Sodium Intake Is associated With Endothelial Damage Biomarkers and Metabolic Dysregulation

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BACKGROUND
Mounting evidence has associated high sodium (HS) intake with hypertension, cardiovascular disease, and stroke. We investigated whether HS intake modulates the parameters of endothelial damage, inflammation, and oxidative stress.

METHODS
We used a cross-sectional study design including 223 Chilean subjects (6.9–65.0 years old). We measured aldosterone, renin activity, cortisol, cortisone, adiponectin, leptin, hsCRP, interleukin 6 (IL-6), tumor necrosis factor-α (TNF-α), plasminogen activator inhibitor type 1 (PAI-1), metalloproteinase (MMP)-9 and MMP-2 activity, and malondialdehyde. Sodium and creatinine were measured in 24-hour urine samples. The subjects were divided by sodium intake, high sodium (HS): ≥150 mEq/day, $n = 118$, and adequate sodium (AS): <150 mEq/day, $n = 105$.

RESULTS
We observed a positive correlation between urinary sodium excretion and blood pressure ($r = 0.1669$, $P = 0.0124$ for systolic and $r = 0.2416$, $P = 0.0003$ for diastolic), glycemia ($r = 0.2660$, $P < 0.0001$), and triglycerides ($r = 0.1604$, $P = 0.0175$) and a highly significant correlation between sodium excretion and PAI-1 ($r = 0.2701$, $P < 0.0001$). An inverse correlation was observed between urinary sodium and HDL-cholesterol ($r = -0.2093$, $P = 0.0018$) and adiponectin ($r = -0.2679$, $P < 0.0001$). In a linear regression model, urinary sodium excretion remained significantly associated with PAI-1 values even after adjusting for age, gender, and BMI. The HS group had higher blood pressure, glycemia, HOMA-IR, atherogenic index of plasma, and PAI-1 values than the group with AS intake.

CONCLUSIONS
HS intake is associated with endothelial damage (high PAI-1) and metabolic dysregulation. On the other hand, inflammation and oxidative stress parameters are not modified by sodium intake.

Keywords: blood pressure; endothelial damage; hypertension; PAI-1; sodium intake.

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Sodium intake has increased in most countries, exceeding the current recommendations by far. Optimal sodium intake has been controversial. The American Heart Association (AHA) indicates <1,500 mg/day,[1] the World Health Organization[2] (WHO) advises <2,000 mg/day for children and adults, assuring an appropriate caloric intake in the pediatric population, and the Institute of Medicine (IOM) recommends 1,000–1,500 mg/day according to the age group.[3]

In Chile, our previous study showed that the mean sodium intake was approximately 3,121 mg (equivalent to 8.0-g salt/day) for children and 4,160 mg (equivalent to 10.6 g of salt/day) for adults.[4] Salt intake is 10 g (3,900-mg sodium) in Vietnam,[5] 13.9 g (5,421-mg sodium) in northern China,[6] and 8.7 g (3,400-mg sodium) in the United States.[7] Despite these studies showing high sodium (HS) intake, the results should be interpreted with caution because they used different methodologies.

In recent decades, a large number of articles have appeared communicating an association between HS intake and an increase in blood pressure in children and adults and in cardiovascular events and mortality. These reports are focused on this issue as a public health problem, analyzing the number

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of incidents of coronary heart disease, stroke, congestive heart failure, and cardiovascular disease in the participants without mentioning the biochemical parameters that triggered them.[8–10]

Recently, we reported that HS intake is related to obesity[4] and chronic diseases, such as insulin resistance and metabolic syndrome.[11] However, currently, there is limited data showing that HS intake may be linked to endothelial damage, inflammation, or oxidative stress.

The aim of the present study was to evaluate whether HS intake modifies endothelial damage, inflammation, or oxidative stress biomarkers.

METHODS

We used a cross-sectional study design inviting 307 Chilean subjects (84 children and 223 adults) between 6.9 and 65.5 years old of both genders. All recruited subjects were evaluated by a pediatric or adult endocrinologist from the Pontificia Universidad Católica de Chile. Patients with confirmed apparent mineralocorticoid excess (n = 2), confirmed familial primary aldosteronism (n = 26), suspected primary hyperaldosteronism (n = 4), suspected Cushing's syndrome (n = 4), treatment with glucocorticoids or nonsteroidal anti-inflammatory drugs (n = 16), and diuretic use (n = 3) were excluded. Women who were using contraceptives were excluded from the analysis of serum cortisol, serum cortisone, and the cortisol/cortisone ratio (n = 21). The participants' salt intake was ad libitum. The evaluation of sodium intake was estimated by measuring 24-hour urinary sodium excretion. Most guidelines advise a daily urinary sodium of approximately 100 mEq/day (approximately 6 g/day of salt intake) and consider a high-salt diet to be urinary sodium excretion >150 mEq/24 hours (approximately 9 g of salt).[12] In addition, new evidence indicates that the association between estimated sodium excretion and cardiovascular events could be J-shaped and that a low sodium excretion of <50 mEq/24 hour (3 g/day) is associated with an increased risk of morbidity and mortality.[13] For these reasons, subjects were divided in two groups, one with HS intake (>150 mEq/24 hour) and the other with adequate sodium intake (AS 44–149 mEq/24 hour) according to urinary sodium excretion as previously reported.[11] All of the participants were instructed to collect complete urine samples over 24 hours. For females, their collection was not performed during menstruation. The participants were asked to discard their first morning urine void (07:00 hours) and to collect all of the urine voided over the following 24 hours up to and including the first morning urine void (07:00 hours) the next day. Upon collection of the bottles, research assistants verified the collection procedure and measured the volume of the 24-hour urine sample. To assure adequate urine collection, we selected the children who excreted >11.3-mg creatinine/kg/24 hour[14] and the adults whose ratio of milligram creatinine 24 hour/(21 × weight in kg) was >0.7.[15]

All subjects visited our outpatient clinic after a 12-hour fasting period, with their 24-hour urine collection done the previous day. Blood samples were obtained between 8:00 and 9:00 am, in a seated position at least 15 minutes, in the following order: serum to measured lipid profile and creatinine; plasma citrate, which was immediately centrifuged (4 °C, 12,000 rpm) to measured plasminogen activator inhibitor type 1 (PAI-1), interleukin 6 (IL-6), and tumor necrosis factor-α (TNF-α) in the supernatant; plasma—fluoride to measure glucose; serum to measure insulin, cortisol, cortisone, aldosterone, adiponectin, leptin, and high-sensitivity C-reactive protein (hsCRP); and plasma EDTA to measure plasma renin activity and matrix metalloproteinase-9 (MMP-9) and MMP-2 activities.

Sodium and creatinine excretion were also measured in 24-hour urinary samples using methods described previously.[4] In brief, sodium was measured in an automated chemistry analyzer (Roche/Hitachi, Kobe, Japan) by indirect potentiometry (coefficient of variation was 4.05% for 63.6 mEq/l and 2.04% for 174.6 mEq/l). Serum and urinary creatinine were measured by the kinetic colorimetric Jaffe method in an automated chemistry analyzer (Roche/Hitachi). The coefficient of variation was 3.6% for 1.2 mg/dl and 2.4% for 5.2 mg/dl for serum and 2.3% for 80.6 mg/dl and 4.0% for 240.9 mg/dl for urine. Serum cortisol and cortisone were measured by HPLC-MS/MS (ABSciex, 4500 QTrap, Foster City, CA), using cortisol-d4 and cortisone-d2 as internal standards as previously reported by our group.[16] Kidney function was estimated using the serum creatinine level.

Serum fasting lipid profiles and glucose concentrations were measured using an automated Roche Hitachi Modular chemistry analyzer (Hitachi, Tokyo, Japan) as previously described.[11] Insulin was measured by electrochemiluminescent immunoassay (Cobas 8000, Roche, Tokyo, Japan).

Aldosterone and plasma renin activity were measured by immunoassay as previously described.[17] Serum cortisol and cortisone were measured by HPLC-MS/MS (ABSciex, 4500 QTrap) using cortisol-d4 and cortisone-d2 as internal standards. The coefficient of variation for three levels of concentration (20, 40, and 80 ng/ml) was <10% for both steroids.

Endothelial dysfunction markers were measured as follows: Plasma PAI-I was measured by enzyme-linked immunosorbent assay (ELISA; HYPHEN BioMed, Neuville-sur-Oise, France), MMP-9 and MMP-2 activities were estimated by zymography as described previously.[18] The results are expressed as the number of changes with respect to the reference plasma used as internal control.

Inflammation biomarkers were measured as follows: hsCRP was measured by nephelometric assay (BN ProSpec Systems, Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany). The interassay coefficients of variation were 3.1% for a concentration of 6.59 mg/l and 4.8% for a concentration of 44.3 mg/l. IL-6 and TNF-α were measured by ELISA using commercial reagents and standards according to the manufacturer's protocols (Bender Med Systems and eBioscience, Vienna, Austria, respectively). Adiponectin was measured by RIA using a commercial kit (Millipore, Billerica, MA). The intra-assay and interassay coefficients of variation were 6.5% and 9.8%, respectively. Serum leptin was measured by radioimmunoassay (Diasource, Nivelles, Belgium), and the intra-assay and interassay coefficients of variation were 4.6% and 6.2%, respectively

Oxidative stress damage was evaluated by malondialdehyde measurement quantifying the thiobarbituric acid-reactive compounds as previously described.[19]

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ETHICS

The protocol was approved by the Ethics Committee of the Faculty of Medicine of the Pontificia Universidad Católica de Chile in accordance with the Helsinki Declaration. All of the parents signed informed consent forms, and subjects who were older than 9 years provided their consent before entering the study.

DATA ANALYSIS

A descriptive analysis was performed. Continuous variables are presented as the mean ± SD. Normality of the parameters was assessed using the Kolmogorov–Smirnov test. BMI-SDS for children was calculated based on the Centers for Disease Control and Prevention (CDC) database, and in adults, it was calculated according to the following formula: (BMI observed − mean BMI for age and gender)/SD for age and gender. The data for BMI and SD for age and gender were obtained from the Chilean health survey, which included 2,303 women and 1,527 men between 18 and 65 years. The mean BMI and SD were 28.005 ± 6.7092 for women and 27.188 ± 4.5253 for men. For regression analyses, BMI-SDS in adults was also calculated. The atherogenic index of plasma was calculated as log (triglyceride/HDL-cholesterol).[20]

Correlations between normally continuous variables were evaluated using Pearson's test. A linear regression was performed with outcomes of interest and sodium intake adjusted by known modulators such as age, gender, and BMI-SDS.

Participants divided by sodium intake criteria were analyzed by Student's *t*-test. In case a variable was not normally distributed, bootstrapping with 1,000 iterations was applied. Malondialdehyde, MMP-9, and MMP-2 were expressed in quartiles and evaluated as ordinal variables.

RESULTS

A total of 307 subjects were invited to participate in this study; of these, 68/84 (82%) children and 155/223 (69%) adults had an adequate 24-hour urinary collection based on their creatinine excretion per kg of body weight. Thus, data from 68 children and 155 adults met the final inclusion criteria. All of the selected participants had normal renal function (serum creatinine was 0.591 ± 0.134 mg/dl for children and 0.7797 ± 0.163 mg/dl for adults). Anthropometric and clinical characteristics of all selected participants are shown in Table 1.

The analysis of the data from all the subjects (n = 223) showed a positive correlation between urinary sodium excretion and age (r = 0.2088, P = 0.0018), blood pressure (r = 0.1669, P = 0.0124 for systolic and r = 0.2416, P = 0.0003 for diastolic blood pressure), glycemia (r = 0.2960, P < 0.0001), triglycerides (r = 0.1604, P = 0.0175), and atherogenic index of plasma (r = 0.2092, P = 0.0020) and a highly significant correlation between sodium excretion and PAI-1 (r = 0.2701, P < 0.0001) (Figure 1, left panel). An inverse correlation was found between urinary sodium and HDL-Cholesterol (r = −0.2093, P = 0.0018) and adiponectin (r = −0.2679, P < 0.0001) (Figure 1, right panel), and a trend toward a negative correlation between plasma aldosterone (r = −0.1115, P = 0.0967) and plasma renin activity (r = −0.1152, P = 0.0868) was also observed. HOMA, cortisol, cortisone, cortisol/cortisone ratio, leptin, TNF-α, IL-6, MMP-9, MMP-2, and hsCRP had no significant correlation with sodium intake (data no shown).

Table 1. Clinical characteristics of the selected subjects

***N* = 223**	
Age (years)	31.7 ± 15.5
Female (%)	55.2
BMI (adults, n = 155)	26.9 ± 3.4
BMI-SDS (children, n = 68)	0.981 ± 0.736
SBP (Hg mm)	126.2 ± 18.0
DBP (Hg mm)	79.7 ± 12.7

BMI, body mass index; BMI-SDS, BMI-SD score; SBP, systolic blood pressure; DBP, diastolic blood pressure.

In a linear regression model with PAI-1 as the response variable, urinary sodium remained significantly associated with PAI-1 values, even when adjusting for age, gender, and BMI-SDS of all participants (partial correlation coefficient = 0.149, P = 0.023). In contrast, adiponectin concentrations maintained a significant association with urinary sodium (partial correlation coefficient = −0.245, P = 0.0001), but this association lost statistical significance when adjusted for age, gender, and BMI-SDS of all participants (partial correlation coefficient = 0.026, P = 0.693).

Anthropometric and clinical characteristics of the selected participants, divided by salt intake, are shown in Table 2.

The HS intake group, compared with the AS intake group, has significant differences in clinical and biochemical characteristics. The first group has higher ages and blood pressure values than those of the second group. In addition, they have higher glycemia, HOMA-IR, triglycerides, and atherogenic index of plasma than the members of the group with AS intake. As expected, subjects with high salt intake had lower aldosterone concentration. Furthermore, subjects with high salt intake had significantly lower adiponectin and higher PAI-1 concentrations. We observed no significant differences in BMI-SDS, plasma renin activity, cortisol, cortisone, leptin, hsCRP, IL-6, TNF-α, malondialdehyde, or MMP-9 and MMP-2 activities.

DISCUSSION

We reported that HS intake was associated with an increased PAI-1 concentration even after adjusting for confounding variables, suggesting that PAI-1 increment may be an early marker of endothelial damage in subjects with HS intake. Increased sodium intake was also associated with increased blood pressure, dyslipidemia, insulin resistance, and hypoadiponectinemia.

In relation to PAI-1, which is the principal inhibitor of plasminogen activation, the increased levels can promote fibrosis and endothelial damage. Moreover, we also found that PAI-1 showed an inverse association with MMP-2 activity (data not shown), an enzyme that degrades collagenous

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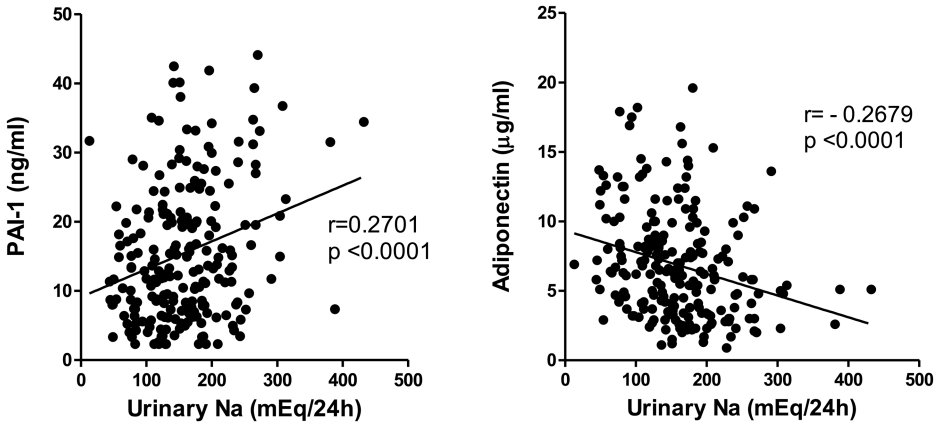

Figure 1. Pearson's correlation between sodium intakes, evaluated as urinary sodium excretion, and PAI-1 (left panel) and adiponectin (right panel).

Table 2. Clinical and biochemical characteristics of selected subjects divided by sodium intake

***N* = 223**	**High sodium intake, (≥150 mEq/24 h) (53%)**	**Adequate sodium intake, (<150 mEq/24 h) (47%)**	***P***
Clinical			
Age (years)	33.9 ± 14.6	29.1 ± 16.2	0.020
Female (%)	39	73.6	
BMI-SDS	0.32 ± 0.8	0.18 ± 0.8	0.2217
SBP (Hg mm)	128.5 ± 17.7	123.5 ± 17.9	0.031
DBP (Hg mm)	81.8 ± 12.6	77.0 ± 11.7	0.003
Blood biochemistry			
Glycemia (mg/dl)	87.6 ± 6.9	84.5 ± 6.8	0.0009
HOMA-IR	2.6 ± 1.6	2.2 ± 1.1	0.0397
Triglycerides (mg/dl)	141.5 ± 107.5	100.6 ± 52.7	0.0006
HDL-cholesterol (mg/dl)	46.6 ± 13.1	53.7 ± 16.2	0.0004
Atherogenic index of plasma	0.41 ± 0.35	0.24 ± 0.28	0.0001
Aldosterone (ng/dl)	9.4 ± 6.1	11.8 ± 10.8	0.037
PRA (ng/ml × hour)	2.0 ± 1.9	2.2 ± 2.0	0.444
Cortisol (µg/dl)	12.1 ± 6.1	11.7 ± 7.3	0.6294
Cortisone (µg/dl)	2.5 ± 0.7	2.6 ± 0.5	0.591
Cortisol/cortisone ratio	4.8 ± 2.1	4.7 ± 2.0	0.595
Leptin (ng/ml)	9.9 ± 8.0	10.5 ± 6.8	0.557
Adiponectin (µg/ml)	6.1 ± 3.8	7.8 ± 3.8	0.001
PAI-1 (ng/ml)	17.6 ± 10.4	13.0 ± 8.7	0.001
hsCRP (mg/l)	2.7 ± 6.6	2.3 ± 4.0	0.552
IL-6 (pg/ml)	5.0 ± 4.0	11.7 ± 35.3	0.127
TNF-alfa (pg/ml)	293.8 ± 700.8	243.8 ± 622.5	0.588
MDA (frequency, % in each quartile)	26.1//23.5//23.5//26.9	23.7//25.8//26.8//23.7	0.877
MMP-9 activity (frequency, % in each quartile)	24.7//25.8//22.5//27	27.1//20.8//29.2//22.9	0.637
MMP-2 activity (frequency, % in each quartile)	31.0//21.6//26.7//20.7	17.7//29.2//22.9//30.2	0.067
Urinary biochemistry			
Sodium excretion (mEq/24 h)	204.6 ± 51.4	104.5 ± 28.8	0.0001

BMI-SDS, BMI-SD score; DBP, diastolic blood pressure; SBP, systolic blood pressure; hsCRP, high-sensitive C-reactive protein; IL-6, interleukin-6; MDA, malondialdehyde; MMP, metalloproteinase; PAI-1, plasminogen activator inhibitor-1; PRA, plasma renin activity; TNF-α, tumoral necrosis factor α; HOMA-IR, homeostatic model assesment for insuline resistance; HDL, high density lipoprotein.

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protein. Thus, PAI-1 could be acting through two mechanisms to induce endothelial damage.

The correlation between sodium intake and blood pressure is well known and mostly secondary to increases in the vascular volume and vascular reactivity. However, we and others had demonstrated that sodium can increase RAC1 expression. Recent studies have revealed a cross-talk between Rac1, a small GTP-binding-protein, and mineralocorticoid receptor activation independent of aldosterone. Thus, the increase in RAC-1 expression mediated by sodium can increase mineralocorticoid receptor, neutrophil gelatinase-1, nuclear factor-kappa B, and heme oxygenase-1 expression, suggesting that RAC1 could be a mediator of cardiovascular damage induced by sodium and may also be useful to identify subjects with different responses to sodium intake.[21]

Sodium intake was associated positively with blood pressure, glycemia, and dyslipidemia and inversely with adiponectin. These findings confirm our previous results observed in another group of patients.[11] The lack of association between sodium intake and HOMA-IR was observed considering all the participants and analyzing the children alone unlike that reported by Kim YM,[22] who found a positive association analyzing 718 Korean children and adolescents. In this study, and in the previous,[11] HOMA-IR was higher, and adiponectin was lower in the group of subjects with HS intake than in the group with AS intake. Adiponectin not only improves insulin sensitivity but also exerts protective effects against inflammation. These effects may be related to its ability to suppress the production of proinflammatory cytokines, such as TNF-α and hsCRP.[20] At cellular levels, adiponectin increases endothelial nitric oxide bioavailability and inhibits oxidative stress.[23]

Recent studies from different authors have shown that sodium modulates immune cell function, making the immune system a possible link between sodium and hypertension.[24,25] Moreover, Yi B[26] carried out a longitudinal study in which healthy subjects were given 12, 9, and 6 g of salt for approximately 50 days at each salt intake level. The authors observed that when subjects ingested 12 g of salt, the number of monocytes and the levels of IL-6 and IL-23 were higher than when subjects ingested 9 g or 6 g, and the levels of TNF-α did not change. In contrast, the level of IL-10 was lower when subjects ingested 12 g of salt than when they ingested 9 and 6 g. In our study, in the group of subjects with HS intake, the mean sodium excretion corresponds to 12.1 g of salt, and in the group with adequate salt intake, the mean sodium excretion corresponds to 6.2 g of salt, and we did not find a difference in TNF-α or IL-6 levels between the two groups of subjects.

Another interesting point is that high salt intake did not affect the activity of the 11β-HSD2 enzyme since serum cortisol/cortisone ratio did not change between the groups of subjects, but its activity changed with age as we previously reported.[27]

One of the strengths of this study is the extensive clinical evaluation, the measurement of several biomarkers and the detailed hormonal profiling in participants (children and adults). We evaluated sodium intake measuring sodium excretion in 24-hour urinary samples and not in isolated urine samples. Another strength is that serum cortisol and cortisone were measured by HPLC-MS/MS which is the gold standard for measuring these parameters.

One of the limitations of this protocol is that it is a cross-sectional study. Since we could not perform an intervention in dietary sodium, we adjusted the analysis for well-established confounders and performed multivariate regression analysis.

In summary, HS intake is associated with high levels of PAI-1 linked to endothelial damage and a high atherogenic index in cardiovascular and cerebrovascular events via more than one pathway. Our results emphasize the impact of HS consumption beyond blood pressure.

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DISCLOSURE

The authors declared no conflict of interest.

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