. Thus, the range of sodium excretions obtained in this experiment might not be uncommon in the real world.

Oral Salt Loading and NO

The association between salt loading and forearm resistance vessel endothelial dysfunction has previously been studied in salt sensitive and salt resistant hypertensives\textsuperscript{24,25} and in normotensive healthy volunteers\textsuperscript{12} with variable results. In animals, salt loading impairs microvascular arterial function in the short term, and in the long-term causes fibrosis, effects that were independent of BP.\textsuperscript{5,6} In humans, Brugalat et al in a group of 50 hypertensive patients have demonstrated a significantly lower forearm vasodilatory response to acetylcholine (endothelium-dependent vasodilation) in 26 salt-sensitive compared to 16 salt-resistant hypertensive patients after oral salt loading.\textsuperscript{25} Similarly, Miyoshi et al also have reported impairment in acetylcholine-induced vasodilation in 6 salt-sensitive essential hypertensive patients compared with 9 salt-resistant hypertensives.\textsuperscript{26} Although the above studies suggest an impairment of the L-arginine–NO pathway as participant of this abnormal endothelial response in salt-sensitive hypertension, the mechanism behind it remains poorly understood. In contrast, Stein et al in a small study of 7 normotensive male subjects found that oral salt loading enhanced vasodilation in response to sodium nitroprusside (endothelium-independent), whereas the response to methacholine was not affected.\textsuperscript{12} Although it is not clear the mechanism that has led to an increase in NO-independent vasodilation after salt loading, it is possible that the small sample size study was insufficient to detect small differences in stimulated NO release.

Nevertheless, the mechanism whereby excess of salt may cause endothelial dysfunction is unclear. In our study, plasma
potassium fell in response to salt supplementation, which could have altered endothelial function. In man, Taddei and colleagues have reported that intraarterial administration of potassium chloride significantly improved ACh induced vasodilation in hypertensive patients. However, it is unlikely, although not impossible, that the small change in potassium alone would explain our results, as large increases in plasma potassium are required to favorably influence the endothelial function. In addition, the change in plasma potassium in our study did not correlate with the change in ACh-induced vasodilation.

Sodium can affect BP. One example is hypertensives with increased aldosterone activity (primary aldosteronism) where hypertension is critically salt-dependent, ie, without salt, hypertension does not occur. Interesting, if treatment of such individuals is discontinued and they are allowed ad libitum salt intake, the BP rise is preceded and maintained by a rise in systemic vascular resistance, rather than by salt-induced volume expansion. However, a rise in BP can itself influence the vascular endothelial function. Paniagua and colleagues recently reported that increases in intravascular blood pressure blunted endothelial dependent vasodilation of resistance arteries of healthy volunteers. These data are experimental, and it is likely that hypertensive blood pressure values had been induced to affect NO bioactivity in healthy volunteers. Conversely, in our study a 4% increase in systolic blood pressure yielded a 30% decrease in NO bioactivity. Taking these findings together, it is not inconceivable that excess salt initiates and perhaps maintains the vicious cycle of endothelial dysfunction and BP rise.

**Oral Salt Loading and Left Ventricular Diastolic Function**

A relationship between salt and impaired LV diastolic dysfunction has been reported before in hypertensive individuals. Our data now show that salt can similarly cause LV diastolic dysfunction even in normotensives with normal renin angiotensin aldosterone systems (RAAS). One possible mechanism is that excess salt increased intracellular calcium which then impaired myocardial relaxation as suggested by the Blaustein hypothesis. In addition to this mechanism we can cautiously speculate that salt loading could also adversely affect coronary NO bioactivity. There is evidence that coronary endothelial NO also plays an important role in determining LV relaxation in humans. Our finding that salt loading decreases vascular NO bioactivity raises the possibility that salt loading has similar effects on ventricular NO bioactivity and that inducible and reversible impairment of NO bioactivity leads to likewise impairment in ventricular diastolic function as evidenced by the reduction in CMMFPV in response to salt. However, it is possible that salt negatively modulates LV stiffness through different and perhaps NO-independent mechanism(s) such as a direct myocyte effect. Nevertheless, although, transmitral diastolic flow indexes did not change significantly, these have poor reproducibility and correlate poorly with LV filling pressures in subjects with normal systolic function. In contrast, the CMMFPV is relatively preload-independent and correlates well with LV intracavitary pressures. This finding might have important implications because impaired ventricular relaxation is thought to be the earliest cardiac abnormality of any disease.

<table>
<thead>
<tr>
<th>Variable</th>
<th>High Salt</th>
<th>Placebo</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVDD, mm</td>
<td>4.9 (0.3)</td>
<td>4.85 (0.4)</td>
<td>0.6</td>
</tr>
<tr>
<td>LVDS, mm</td>
<td>3.6 (0.3)</td>
<td>3.5 (0.4)</td>
<td>0.6</td>
</tr>
<tr>
<td>IVSD, cm</td>
<td>1.10 (0.1)</td>
<td>1.06 (0.1)</td>
<td>0.3</td>
</tr>
<tr>
<td>PWD, cm</td>
<td>0.8 (0.1)</td>
<td>0.9 (0.1)</td>
<td>0.2</td>
</tr>
<tr>
<td>LA, cm</td>
<td>2.9 (0.3)</td>
<td>3 (0.6)</td>
<td>0.5</td>
</tr>
<tr>
<td>Emax, cm/s</td>
<td>75 (14)</td>
<td>70 (17)</td>
<td>0.1</td>
</tr>
<tr>
<td>Amax, cm/s</td>
<td>46 (9)</td>
<td>42 (7)</td>
<td>0.2</td>
</tr>
<tr>
<td>E/A ratio</td>
<td>1.6 (0.3)</td>
<td>1.7 (0.5)</td>
<td>0.1</td>
</tr>
<tr>
<td>E DT, cm/s</td>
<td>189 (33)</td>
<td>171 (15)</td>
<td>0.1</td>
</tr>
<tr>
<td>CMMFPV, cm/s</td>
<td>44 (9)</td>
<td>64 (6)†</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>QT dispersion, ms</td>
<td>59 (11)</td>
<td>49 (12)*</td>
<td>0.02</td>
</tr>
<tr>
<td>QT interval, ms</td>
<td>416 (26)</td>
<td>408 (20)</td>
<td>0.2</td>
</tr>
</tbody>
</table>

LVDD and LVDS indicate left ventricular internal diastolic and systolic dimensions, respectively; IVSD and PWD, interventricular septal and posterior wall dimensions, respectively; LA, left atrium; E A/E ratio and DT refer to peak velocities of the early E and late A waves and E/A ratio and E deceleration time; CMMFPV, color M-mode flow propagation velocity index; PRA, plasma renin activity. Values are expressed as mean (SD).

*P<0.05, †P<0.01.
Oral Salt Loading and QT Dispersion

We have previously reported that salt loading in hypertensive individuals with excess aldosterone activity increased QT dispersion.11 Although the magnitude of rise in QT dispersion in the present study was smaller, an effect was still seen in normal healthy young adults. The mechanism for this effect of salt is unclear. It could be an electric phenomenon attributable to impaired LV relaxation consistent with the electromechanical feedback mechanism, or it could be attributable to increased afterload secondary to salt-induced endothelial dysfunction.37 Nevertheless, increased QT dispersion represents an increased risk for cardiac death, especially in apparent normal populations.38 In the recently published Strong Heart Study, a QT dispersion of >58 ms was associated with a 2.8-fold increased risk of cardiovascular mortality in apparently healthy American Indians.39

Study Limitations

Our study has important limitations. We focused on the short-term rather long-term cardiovascular effects of salt loading, in a relatively small number of volunteers and in males only. In addition, we did not directly assess salt sensitivity or resistance, which could have influenced the vascular responses. Although our study is the biggest of its kind, it would be interesting to investigate whether our findings would be also reproduced in a larger group of subjects perhaps comprising also females and with diverse ethnicities with a longer chronological follow-up. Part of our observations might be partially attributed to a lower plasma potassium concentration because we did not add potassium as oral supplementation. Finally, we used color M-mode echocardiography as left ventricular preload independent index of diastolic function. It is conceivable that more recently introduced echocardiographic imaging tools such as tissue Doppler imaging and strain rate could have provided additional valuable information on myocardial stiffness; however, those parameters were not available on the echocardiographic platform used in our studies.

In conclusion, we have demonstrated that oral salt loading impaired vascular endothelial function, LV filling indices of diastolic dysfunction, and increased QT dispersion. Thus, in persons without hypertension, the adverse effects of sodium, rather than limited to a rise in BP, may be substantial and related to several deleterious changes in vascular endothelial function.

Perspectives

We investigated the effects of salt on vascular endothelial function, left ventricular diastolic function, and QT dispersion in normal individuals during 5-day high-salt and 5-day placebo diets. We found that moderate oral salt loading in young normotensive individuals devoid of cardiovascular risk factors was associated with reduced vascular NO bioactivity, impaired left ventricular myocardial relaxation, and increased QT dispersion. Our findings provide evidence of a multifaceted negative interaction between excess of dietary salt and cardiovascular system, thus recalling the need of a more balanced dietary salt intake.

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Disclosures

None.

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