**Effect of salt intake on endothelium-derived factors in a group of patients with essential hypertension**

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**ABSTRACT**

The aim of the present study was to evaluate the effects of the level of salt intake on endothelium-derived factors in a group of patients with essential hypertension. A group of 50 patients with essential hypertension who had never been treated for the condition were placed on a low-sodium (50 mmol/day), low-nitrate (400 µmol/day) diet, which was supplemented, in a single-blind fashion, with placebo tablets for the first 7 days and then with NaCl tablets (200 mmol/day) for a further 7 days (total sodium intake 250 mmol/day). At the end of both periods, 24-h ambulatory blood pressure monitoring was performed. In addition, plasma levels and 24-h urinary excretion of nitrites plus nitrates and cGMP were measured, along with plasma levels of endothelin. A high salt intake promoted significant decreases in plasma levels of nitrites plus nitrates (from 41.0 + 2.1 to 32.8 + 1.8 nmol/ml; P < 0.001), 24-h urinary excretion of nitrites plus nitrates (from 417 + 36 to 334 + 37 µmol/24 h; P = 0.045) and plasma endothelin levels (from 5.6 + 0.3 to 4.6 + 0.3 pg/ml; P = 0.007). The plasma concentration and 24-h urinary excretion of cGMP were not altered significantly by a high salt intake. We did not find any relationship between endothelium-derived products and 24-h mean blood pressure, at either low or high salt intakes, or between changes induced by the high-salt diet. A high salt intake also induced significant decreases in plasma renin activity, angiotensin II and aldosterone, and a significant increase in atrial natriuretic peptide. We conclude that a high salt intake decreases the plasma concentration and urinary excretion of nitrites and plasma levels of endothelin in patients with essential hypertension, suggesting that the level of salt intake may affect endothelial cell function. However, these alterations are not correlated with changes in blood pressure induced by the high salt intake.

**INTRODUCTION**

The relationship between a high salt intake and hypertension is still a matter of controversy [1]. Although NaCl has been considered to be an important factor in the development of hypertension, the blood pressure response to NaCl loading varies among individuals [2]. Several abnormalities in the renin/angiotensin system [3], sympathetic nervous system [4] and transmembrane sodium transport [5] have been suggested to be involved in the salt-induced pressor effect.

It has been clearly demonstrated that endothelial cells play a critical role in the maintenance of vascular tone, and thus may represent a potential target for the pressor

**Key words:** dietary salt, endothelium, hypertension, nitric oxide.

**Abbreviations:** ABPM, ambulatory blood pressure monitoring; ANP, atrial natriuretic peptide; NOₓ, nitrites plus nitrates.

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effect of salt. NaCl loading promotes changes in endothelial cell function in experimental models of hypertension. In fact, increases in the plasma and urinary concentrations of nitrates plus nitrites (NO$_3$) have been demonstrated in Sprague–Dawley rats after 8 weeks of a high salt intake [6]. Moreover, in Dahl salt-sensitive rats, the elevation in blood pressure induced by a high NaCl intake can be prevented by the concomitant administration of the NO precursor l-arginine [7]. This latter intake can be prevented by the concomitant administration of the NO precursor l-arginine [7]. This latter observation suggests that changes in endothelial cell function may participate in the effect of salt on blood pressure. In humans, decreased NO production has been reported in patients with essential hypertension compared with normotensive controls [8,9]. Likewise, a high salt intake has been shown to decrease plasma levels of NO$_3$ in Afro-American [10] and Japanese [11] patients with hypertension.

The aim of the present study was to evaluate the effects of two different levels of salt intake on endothelium-derived products, by measuring plasma and urinary levels of NO$_x$ and cGMP, and plasma levels of endothelin.

**METHODS**

**Patient selection**
The study population included 50 patients with essential hypertension, who had never been treated for the condition, recruited consecutively from the Hypertension Unit of the Department of Internal Medicine, Hospital Clinic, Barcelona, Spain. The group comprised 31 men and 19 women, with a mean age of 43 years (range 29–69 years). The diagnosis of essential hypertension was considered if seated arterial blood pressure (after 10 min of rest), measured by a mercury sphygmomanometer, was consistently higher than 140/90 mmHg. Secondary forms of hypertension were excluded by routine diagnostic procedures. Subjects with hypercholesterolaemia (total cholesterol > 6.5 mmol/l (250 mg/dl)), diabetes mellitus, impaired renal function (serum creatinine > 132 µmol/l (1.5 mg/dl)) or a previous history of coronary or cerebrovascular diseases were excluded from the study. Moreover, patients smoking more than five cigarettes per day and/or consuming more than 40 g of pure ethanol per day, as well as women taking oral contraceptives or oestrogen replacement therapy, were also excluded.

**Study design**
All patients gave informed consent. The protocol was approved by the Ethics Committee of the Hospital and by the Spanish Health Authority (Protocol FIS 00/0435).

The patients were placed on a baseline low-sodium diet containing 50 mmol of Na$^+$ for 14 days. This diet was supplemented, in a single-blind fashion, with placebo tablets for the first 7 days (low-salt period) and with NaCl tablets (200 mmol/day) (high-salt period) for a further 7 days. Thus the total NaCl intake during the high-salt period was 250 mmol/day. The amount of nitrates in the diet was also adjusted to a total daily intake of less than 400 µmol/day. This was achieved by excluding food items that contain a high nitrate concentration, i.e. cured meat, cheese and green leafy vegetables, as has been described previously [12].

Compliance with the diet was assessed twice weekly by measuring 24-h urinary Na$^+$ excretion throughout the study.

On the final day of both the low- and high-salt periods, 24-h ambulatory blood pressure monitoring (ABPM) was performed using an automated, non-invasive oscillometric device (Space Labs 90207; SpaceLabs Inc., Redmon, WA, U.S.A.). Blood pressure was registered automatically at 15-min intervals for 24 h.

**Laboratory measurements**
A venous blood sample was obtained on the final day of both the low-salt and high-salt periods, after 12 h of fasting and 1 h of bed rest, with the patient in the recumbent position. A prolonged fasting period of between 12 and 18 h has been shown to be necessary for a meaningful measurement of plasma NO$_x$ concentration [13]. In fact, in a previous paper, Node et al. [9] reported a complete clearance of dietary nitrate after a fasting period of 12 h.

Routine biochemical determinations were obtained by means of a Technicon Dax-72 instrument using standard laboratory methods. Plasma renin activity, aldosterone, angiotensin II and atrial natriuretic peptide (ANP) were measured using previously described methods [4].

To measure serum and 24-h urine concentrations of NO$_x$, samples were ultrafiltered (PL-10 Ultrafree-MC centrifugal filter units; Millipore Corp., Bedford, MA, U.S.A.) at 1200 g for 1 h to remove proteins before analysis. The NO$_x$ concentration in the filtered samples was then determined by the fluorimetric method of Misko et al. [14]. The fluorescent signal was measured in a fluorimeter (Perkin Elmer, Foster City, CA, U.S.A.) at excitation and emission wavelengths of 365 and 425 nm respectively. Intra-assay and inter-assay coefficients of variation were 8.4% and 13.8% respectively.

To prevent contamination by endogenous phosphodiesterases, cGMP levels in plasma were assessed after acetylation. Plasma samples were brought to 0.5 mM isobutylmethylxanthine, cooled in an ice bath and assayed immediately for cGMP concentration. Urinary measurements were carried out using non-acetylated samples diluted 1:100 (v/v). The concentration of cGMP was determined by RIA (Biomedical Technologies Inc., Stoughton, MA, U.S.A.). Intra-assay and inter-assay coefficients of variation were 3.9% and 11.2%, respectively.
The endothelin concentration was measured by RIA (Nichols Institute Diagnostics B.V., Wijchen, The Netherlands) after extraction on Sep-Pack C18 cartridges (Waters Associates, Milford, MA, U.S.A.). For this purpose, plasma samples (1 ml) were acidified with 4% (v/v) acetic acid (4.5 ml) and applied to cartridges preactivated with methanol, distilled water and 4% (v/v) acetic acid. The cartridges were then washed with distilled water and 25% (v/v) ethanol, and immunoreactive endothelin was eluted twice with 1 ml of 4% (v/v) acetic acid in 86% (v/v) ethanol. The eluted endothelin was then concentrated to dryness (Speed Vac Concentrator; Savant Instruments Inc., Framingdale, NY, U.S.A.) and reconstituted for RIA. The recovery rate for the ex- traction procedure was 85%, as determined by the addition of labelled endothelin-1, -2 and -3 and big endothelin was 100%, 52%, 96% and 7% respectively. Intra-assay and inter-assay coefficients of variation were 6.9% and 12.1% respectively.

Statistical analysis
Values are expressed as means ± S.E.M. for normally distributed variables, or as median (range) for variables that deviated from a normal distribution. Changes in various parameters induced by the level of salt intake were analysed by means of the paired Student’s t-test or the Wilcoxon test, as appropriate. Possible relationships between endothelium-derived factors and blood pressure at different levels of salt intake, or between changes induced by a high-salt diet, were analysed by means of Pearson’s correlation coefficient.

RESULTS
A high salt intake promoted a significant increase in the 24 h systolic (from 135.4 ± 2.4 to 142.0 ± 2.5 mmHg; P < 0.001), mean (from 103.0 ± 1.8 to 107.2 ± 2.0 mmHg; P < 0.001) and diastolic (from 85.0 ± 1.6 to 88.8 ± 1.9 mmHg; P < 0.001) blood pressures, as assessed by means of ABPM (Table 1).

Table 1 Blood pressure and heart rate in patients with essential hypertension during low and high salt intakes

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Low salt</th>
<th>High salt</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>24-h systolic blood pressure (mmHg)</td>
<td>135.4 ± 2.4</td>
<td>142.0 ± 2.5</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>24-h diastolic blood pressure (mmHg)</td>
<td>85.0 ± 1.6</td>
<td>88.8 ± 1.9</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>24-h mean blood pressure (mmHg)</td>
<td>103.0 ± 1.8</td>
<td>107.2 ± 2.0</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>24-h heart rate (beats/min)</td>
<td>77.2 ± 1.3</td>
<td>76.0 ± 1.3</td>
<td>0.143</td>
</tr>
</tbody>
</table>

The patients showed significant decreases in plasma NO levels (from 41.0 ± 2.1 to 32.8 ± 1.8 nmol/ml; P < 0.001) and in the 24-h urinary excretion of NO, (from 417 ± 36 to 334 ± 37 μmol/24 h; P = 0.045) when they switched from a low to a high salt intake (Table 2). Moreover, a high salt intake significantly decreased mean levels of plasma endothelin (from 5.6 ± 0.3 to 4.6 ± 0.3 pg/ml; P = 0.007), whereas both the plasma concentration and the 24-h urinary excretion of cGMP were not changed significantly by a high salt intake. Values of endothelium-derived factors obtained at low and high salt intakes did not correlate with 24-h mean blood pressure obtained simultaneously. Likewise, we did not find any correlation between salt-induced changes in both blood pressure and endothelial factors (Table 3).

A high salt intake also promoted significant decreases in serum uric acid (from 316 ± 11 to 284 ± 11 μmol/l; P < 0.001), plasma renin activity [from 0.36 (range 0.01–1.54) to 0.12 (0.01–1.61) pmol·h⁻¹·ml⁻¹; P = 0.019], plasma angiotensin II (from 24.3 ± 1.6 to 18.8 ± 1.6 pg/ml; P = 0.004) and serum aldosterone (from 489 ± 41 to 296 ± 24 pmol/l; P < 0.001), and a significant increase in plasma ANP [from 28.3 (8–107) to 32.2 (6–169) fmol/l; P = 0.044] (Table 4).

DISCUSSION
The present study shows that a high salt intake promotes significant decreases in both plasma levels and the 24-h urinary excretion of NO, as well as in plasma endothelin, in patients with essential hypertension, thus suggesting that the level of salt intake can affect endothelial cell function. However, the relationship of these alterations with salt-sensitive human hypertension remains uncertain, due to the lack of correlation between salt-induced changes in endothelium-derived products and in 24-h mean blood pressure.

Several observations have suggested that the endogenous mediator NO plays an important role in the modulation of both vascular tone and renal sodium excretion [6,15,16]. Shultz and Tolins [6] first demonstrated that a high dietary salt intake was associated with an increase in the plasma levels and urinary excretion of...
Table 3  Correlation coefficients for the relationships between endothelium-derived factors and 24-h mean blood pressure measured at low and high salt intakes, and the variations induced by high salt intake

MBP, mean blood pressure. None of the correlation coefficients are statistically significant.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Low salt 24-h MBP</th>
<th>High salt 24-h MBP</th>
<th>Change in 24-h MBP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma NO&lt;sub&gt;x&lt;/sub&gt;</td>
<td>0.041</td>
<td>0.047</td>
<td>-0.212</td>
</tr>
<tr>
<td>Urine NO&lt;sub&gt;x&lt;/sub&gt;</td>
<td>-0.130</td>
<td>0.015</td>
<td>-0.101</td>
</tr>
<tr>
<td>Plasma cGMP</td>
<td>-0.016</td>
<td>0.244</td>
<td>0.155</td>
</tr>
<tr>
<td>Urine cGMP</td>
<td>0.039</td>
<td>0.087</td>
<td>0.026</td>
</tr>
<tr>
<td>Plasma endothelin</td>
<td>0.167</td>
<td>-0.063</td>
<td>-0.158</td>
</tr>
</tbody>
</table>

Table 4  Biochemical and hormonal parameters in patients with essential hypertension during low and high salt intakes

Values are means ± S.E.M. or median (range). LDL, low-density lipoprotein; HDL, high-density lipoprotein.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Low salt</th>
<th>High salt</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma Creatinine</td>
<td>86.8 ± 2.7</td>
<td>84.0 ± 3.2</td>
<td>0.223</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>5.3 ± 0.2</td>
<td>5.3 ± 0.1</td>
<td>0.588</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>5.1 ± 0.1</td>
<td>4.9 ± 0.1</td>
<td>0.099</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/l)</td>
<td>3.1 ± 0.1</td>
<td>2.9 ± 0.1</td>
<td>0.066</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>1.3 ± 0.1</td>
<td>1.3 ± 0.1</td>
<td>0.130</td>
</tr>
<tr>
<td>Triacylglycerols (mmol/l)</td>
<td>1.3 ± 0.1</td>
<td>1.3 ± 0.1</td>
<td>0.427</td>
</tr>
<tr>
<td>Uric acid (µmol/l)</td>
<td>316 ± 11</td>
<td>284 ± 11</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Haematocrit (l/l)</td>
<td>0.41 ± 0.05</td>
<td>0.40 ± 0.05</td>
<td>0.103</td>
</tr>
<tr>
<td>Plasma renin activity (pmol·h&lt;sup&gt;-1&lt;/sup&gt;·ml&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.36 (0.01–1.54)</td>
<td>0.12 (0.01–1.61)</td>
<td>0.019</td>
</tr>
<tr>
<td>Angiotensin II (pg/ml)</td>
<td>24.3 ± 1.6</td>
<td>18.8 ± 1.6</td>
<td>0.004</td>
</tr>
<tr>
<td>Aldosterone (pg/ml)</td>
<td>489 ± 41</td>
<td>294 ± 24</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>ANP (fmol/l)</td>
<td>28.3 (8–107)</td>
<td>32.2 (6–169)</td>
<td>0.044</td>
</tr>
<tr>
<td>Urine Na&lt;sup&gt;+&lt;/sup&gt; (mmol/24 h)</td>
<td>63 ± 4.9</td>
<td>227 ± 11</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Urine K&lt;sup&gt;+&lt;/sup&gt; (mmol/24 h)</td>
<td>69 ± 3.7</td>
<td>70 ± 3.6</td>
<td>0.717</td>
</tr>
</tbody>
</table>

NO<sub>x</sub>, as well as an increase in cGMP excretion, in normotensive Sprague–Dawley rats. Additionally, they found a significant correlation between urinary sodium and NO<sub>x</sub> excretion rate. Based on these results, the authors concluded that exposure to high dietary salt resulted in increased endogenous NO production, and that this could contribute to the regulation of blood pressure by facilitating sodium excretion after an NaCl load. Another study from the same group [15] confirmed this hypothesis by demonstrating that the same rats became sensitive to the pressor effect of salt when the expected increase in NO production was blocked by administration of an exogenous NO synthase inhibitor.

Chen and Sanders [7] also suggested that a defect in NO synthesis may underlie the phenomenon of salt-sensitive hypertension in rats. These authors reported that dietary salt loading resulted in increased urinary excretion of cGMP in salt-resistant Dahl rats, whereas this response was blunted in salt-sensitive rats. In addition, the administration of L-arginine decreased blood pressure to normotensive levels and increased the urinary excretion of cGMP in salt-sensitive Dahl rats made hypertensive by consumption of a high-salt diet for 2 weeks [7]. Other groups have also suggested that the development of salt-sensitive hypertension may be secondary to an inability to enhance NO synthesis in response to increased dietary salt intake [17–19].

In humans, it has been suggested that essential hypertension may be linked to decreased NO production. In this regard, Forte et al. [8] and Node et al. [9] reported that patients with essential hypertension exhibited decreased baseline plasma and urinary concentrations of NO<sub>x</sub> compared with normotensive controls. Endothelium-derived factors have also been measured in
In agreement with the present results, Hwang et al. [30] decreased in the production of both NO and endothelin secretion, and the results of this inhibitory effect are exerts a general inhibitory effect on endothelial cell salt intake, perhaps through an increase in plasma volume, on NO-derived products. We can speculate that a high plasma endothelin concentration, in addition to its effect for this inhibitory effect of a high salt intake on the statistically significant. We do not have a clear explanation hypothetically decreases in plasma endothelin observed in our group. However, it is important to note that plasma endothelin concentrations are a poor reflection of endothelial cell secretion, due to the predominantly abluminal transport of endothelin from endothelial cells to smooth muscle cells.

We did not observe any significant change in plasma or urinary concentrations of cGMP in response to a high salt intake. In this sense, it might be expected that cGMP changes would parallel those observed in NO production. However, it is important to note, as discussed above, that ANP was significantly increased by a high salt intake, and it is known that cGMP is the cellular mediator of the actions of ANP. Thus the increase in ANP could counteract a hypothetically decreases in cGMP due to the reduction in endothelial NO production [32].

We have not observed any correlation between changes in endothelium-derived factors and changes in 24-h blood pressure induced by a high salt intake. Moreover, values of endothelial products obtained at the end of the low- and high-salt periods did not correlate with blood pressure changes measured simultaneously. Fujiwara et al. [11] recently reported that variations in nitrate induced by changes in salt intake were correlated with the increase in blood pressure. However, the correlation was only statistically significant with salt loading, but not with salt restriction. Moreover, two studies measuring the plasma and urinary excretion of endothelin in relation to salt sensitivity have shown contradictory results. Ferri et al. [28] reported an increase in plasma endothelin levels with high salt intake in salt-sensitive patients with essential hypertension, whereas Hoffman et al. [29] described low urinary endothelin excretion in salt-sensitive hypertensive subjects, irrespective of the level of salt intake. We have not confirmed these results. In fact, although we did not categorize patients with essential hypertension into salt-sensitive and salt-resistant groups, the lack of a correlation between blood pressure and endothelial-derived factors in response to a high salt intake observed in the present study does not support the hypothesis that salt-induced changes in endothelium-derived factors may be involved in the pathogenesis of salt-sensitive hypertension. Nevertheless, it is important to note that plasma and urinary measurements of NO, cGMP and endothelin are poor indicators of endothelial cell secretion.

We conclude that, in patients with essential hypertension, a high salt intake promotes significant decreases in the plasma levels and urinary excretion of NO, as well as in plasma endothelin levels. This may indicate that a high salt intake impairs endothelial cell function. How-
ever, the lack of a correlation between these changes and variations in blood pressure does not support a patho-
genic role for such changes in salt-sensitive hypertension.

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