Salt-Sensitivity

Salt Sensitivity in Blacks

Evidence That the Initial Pressor Effect of NaCl Involves Inhibition of Vasodilatation by Asymmetrical Dimethylarginine

Olga Schmidlin, Alex Forman, Anna Leone, Anthony Sebastian, R. Curtis Morris, Jr

Abstract—In healthy, mostly normotensive blacks, 19 salt-sensitive (SS) and 18 salt-resistant (SR), we tested the hypothesis that, in SS subjects, dietary NaCl loading induces its initial pressor effect by inducing a normal increase of cardiac output, while failing to induce a normal pressor-offsetting vasodilatation, consequent to its inhibition by asymmetrical dimethylarginine that is abnormally increased by NaCl. In SS and SR subjects, dietary NaCl loading, 250 from 30 mmol/d, over a 7-day period, induced similar, immediate increases in external Na⁺ balance (by day 2, ≈360 mmol), plasma volume (+11%), and cardiac output (+8%). In SR subjects, from day 1, transient decreases occurred in both systemic vascular resistance (nadir: −13%, day 2) and mean arterial pressure (nadir: −5%, day 2). In SS subjects, systemic vascular resistance did not change over days 1 to 3, whereas mean arterial pressure increased progressively after day 1, ultimately by 10 mm Hg. Failure of systemic vascular resistance to normally decrease, while cardiac output normally increased, accounted for salt’s initial pressor effect in the SS subjects. In SS subjects, baseline plasma levels of asymmetrical dimethylarginine (0.76 μmol/L) and symmetrical dimethylarginine (0.60 μmol/L), which does not affect vasodilatation, approximated those in SR subjects. In SS but not SR subjects, NaCl loading induced increases in asymmetrical dimethylarginine on both days 2 (+38%, median) and 7 (+14%, median). Symmetrical dimethylarginine changed in neither group. For all of the subjects combined, changes in asymmetrical dimethylarginine on day 2 predicted changes in systemic vascular resistance (R=0.751; P<0.001) and mean arterial pressure (R=0.527; P=0.006) on day 2 and similarly on day 7. These observations support the hypothesis tested. (Hypertension. 2011;58:380-385.) ● Online Data Supplement

Key Words: blood pressure ▪ blacks ▪ sodium chloride, dietary ▪ asymmetrical dimethylarginine ▪ symmetrical dimethylarginine

Blood pressure (BP) that is, or is not, increased by dietary NaCl loading is deemed salt-sensitive (SS) or salt-resistant (SR), as are those so affected. Hypertension and fatal cardiovascular disease occur more frequently in the SS than in the SR.1–3 In the traditionally formulated pathophysiological initiation of salt sensitivity, an abnormally enhanced renal reclamation of NaCl and commensurate water induces intravascular “volume loading” which leads to an excessive increase of cardiac output (CO) that alone initiates salt’s pressor effect.4,5 However, recent observations in SS blacks6 suggest that the pressor effect of dietary NaCl loading might be initiated by NaCl’s induction of a normal increase of CO, whose direct pressor effect fails to be offset by normal vasodilatation but instead is amplified by inhibition of this vasodilatation. Asymmetrical dimethylarginine (ADMA) is a major endogenous inhibitor of vasodilatation by inhibiting the endothelial synthesis of NO, which relaxes vascular smooth muscle.7,8 In isolated rat arterioles,9 and likely in small resistance arteries,10 ADMA inhibits vasodilatation otherwise induced by increased flow/shear stress. Intravenously administered ADMA acutely increases systemic vascular resistance (SVR) and BP in humans and animals.11–13 Thus, in SS but not SR blacks, an increased formation of ADMA induced by NaCl loading might render pressor its normal transient increase of CO by inhibiting an otherwise offsetting vasodilatation, as judged by that induced in the SR subjects. We report observations supporting this hypothesis.

Methods

Participants and Setting

We studied 37 healthy black (African American) men and women with screening BPs of 115 to 155/70 to 95 mm Hg and no history or evidence of renal disease, ischemic heart disease, stroke, or diabetes mellitus. BP and laboratory screening were usually performed 1 to 2 weeks before admission. The study was approved by the University of California, San Francisco, Committee on Human Research. All of...
the procedures followed were in accordance with institutional guidelines. All of the participants gave written informed consent.

Participants were admitted to the General Clinical Research Center at the University of California, San Francisco, for a 2-week course of study. On admission, that is, on their usual salt intake, 3 of the 37 participants were mildly hypertensive, with BP ranging from 142/79 to 157/95 mm Hg. Throughout the study, participants ate a eucaloric metabolic diet, as described before.6 Physical activity was limited to daily walks on the center’s one floor.

Intervention (NaCl Load)
Week 1 served as the low NaCl (LS) baseline period. Throughout week 2, the high NaCl (HS) period, NaCl was supplemented with the diet. Average NaCl intake during the LS period was (in millimoles per day), 33 (SD 7) in SS and 36 (SD 6) in SR (P=0.21) subjects. During the HS period, intakes were 270 (SD 36) and 281 (SD 30) in SS and SR subjects, respectively (P=0.31).

Assessment of Salt Sensitivity
To determine salt sensitivity, the average mean arterial pressure (MAP) of days 5 to 7 of the LS period was subtracted from the average MAP of days 5 to 7 of the HS period. Salt sensitivity was defined as an NaCl-induced increase in MAP of ≈5 mm Hg and salt resistance as an increase of <5 mm Hg.6

Hemodynamic and Metabolic Outcomes
A detailed Methods section describing measurements of hemodynamic and metabolic variables is supplied in the online Data Supplement (please see http://hyper.ahajournals.org).

Data Analysis
Data are presented as mean and SD, mean and 95% CI, or median and 25th/75th percentile, as appropriate. The null hypothesis was rejected at P<0.05 (P values were adjusted for multiple comparisons using the Bonferroni method). Effects of NaCl loading were calculated as the percentage of change from the average value of days 5 to 7 of the LS period. We used paired t tests for within-group (NaCl effect) comparisons and unpaired t tests for between-group (SS versus SR) comparisons. When variances were unequal or observations were not normally distributed, we used nonparametric test equivalents. We explored relationships between variables using linear regression analysis and Spearman rank order correlation, as appropriate, and assessed homogeneity of regression slopes in SS versus SR using ANCOVA. Analyses were carried out using Statistica (Statsoft, Inc, Tulsa, OK).

Results
Characteristics of SS and SR Subjects
Nineteen of 37 subjects (51%) were SS with average NaCl-induced increase in MAP on days 5 to 7 of 10 mm Hg (95% CI: 0.001 compared with LS). Eighteen (51%) were SS, with average NaCl-induced change in MAP of 0.3 mm Hg (95% CI: 0.001 compared with LS). Hemodynamic and metabolic data of a subset (n≈23) of the currently studied subjects have been reported recently.6 SS subjects were slightly older than SR subjects, but SS and SR did not differ from each other with respect to initial BP, serum electrolytes, and creatinine (Table S1, available in the online Data Supplement at http://hyper.ahajournals.org). All subjects had a body mass index of <31 and a calculated glomerular filtration rate of >60 mL/min per 1.73 m².

Hemodynamic Effects of LS and HS Diets
At the end of the LS period (average of days 5 to 7), SS and SR subjects did not differ with respect to MAP (SS: 15.4 mm Hg [SD 1.25 mm Hg]; SR: 15.2 mm Hg [SD 1.17 mm Hg]). In the SR subjects, NaCl loading induced within 9 hours (ie, at 6:00 PM of day 1) a decrease in MAP of −5% (95% CI: −7% to −4%; Figure 1), a decrease persisting through day 4;
thereafter, MAP increased progressively to values not different from those on LS. In the SS subjects, MAP increased progressively from day 2 of NaCl loading. NaCl-induced changes in MAP differed significantly between SS and SR subjects within 9 hours of initiating NaCl loading and remained significantly different throughout the 7-day HS period (Figure 1 and Table S2).

In SR but not SS subjects, NaCl loading induced a decrease in SVR (Figure 1) by 6:00 PM of day 1 of $-7.0%$ (95% CI: $-13.0\%$ to $-1.5\%$), a decrease reaching its nadir on day 2 of $-13.0\%$ (95% CI: $-16.0\%$ to $-10.0\%$), and the decrease persisting through day 5; thereafter, SVR returned to levels not different from those on LS. By contrast, in SS subjects, SVR rose progressively from day 4 to a maximum of $10\%$ (95% CI: 6% to 13%) above LS baseline by day 7. On all days but day 1, changes in SVR differed significantly between SR and SS subjects (Table S2).

In both SS and SR subjects, NaCl loading induced similar, transient increases in CO from day 1 (Figure 1 and Table S2). Average CO on day 1 increased significantly both in SS subjects by $+5\%$ (95% CI: 2% to 7%) and SR subjects by $+4\%$ (95% CI: 2% to 7%). In most subjects, the maximum average daily increase occurred on day 2 or 3 and amounted in SS subjects to $12\%$ (95% CI: 9% to 14%) above baseline and in SR subjects to $10\%$ (95% CI: 7% to 14%; SS versus SR, $P=0.6$). In both SS and SR subjects, values of CO on day 7 of HS did not differ significantly from those on LS.

NaCl-induced changes in MAP from day 1 and in SVR from day 2 predicted average changes in MAP on days 5 to 7 of HS. Figure S1A and S1B show the relationship between NaCl-induced changes in MAP ($R=0.767; P<0.001; n=37$) and SVR ($R=0.647; P<0.001; n=37$), respectively, on day 2 of HS and changes in MAP on days 5 to 7.

### Metabolic Effects of NaCl Loading

NaCl loading induced in SS and SR subjects the same increase in body weight (Figure S2, top left). Throughout the 7-day HS period, net cumulative Na$^+$ balance (Figure S2, bottom left) did not differ between SS and SR subjects. In both SS and SR subjects, increases in body weight and external Na$^+$ balance were largest in the first 2 days of, and persisted throughout, the HS period.

As judged by decreases in both serum total protein (Figure S2, top right) and hematocrit values, NaCl loading induced in both SS and SR subjects significant increases in plasma volume (PV) of $>10\%$ by day 2 that persisted through day 7. Changes in serum total protein on day 2 of HS varied directly with changes in SVR (Figure 2), both for all subjects combined and for SS and SR groups individually, suggesting that, in both SS and SR groups, the extent of PV expansion, or some function of that expansion, is a determinant of the initial decrease in SVR. Regression lines for SS and SR groups were parallel (homogeneity test: $P=0.92$).

NaCl loading induced similar increases in extracellular volume both on days 2 (Figure S2, bottom right, and Table S2) and 7 of HS. Serum creatinine levels and creatinine clearance did not differ between SS and SR subjects at baseline or during NaCl loading. NaCl loading had no effect on creatinine levels or clearance values in either group. For additional hemodynamic and metabolic results, see the online Data Supplement.

### Effects of NaCl Loading on Plasma Levels of ADMA and Symmetrical Dimethylarginine

Baseline levels of ADMA (median and 25th/75th percentile) were similar in SS (0.76 μmol/L, 0.64/0.82) and SR (0.78 μmol/L, 0.63/0.80) subjects (SS versus SR, $P=0.69$; Figure S3). These levels are at the high end of a reference range obtained in healthy whites (0.40 to 0.77 μmol/L).14–16

In SS but not in SR subjects, NaCl loading induced significant increases in ADMA on both days 2 and 7 (Figure 3). In SS subjects, with NaCl loading, median ADMA levels (1.00 μmol/L, 25th/75th percentile 0.85/1.10) were above the reference range.14–16 On both days 2 and 7, NaCl-induced changes in ADMA were significantly greater in SS than in SR subjects. For all subjects combined, changes in ADMA on day 2 predicted changes in SVR and MAP throughout the HS period (Figure 4). Baseline levels of symmetrical dimethylarginine (SDMA [median, 25th/75th percentile]) were similar in SS (0.60 μmol/L, 0.52/0.68) and SR (0.60 μmol/L, 0.48/0.68) subjects and did not change with NaCl loading in either group (Figure S3). Accordingly, the ADMA:SDMA ratio did not change in SS subjects but increased in SS subjects from (median, 25th/75th percentile) 1.2 (1.0/1.5) on LS to 1.7 (1.3/1.8) on day 2 of HS ($P=0.002$) and to 1.5 (1.3/1.8) on day 7 of HS ($P=0.002$).

### Discussion

The traditional renal mechanism of salt sensitivity dictates that, in SS subjects, NaCl loading initiates a pressor effect entirely by inducing sequential increases in external Na$^+$ balance, extracellular volume, PV, and CO greater than any induced in SR subjects.4,5 But in the current study, as in a
previous one, NaCl loading induced in the SS subjects increases in these variables indistinguishable from those induced in the SR. By contrast, on the first day of NaCl loading, while MAP and SVR changed little in the SS subjects, both decreased sharply in the SR. The decrease in MAP resulted entirely from that in SVR. On the third day of NaCl loading, despite CO peaking in both the SS and SR subjects, SVR and MAP stayed nadiral in the SR, whereas in the SS, MAP increased without significant change in SVR. Thus, this failure of SVR to decrease normally with NaCl loading rendered pressor its normal increase of CO, the failure, if not NaCl’s pressor effect, beginning on the first day of NaCl loading.

On the second day of NaCl loading in both the SS and SR subjects, the changes induced in SVR varied directly with those in plasma protein concentration. Thus, in both the SS and SR, the greater the initial NaCl-induced increase in PV, the greater the initial decrease in SVR. The line describing the relationship between the change in plasma protein concentration and that in SVR in the SS subjects was parallel to that in the SR, and shifted to the left. This relationship demonstrates that (1) a differing extent of PV expansion cannot account for the differing decreases in SVR initially induced by NaCl in the SR and SS, and (2) systemic vasodilatation physiologically elicited by similar initial PV expansion in the SR and SS is somehow constrained in the SS. For all subjects combined, the changes induced in SVR and MAP on day 2 of NaCl loading predicted changes induced in MAP on days 5 to 7. Thus, subjects were predictably either SR or SS on day 7 of NaCl loading, depending on whether, on day 2, NaCl loading did or did not induce a decrease in SVR great enough to offset a pressor effect otherwise induced by NaCl’s increase of CO.

The current observations and interpretations are not without some precedent. Greene et al compared the archetypal animal model of genetic salt-sensitive hypertension, the Dahl SS rat (DS), with the Dahl SR rat (DR) and the Sprague-Dawley rat (SD), with respect to metabolic and hemodynamic

Figure 3. NaCl-induced changes in plasma asymmetrical dimethylarginine (ADMA) concentration in salt-sensitive (SS; n=13) and salt-resistant (SR; n=13) subjects. In SS but not in SR subjects, NaCl induced significant increases in ADMA levels both on day 2 and on day 7 of its loading. On both days 2 and 7, NaCl-induced changes in ADMA were significantly greater in SS than in SR subjects. For within-group comparison we used the Wilcoxon matched-pairs test, for between-group comparisons the Mann-Whitney U test. Values are median.

Figure 4. Relationship between initial NaCl-induced changes in plasma levels of asymmetrical dimethylarginine (ADMA) and changes in systemic vascular resistance (SVR) and mean arterial pressure (MAP). Graphs show the relationship between the percentage changes in ADMA on day 2 of high-salt diet (HS) and the percentage change in SVR and MAP on days 2 (A and B) and days 5 to 7 (average; C and D), respectively, of HS. Data of 1 salt-sensitive (SS) subject who was included in the analysis, but whose change in ADMA was extremely large (>400%), is not represented in the graphs. R and P values represent those from Spearman rank correlations on all 26 subjects (13 SS and 13 salt-resistant [SR] subjects). SS subjects are represented by closed, SR by open circles.
responses to intravenous NaCl loading over a 4 day-period, after a low NaCl diet from weaning had prevented the occurrence of hypertension in the DS. NaCl loading induced in the DS, DR, and SD immediate, similar, sustained increases in Na⁺ retention, blood volume, body weight, and CO. Furthermore, when CO increased, SVR decreased in both the DR and SD, causing a sustained decrease in MAP, whereas in the DS, SVR “did not change leading to the increase in MAP.” Indeed, when sought early in the course of NaCl loading studies comparing normotensive SS and SR animals17–19 and humans,20 a pressor-offsetting decrease in SVR has repeatedly been observed in the SR but never reported in the SS.

In the current study, NaCl loading induced in the SS subjects within 24 hours a 38% increase in plasma ADMA, an immediacy of increase with NaCl loading previously unreported, and a lesser increase on day 7. Plasma ADMA did not change with NaCl loading in the SR subjects; SDMA did not change in either SS or SR. ADMA, but not SDMA, is a major endogenous inhibitor of vasodilatation by competitively inhibiting NO synthase (NOS), which catalyzes the endothelial formation of NO from L-arginine.7 NO diffuses into vascular smooth muscle cells, where it activates soluble guanylate cyclase to form cGMP,21 which normally determines a state of relaxed arteriolar tone and vasodilatation in humans.22 In healthy humans23 and SDs,24 NaCl loading induces a robust increase of NOS activity, as judged by the increase in apparent rates of formation of NO and cGMP. There is substantial evidence that an L-arginine–reversible inhibition of NOS is critical to the causation of the pressor effect induced by NaCl loading in both DSS,25 and in DRs and SDs rendered SS by L-arginine analogues.24,25 This evidence accords with the current observation that the NaCl-induced changes in plasma ADMA predicted both the initial and later NaCl-induced changes in SVR and MAP, which suggest that the changes in ADMA are causally related to the NaCl-induced changes in BP through those in SVR. The increase in plasma ADMA currently observed on day 2 of NaCl loading is similar to that reported in other studies of SS humans after a week of NaCl loading.26,27 The current observations support the hypothesis that, in the SS subjects studied, NaCl loading induced its initial pressor effect by inducing a near immediate increase in the net cellular production of ADMA, which, by inhibiting the vasodilatation normally induced by dietary NaCl loading, rendered pressor its normal increase of CO.

Whereas SDMA is eliminated almost entirely by simple renal excretion, ADMA is largely eliminated by metabolic degradation that is catalyzed by dimethylarginine dimethylaminohydrolase (DDAH), whose activity is a major determinant of the cellular concentration of ADMA and, hence, also of its plasma concentration.28 Inhibition of DDAH can induce within hours increases in plasma ADMA and SVR,8 increases in ADMA somewhat like those currently induced in the SS subjects. Because ADMA, but not SDMA, is metabolized by DDAH, the ADMA:SDMA ratio can be used as a surrogate measure of DDAH activity.20 Accordingly, the currently observed NaCl-induced selective increase in plasma ADMA could well reflect inhibition of DDAH. DDAH activity is exquisitely sensitive to inhibition by oxidative stress,29,30 as can occur when NaCl loading induces an increased vascular production of superoxide anion (O₂⁻), a major component of oxidative stress.30,31 O₂⁻ scavenges NO, which, by reacting with O₂⁻, is a major endogenous antioxidant.30,32 Thus, an ADMA-determined decrease in NO formation increases oxidative stress. The increase is self-amplifying in that it can “uncouple” NO synthase (NOS) so that it yields not NO but O₂⁻.32 Indeed, in SDs33 and C57BL/6j mice,34 NaCl loading reduced endothelium-dependent dilatation of muscle arterioles apparently via O₂⁻ generated by NOS. In mesenteric resistance arteries of SDs fed a high NaCl diet for 3 days, O₂⁻ production increased, NO release decreased, and endothelium-dependent relaxation became impaired.35 Thus, in the currently studied SS subjects, NaCl loading that induced an increase of cellular ADMA could impair vasodilatation by reducing vascular bioavailability of NO, not only by decreasing its NOS-catalyzed formation, but also by increasing its inactivation by O₂⁻ that is increased both by the decrease in NO and by NOS-catalyzed (“uncoupled”) formation of O₂⁻.

**Perspectives**

In the currently studied NaCl-loaded normotensive blacks, the expression in half of salt sensitivity, which NaCl initiated by a near immediately impaired vasodilatation attended by an increase in plasma ADMA, suggests an endothelial dysfunction that might account for at least part of the enhanced cardiovascular risk conferred by normotensive salt sensitivity.1–3 In addition to its capacity to promote the occurrence of hypertension by inhibiting vasodilatation, an ADMA-determined reduction of endothelial NO bioavailability could impair its vasoprotective function and thereby contribute to the pathogenesis of both hypertension and atherosclerosis and consequent cardiovascular morbidity and mortality.30 The current observations suggest that normotensive salt sensitivity accompanied by NaCl-induced increase in plasma ADMA might constitute a pathogenic state worthy of preventive intervention, as with supplemental dietary potassium,27 particularly in those who are potassium deficient, as blacks often are.36,37

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**Disclosures**

A.L. is the founder and chief operating officer of OXONON BioAnalysis, Emeryville, CA.
References


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Salt Sensitivity in Blacks
Evidence That the Initial Pressor Effect of NaCl Involves Inhibition of Vasodilatation by Asymmetrical Dimethylarginine

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Short Title: ADMA and Salt Sensitivity in Blacks
Methods

Diet: Throughout the study, participants ate a eucaloric metabolic diet as described before.\(^1\) Per 70 kg of body weight (BW) the basal diet provided 30 mmol Na\(^+\) and 45 mmol K\(^+\). Week 1 served as low NaCl (LS) baseline period. Throughout week 2, the high NaCl (HS) period, the basal diet was supplemented with NaCl as table salt and broth for a total daily NaCl intake of 250 mmol per 70 kg per day (but no more than 300 mmol per day). Average NaCl intake during the LS period was mmol/d, 33 (SD 7) in salt-sensitive (SS) and 36 (SD 6) in salt-resistant (SR) subjects, \(P=0.21\). During the HS period intakes were 270 (SD 36) and 281 (SD 30) in SS and SR, respectively, \(P=0.31\).

Hemodynamic Outcomes: Programmed to obtain 5 readings within 5 minutes, blood pressure (BP) was measured daily every 4 hours after 5 minutes of supine rest using an oscillometric device (Dinamap, Criticon Inc. Tampa, Florida); average daily BP was calculated.

Throughout days 5-7 of the LS and throughout the 7-day HS period, between 6AM and 10PM, immediately following BP measurements, cardiac output (CO) was measured at 4-hour intervals using impedance cardiography (BioZ ICG monitor, Cardiodynamics, San Diego). Systemic vascular resistance (SVR) was calculated from CO and mean arterial pressure (MAP). Daily averages of CO and SVR were calculated. Details have been described previously.\(^1\)

Metabolic Outcomes: BW was measured daily at 6AM. Spontaneously voided urine was collected daily over 24-hour periods and analyzed for Na\(^+\) and creatinine. Daily net external balance of Na\(^+\) was calculated from its dietary intake and urinary output. On day 6 of LS and days 1 and 6 of HS 24-hour creatinine clearance was measured. On day 7 of the LS period, and on days 2 and 7 of the HS period, blood samples were obtained at 9AM to determine levels of electrolytes, creatinine, serum total protein and hematocrit by standard techniques. Changes in plasma volume (PV) were estimated from NaCl-induced changes in serum total protein and hematocrit values.

In 9 SS and 11 SR subjects changes in extracellular fluid volume (ECV) were determined with bio-impedance spectroscopy using a tetrapolar bioimpedance spectroscop (Impedimed Imp\(^\text{TM}\) SFB7, Impedimed Ltd., Mansfield, Australia).\(^2,3\)

From a total of 32 (of 37) subjects for whom sufficient plasma samples were available, 13 SR and 13 SS subjects were selected for measurements of plasma levels of asymmetrical dimethylarginine (ADMA) and symmetrical dimethylarginine (SDMA). Selection was based on the NaCl-induced changes in MAP (average of days 5 to 7) to include those with the smallest (SR, -4 to 2 mmHg) and largest (SS, 6 to 21 mmHg) changes, respectively. ΔMAP values in the 6 subjects (3 SS and 3 SR) not selected for ADMA measurements ranged from 2.3 to 5.4 mmHg. There was no obvious bi-modal distribution of ΔMAP data (average of days 5 to 7) in the group of subjects selected for ADMA and SDMA measurements. Characteristics of all SS and SR subjects and of the 26 SS and SR subjects in whom plasma ADMA and SDMA were measured are shown in Table S1 below. Measurements of ADMA and SDMA were made on day 7 of LS and days 2 and 7 of HS using high pressure liquid chromatography (HPLC) as described previously.\(^4\)
Results

Effects of NaCl loading on stroke volume (SV) and heart rate (HR). In both SS and SR, the increase in CO resulted from an increase in SV which, in both SS and SR, increased progressively during the initial 3-day period of NaCl loading and thereafter remained increased above baseline by 9% (95% C.I. 5 to 12) in SS and by 13% (95% C.I. 7 to 19) in SR; SS vs. SR, P=0.17. With NaCl loading in both groups, HR decreased progressively over time by -4% [95% C.I. -1 to -7] in SS and by -8% [95% C.I. -4 to -12] in SR; SR vs. SS, P=0.09), the decrease in HR reversing the SV-induced increase in CO. Values reported above reflect average changes of days 5-7.

Effects of NaCl loading on serum total protein concentration, hematocrit and ECV. Total protein concentration decreased by 11% on day 2 in both SS (95% C.I., -13 to -8) and SR (95% C.I., -12 to -9) and remained reduced by day 7, in SS -12% (95% C.I., -14 to -8) and in SR -11% (95% C.I., -15 to -9); SS vs. SR day 2, P=0.98; day 7, P=0.64. Hematocrit values decreased on day 2 in SS by 9% (95% C.I. -10 to -7) and in SR by 8% (95% C.I. -9 to -6), and remained reduced by day 7, -11%, in both SS (95% C.I. -13 to -9) and SR (95% C.I. -14 to -9); SS vs. SR day 2, P=0.53; day 7, P=0.71. In SS and SR, NaCl loading induced similar increases in ECV both on days 2 (Figure S2, bottom right) and 7 of HS. Increases from LS, were on day 2 in SS 8% (95% C.I. 6 to 9) and in SR 9% (95% C.I. 6 to 11); and on day 7, in SS 10% (95% C.I. 6 to 14) and in SR 11% (95% C.I. 8 to 14); SS vs. SR day 2, P=0.49; day 7, P=0.72.

Effects of NaCl loading on renal function. Serum creatinine levels and creatinine clearance did not differ between SS and SR at baseline or during NaCl loading. NaCl loading had no effect on creatinine levels or clearance values in either group. In SS, average creatinine clearance at baseline and on days 1 and 6 of NaCl loading, respectively, was (mL min\(^{-1}\) per 1.73m\(^2\)): 110 (SD 22), 110 (SD 22) and 109 (SD 25). Corresponding values in SR were: 117 (SD 21), 114 (SD 20) and 120 (SD 22).

References


Table S1. Characteristics of salt-sensitive (SS) and salt-resistant (SR) subjects

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<th>Variable</th>
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<td>72 (8.0)</td>
<td>0.052</td>
<td>0.052</td>
</tr>
<tr>
<td>Initial MAP, mmHg</td>
<td>92 (8.6)</td>
<td>89 (10)</td>
<td>0.37</td>
<td>95 (8.1)</td>
<td>89 (9.7)</td>
<td>0.072</td>
<td>0.072</td>
</tr>
<tr>
<td>Initial Heart Rate, bpm</td>
<td>65 (8.2)</td>
<td>69 (14)</td>
<td>0.29</td>
<td>66 (8.3)</td>
<td>67 (12)</td>
<td>0.91</td>
<td>0.91</td>
</tr>
<tr>
<td>Serum Creatinine, mg/dL</td>
<td>1.0 (0.2)</td>
<td>1.0 (0.2)</td>
<td>0.76</td>
<td>1.0 (0.2)</td>
<td>1.1 (0.2)</td>
<td>0.55</td>
<td>0.55</td>
</tr>
<tr>
<td>cGFR, mL/min/1.73m² †</td>
<td>97 (13)</td>
<td>103 (18)</td>
<td>0.24</td>
<td>98 (15)</td>
<td>101 (16)</td>
<td>0.67</td>
<td>0.67</td>
</tr>
<tr>
<td>Serum Na⁺, mmol/L</td>
<td>140 (1.6)</td>
<td>141 (2.3)</td>
<td>0.71</td>
<td>140 (1.9)</td>
<td>140 (2.0)</td>
<td>0.48</td>
<td>0.48</td>
</tr>
<tr>
<td>Serum K⁺, mmol/L</td>
<td>4.2 (0.3)</td>
<td>4.2 (0.3)</td>
<td>0.52</td>
<td>4.2 (0.3)</td>
<td>4.2 (0.4)</td>
<td>0.69</td>
<td>0.69</td>
</tr>
<tr>
<td>Serum Cl⁻, mmol/L</td>
<td>105 (2.5)</td>
<td>107 (2.9)</td>
<td>0.15</td>
<td>105 (2.6)</td>
<td>107 (3.3)</td>
<td>0.16</td>
<td>0.16</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>42 (4.3)</td>
<td>42 (2.4)</td>
<td>0.58</td>
<td>42 (3.7)</td>
<td>43 (1.9)</td>
<td>0.67</td>
<td>0.67</td>
</tr>
<tr>
<td>NaCl-induced ΔMAP, mmHg</td>
<td>10 (8 to 12)</td>
<td>0.3 (-1 to 1.5)</td>
<td>0.001</td>
<td>11 (8 to 13)</td>
<td>0 (-1 to 1)</td>
<td>0.001</td>
<td></td>
</tr>
</tbody>
</table>

Data is presented as mean and (SD) except where otherwise noted. Values of subjects in the “ADMA subgroup” do not differ from those in the complete sample except that in the subgroup the difference in age between SS and SR does not reach significance whereas the difference in BW does. Metabolic data was collected during screening visits, in general 2 weeks prior to admission. * “Initial” BP and heart rate were obtained at 2 PM on the 1st day of study, shortly after subjects were admitted, using the same standardized procedure as throughout the study; † cGFR denotes calculated GFR using the equation developed by the Chronic Kidney Disease Epidemiology (CKD-EPI) research group. ‡ Values are mean and (95% C.I.)
Table S2. Effect of dietary NaCl on hemodynamic variables, body weight, net external Na\(^+\) balance, hematocrit, serum protein and electrolyte concentrations and body fluid volumes during 7 consecutive days of NaCl loading: Difference between salt-sensitive (SS, n=19) and salt-resistant (SR, n=18) subjects.

<table>
<thead>
<tr>
<th>Day</th>
<th>ΔMAP (mmHg)</th>
<th>ΔSVR (mmHg/min-L(^{-1}))</th>
<th>ΔCardiac Output (L/min(^{-1}))</th>
<th>ΔHeart Rate (bpm)</th>
<th>ΔStroke Volume (mL)</th>
<th>ΔBody Weight (kg)</th>
<th>Daily Na(^+) Balance (mmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3 (1/4) †</td>
<td>0.5 (-0.2/1.1) †</td>
<td>0.0 (-0.2/0.2)</td>
<td>2 (-1/4)</td>
<td>1 (-5/3)</td>
<td>0.1 (-0.1/0.3)</td>
<td>-15 (-40/11)</td>
</tr>
<tr>
<td>2 *</td>
<td>7 (4/9) †</td>
<td>1.5 (0.7/2.2) †</td>
<td>-0.1 (-0.4/0.1)</td>
<td>2 (-1/5)</td>
<td>-6 (-11/0)</td>
<td>0.1 (-0.3/0.5)</td>
<td>-24 (-55/6)</td>
</tr>
<tr>
<td>3</td>
<td>8 (5/11) †</td>
<td>1.5 (0.7/2.4) †</td>
<td>-0.1 (-0.3/0.2)</td>
<td>2 (-1/6)</td>
<td>-5 (-11/2)</td>
<td>0.1 (-0.3/0.5)</td>
<td>-25 (-62/11)</td>
</tr>
<tr>
<td>4</td>
<td>10 (8/12) †</td>
<td>1.4 (0.7/2.2) †</td>
<td>0.0 (-0.3/0.3)</td>
<td>4 (0/7)</td>
<td>-4 (-11/3)</td>
<td>0.0 (-0.5/0.5)</td>
<td>-15 (-44/15)</td>
</tr>
<tr>
<td>5</td>
<td>10 (8/13) †</td>
<td>2.0 (1.2/2.8) †</td>
<td>0.0 (-0.3/0.2)</td>
<td>3 (-1/7)</td>
<td>-4 (-10/2)</td>
<td>-0.1 (-0.7/0.6)</td>
<td>-23 (-52/7)</td>
</tr>
<tr>
<td>6</td>
<td>9 (6/12) †</td>
<td>1.8 (1.0/2.6) †</td>
<td>0.0 (-0.2/0.3)</td>
<td>3 (-1/7)</td>
<td>-4 (-11/3)</td>
<td>-0.1 (-0.7/0.5)</td>
<td>-15 (-46/16)</td>
</tr>
<tr>
<td>7 *</td>
<td>10 (7/13) †</td>
<td>2.0 (1.0/3.0) †</td>
<td>0.0 (-0.3/0.3)</td>
<td>3 (-1/8)</td>
<td>-4 (-11/3)</td>
<td>-0.2 (-0.8/0.5)</td>
<td>-43 (-74/-12)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Day</th>
<th>ΔSerum Na(^+) (mmol-L(^{-1}))</th>
<th>ΔSerum Cl(^-) (mmol-L(^{-1}))</th>
<th>ΔSerum K(^+) (mmol-L(^{-1}))</th>
<th>ΔSerum Protein (g-dL(^{-1}))</th>
<th>ΔHematocrit (%)</th>
<th>ΔECV (L) n=20</th>
<th>ΔICV (L) n=20</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 *</td>
<td>-1 (-3/1)</td>
<td>-1 (-3/0)</td>
<td>-0.1 (-0.3/0.3)</td>
<td>-0.3 (-0.5/1.1)</td>
<td>0 (-1/1)</td>
<td>-0.4 (-0.8/0.1)</td>
<td>0.1 (-0.7/0.9)</td>
</tr>
<tr>
<td>7 *</td>
<td>-2 (-4/0)</td>
<td>-3 (-4/-1) †</td>
<td>-0.3 (-0.9/0.2)</td>
<td>0.1 (-0.2/0.4)</td>
<td>1 (-1/2)</td>
<td>-0.4 (-1.4/0.5)</td>
<td>0.2 (-0.7/1.2)</td>
</tr>
</tbody>
</table>

Data is displayed as difference of differences, \(\bar{y}_{SS} - \bar{y}_{SR}\) (ie, \([SS-HS \text{ – SS-LS}] – [SR-HS \text{ – SR-LS}]\)), whereby \(\bar{y}_{SS}\) and \(\bar{y}_{SR}\) denote the mean NaCl-induced difference from baseline (ie, average of the last 3 days of low NaCl [LS] diet) in SS and SR subjects, respectively, on days 1 through 7 of NaCl loading (HS). 95% lower and upper confidence limits, shown in parenthesis, were calculated from the pooled SD. MAP, mean arterial pressure; SVR, systemic vascular resistance. * Hemodynamic and metabolic differences on days 2 and 7 of NaCl-loading were predetermined as primary outcomes; † denotes significant difference between SS and SR, whereby \(P\) values are adjusted according to Bonferroni.
FIGURE S1.

Relationship between NaCl-induced changes in mean arterial pressure (MAP) on days 5-7 (average) of high-salt (HS) diet and changes in MAP and systemic vascular resistance (SVR) on day 2 of HS diet, n=37. NaCl-induced changes in MAP (panel A) and SVR (panel B) on day 2 of the HS diet are highly predictive of NaCl-induced changes in average MAP of days 5-7. Values are shown as percent change from the low-salt period (average of days 5-7).
FIGURE S2.

**Left panels:** 7-day time course of NaCl-induced changes in body weight (BW), top, and cumulative external Na\(^+\) balance, bottom, in salt-resistant (SR) (○) and salt-sensitive (SS) (●) subjects. Values for BW are shown as percentage change from the low-salt (LS) period (average of days 5-7). Cumulative Na\(^+\) balance is presented in mmol. Values are means and 95% C.I. *, P<0.01, and ‡, P<0.001, respectively, compared to LS. Throughout the 7-day high-salt (HS) period net cumulative Na\(^+\) balance did not differ between SR and SS.

**Right panels:** NaCl-induced changes in extracellular volume (ECV), top, and serum total protein concentration, bottom, in SR and SS subjects 24 hours (day 2) after initiating the HS diet. Changes on day 7 were similar to those on day 2. Values are means and 95% C.I.
FIGURE S3.

Plasma levels of ADMA and SDMA before and during NaCl loading in salt-sensitive (SS) and salt-resistant (SR) subjects. (A) After 7 days of low-salt diet (Day 0) ADMA levels were the same in SR and SS subjects. In SS, but not in SR, NaCl induced significant increases in ADMA levels both on day 2 (*P=0.002 compared to D0) and on day 7 (*P=0.003 compared to D0) of its loading. *P values for between-group comparisons (SS versus SR) are shown in the graph. (B) Plasma levels of SDMA did not differ between SR and SS subjects throughout the study. NaCl loading did not induce changes in SDMA levels in either group.

Values are median, SS, n=13, SR, n=13. For within-group comparisons the Wilcoxon matched pairs test was used. For between-group comparisons the Mann-Whithey U-test was used.