

Hemodynamic and Autonomic Response to Different Salt Intakes in Normotensive Individuals

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Background—Even if sodium sensitivity represents a risk factor at any blood pressure (BP) level, limited evidence is available that it may influence cardiovascular control in normotensives, particularly in white individuals. Therefore, the aim of the study was to investigate whether sodium sensitivity alters hemodynamic or autonomic responses to salt in normotensives.

Methods and Results—We evaluated the Sodium-Sensitivity Index (SS-Index) in 71 white normotensives after 5 days of high- and low-sodium diets. We measured BP continuously at the end of each period, estimating hemodynamic indices from BP waveform analysis, and autonomic indices from heart rate (HR) and BP variability. According to the SS-Index distribution, we defined 1 sodium-sensitive group (SS, with SS-Index >15 mm Hg/[mmol·day]), 1 sodium-resistant group, (unresponsive to sodium load with $-15 \leq$ SS-Index $\leq +15$), and 1 inverse sodium-sensitive group, responsive to sodium by decreasing BP, with SS-Index <-15). We compared the effects of the diets among groups, and correlated autonomic/hemodynamic indices with the SS-Index. After sodium loading, a significant decrease in systemic peripheral resistances, HR, spectral indices of BP modulation, and a significant increase of indices of HR vagal modulation were found in the inverse sodium-sensitive group but not in SS normotensives. Moreover, the highest SS-Indices were associated with the lesser vagal HR decelerations.

Conclusions—Our data suggest that salt sensitivity in white normotensive individuals is associated with impaired vasodilation and altered autonomic response to dietary salt. Such dysfunction may critically contribute to induce a BP response to dietary salt. (*J Am Heart Assoc.* 2016;5:e003736 doi: 10.1161/JAHA.116.003736)

Key Words: autonomic function • baroreflex • blood pressure spectral analysis • heart rate variability • peripheral resistance • salt intake • salt-sensitive

Salt-sensitive hypertensive individuals display a higher rate of cardiovascular events than salt-resistant hypertensive individuals.¹ A similar trend characterizes salt-sensitive normotensives, who also have a significant increase in mortality

rate over time.² However, few studies investigated the mechanisms relating the increased cardiovascular risk to sodium sensitivity. Sodium loading/depletion maneuvers may unveil alterations in cardiovascular control associated with sodium sensitivity possibly involved in the increased rate of cardiovascular events. In this regard, while it has been shown that salt-sensitive hypertensive patients respond to sodium loading with either a blunted sympathetic deactivation³ or an impaired cardiac parasympathetic activation,^{4,5} limited evidence of an altered autonomic cardiovascular modulation is available in normotensive subjects.

A study in mostly normotensive black Americans, undergoing a few days of dietary salt loading, showed a significant decrease in systemic vascular resistance in sodium-resistant individuals, while such a response was largely impaired in sodium-sensitive individuals.⁶ This finding suggests the hypothesis that an autonomic-mediated reduction in peripheral vascular resistance, in response to sodium loading, might be weakened or absent in sodium-sensitive normotensives. However, to the best of our knowledge, no data are available on the dependence of hemodynamic or autonomic responses

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Accompanying Data S1 through S3, Table S1, and Figure S1 are available at <http://jaha.ahajournals.org/content/5/8/e003736/DC1/embed/inline-supplementary-material-1.pdf>

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to salt loading on the degree of sodium sensitivity in white normotensive individuals.

The present study specifically addressed the above open issue. We tested the hypothesis that an altered hemodynamic or autonomic response to dietary salt manipulation is detectable in white normotensive individuals as a function of their sensitivity to sodium. This was done by deriving information on hemodynamic control from pulse waveform analysis and by assessing spontaneous heart rate (HR) and blood pressure (BP) variability during different levels of sodium intake in healthy normotensives with different degrees of sodium sensitivity, as quantified in a continuous fashion by the sodium sensitivity index (SS-Index).

Methods

Subjects and Data Collection

The experimental protocol conformed to the Declaration of Helsinki and was approved by the ethics committee of Don C. Gnocchi Foundation. Seventy-one normotensive volunteers (45 females), recruited among medical students and residents attending our University Hospital, were included. Inclusion criteria were a normotensive status, confirmed by clinic BP measurements and 24-hour ambulatory BP monitoring, and the absence of history and of any physical or laboratory evidence of cardiovascular disease. No endurance or professional athletes were enrolled. Volunteers received a detailed explanation of the study and gave informed consent. All participants followed a low-sodium (30 mmol NaCl per day) and a high-sodium (200 mmol NaCl per day) diet, each for 5 days, in random order. They were instructed on how to prepare their meals at home, and received the total amount of sodium to be used in their food during the high-sodium diet from the hospital pharmacy, in separate 1-g sachets. During both diets, they were asked to refrain from intense physical exercise. On the last day of each diet, the 24-hour urinary sodium excretion rate (UNaV) was quantified. In the morning of the same day, body weight was assessed and brachial BP was measured on the right arm with subjects in a sitting position for 2 hours, with readings every 15 minutes with an oscillometric ambulatory BP monitoring device (Spacelabs 90207; Spacelabs Healthcare, Redmond, WA). Mean arterial pressure (MAP) was averaged over the 2-hour recording period. The difference between average MAP values at the end of the high-salt and low-salt diets (Δ MAP) was divided by the difference between the corresponding urinary sodium excretion rates (Δ UNaV) to obtain the SS-Index,⁷ defined as Δ MAP/ Δ UNaV ratio and expressed in mm Hg/(mol-day).

Finger BP was recorded beat-by-beat for 2 hours from the mid finger of the left hand by the Portapres model-2 device (Finapres Medical Systems B.V., Amsterdam, the Netherlands)

simultaneously with the brachial right-arm cuff. Finger BP was obtained in 68 of the 71 volunteers; technical problems prevented the recording in 3 cases. Finger BP waveforms were sampled at 100 Hz. Beat-by-beat values of systolic BP, diastolic BP, and pulse interval (PI) were derived from the finger BP waveforms. Since we previously observed a significant proportional bias between finger and brachial BP measures associated with the sodium depletion maneuver,⁸ the brachial measures of systolic and diastolic BP, averaged over the 2-hour recordings, were used to calibrate the finger BP waveform. After such calibration, the average of systolic and diastolic beat-by-beat BP series from Portapres coincided with the corresponding brachial BP measures. The beat-by-beat BP series were visually inspected by an expert operator to remove premature beats and artifacts, and the recordings of 1 subject were discarded because a high number of artifacts made them unsuitable for spectral analysis. Thus, data of 67 volunteers (41 females) were available for BP waveform and for HR and BP variability analysis.

Hemodynamic parameters were derived from BP waveform analysis through Model-Flow analysis (see details of the Model-Flow method and of validation studies versus “gold standard” methods in Data S1). Hemodynamic parameters were the following: left ventricular ejection time, systemic vascular resistances (SVR), aortic characteristic impedance, arterial compliance, stroke volume, and cardiac output. Autonomic indices were calculated from the analysis of HR and BP variability (Data S1). Frequency-domain indices of HR variability were the high-frequency power of PI (PI HF), index of vagal modulations of HR, and the ratio between low-frequency (LF) and HF powers of PI (PI LF/HF powers ratio), which is an index of the cardiac sympatho/vagal balance.⁹ Frequency-domain indices of BP variability were the LF power of diastolic BP, reported to reflect sympathetically mediated vasomotor oscillations generally assumed to be induced by baroreflex resonance^{10,11}; the very-low frequency power of diastolic BP that quantifies long-term fluctuations mainly of vasomotor origin; and the sensitivity of baroreflex control of HR estimated by the transfer function method over the LF (BRS LF) and HF (BRS HF) bands. Additionally, we calculated the number of PI increases per minute larger than 50 ms (NN50+, time-domain index of cardiac parasympathetic modulation under the hypothesis that bursts of vagal outflow on the sinus node produce PI lengthening of more than 50 ms)¹²; and the short-term scale coefficient α_1 through detrended fluctuation analysis of PI, which is a complexity-domain index of cardiac sympatho/vagal balance.^{13,14}

Statistics

The association between SS-Index and hemodynamic or autonomic indices was assessed by the nonparametric

Kendall's rank correlation coefficient, τ , separately after each diet.

Traditionally, individuals are classified on the basis of their response to sodium tests in those that increase BP, or sodium sensitive (SS), and in those that do not increase BP, even if this latter group has a heterogeneous response, with some individuals presenting even a marked depressor response to salt loading.¹⁵ Table S1 (see Data S2) summarizes results following this dichotomous approach. More recently, it has been proposed to further divide individuals who do not increase BP after sodium loading in a genuine sodium-resistant (SR) group, actually unresponsive to sodium, and in an inverse sodium-sensitive (ISS) group, that respond to high dietary salt decreasing BP.¹⁶ Following this trichotomous approach, we defined SR as those individuals with SS-Index between -15 and $+15$ mm Hg/(mol-day), ISS those with SS-Index lower than -15 mm Hg/(mol-day), and SS those with SS-Index greater than 15 mm Hg/(mol-day). Figure S1 (see Data S3) illustrates the SS-Index distribution and the 3 sodium-sensitivity classes in our normotensive population.

The effects of sodium loading on ISS, SR, and SS groups were assessed by longitudinal analysis of mean response profiles, assuming no specific covariance structure of the repeated measures.¹⁷ Analysis factors were the "SS condition" (ie, ISS, SR, and SS) and "diet," the latter with 2 repeated measures, 1 at the low-salt diet and 1 at the high-salt diet. Statistical significance of the difference between diets was evaluated with "a posteriori" contrasts after correction for multiple comparisons with the false discovery rate procedure.¹⁸ A proper transformation was applied if the hypothesis of normality was rejected (Shapiro–Wilk test). Frequency

domain indices were log-transformed, reducing their skewness.¹⁹ Cardiac output and stroke volume were also log-transformed. No transformation was required for NN50+ and α_1 , as previously reported in healthy volunteers.²⁰ Analysis was performed with "R: A Language and Environment for Statistical Computing" software package (R Core Team, R Foundation for Statistical Computing, Vienna, Austria, 2015), setting the statistical significance threshold at $P=0.05$.

Results

Table 1 reports the general characteristics of our population. The 3 groups had similar age and 24-hour ambulatory BP level during habitual diet. Female sex was more common, with their prevalence at 69%, 60%, and 59% in the ISS, SR, and SS groups, respectively, without significant differences among groups. The body mass index was slightly higher in SS than in ISS. The percentage of individuals with positive family history for hypertension was almost double in the SS (41%) than in the ISS (21%) group, although this difference did not reach statistical significance.

Figure 1 shows the effect of the sodium loading/depletion maneuver on BP and HR. With respect to the low-salt diet, the high-salt diet decreased BP in the ISS group (systolic BP: from 115.3 ± 11.0 to 108.5 ± 11.2 ; MAP: from 85.1 ± 9.3 to 78.7 ± 9.2 ; diastolic BP: from 71.3 ± 9.5 to 64.9 ± 8.3 mm Hg, mean \pm SD), increased BP in the SS group (systolic BP: from 112.8 ± 9.2 to 119.0 ± 9.2 ; MAP: from 80.7 ± 7.7 to 87.2 ± 8.2 ; diastolic BP: from 67.0 ± 6.9 to 72.6 ± 7.6), and did not affect the SR group (systolic BP: from 114.7 ± 10.4 to 114.2 ± 11.4 ; MAP: from 82.3 ± 7.2 to 82.2 ± 7.8 ; diastolic BP: from

Table 1. General Characteristics of Normotensive Volunteers by SS-Index Classes: Mean (SD)

Classes	ISS (N=29)	SR (N=25)	SS (N=17)	P Value
SS-Index range, mm Hg/(mol-day)	-98.2: -15.3	-14.5: +14.3	+15.1: +123.0	
Δ MAP, mm Hg*	-6.4 (3.7)	0.0 (2.0)	6.5 (3.9)	
Sex, F/M	20/9	15/10	10/7	0.72
Age, y	27.3 (6.4)	26.0 (3.8)	29.6 (6.8)	0.053
Body mass index, kg/m ^{2†}	21.4 (2.6) [‡]	22.1 (2.4)	23.9 (2.5)	<0.01
Δ Weight, kg [§]	1.4 (1.1)	1.2 (1.6)	0.9 (1.4)	0.34
Family history of hypertension	21%	36%	41%	0.28
24-h systolic BP, mm Hg	115.0 (7.7)	116.5 (8.6)	116.2 (11.7)	0.82
24-h diastolic BP, mm Hg	68.6 (5.6)	69.2 (6.1)	69.8 (8.6)	0.84
24-h HR, bpm	73.5 (9.0)	70.7 (9.2)	71.5 (10.5)	0.53

BP indicates blood pressure; HR, heart rate; ISS, inverse sodium-sensitive; SR, sodium resistant; SS, sodium sensitive.

* Δ MAP, difference between high-salt and low-salt conditions in mean arterial pressure averaged over the 2-h recording period at the end of the diet.

[†]Measures at the end of the low-sodium diet.

[‡]Means a significant difference vs SS (Tukey's post-hoc).

[§] Δ Weight, difference between body weights at the end of the high-salt and low-salt diets.

^{||}Data from 24-h ambulatory BP monitoring during habitual diet; P calculated by χ^2 test for sex and family history of hypertension, by 1-way ANOVA for all the other variables.

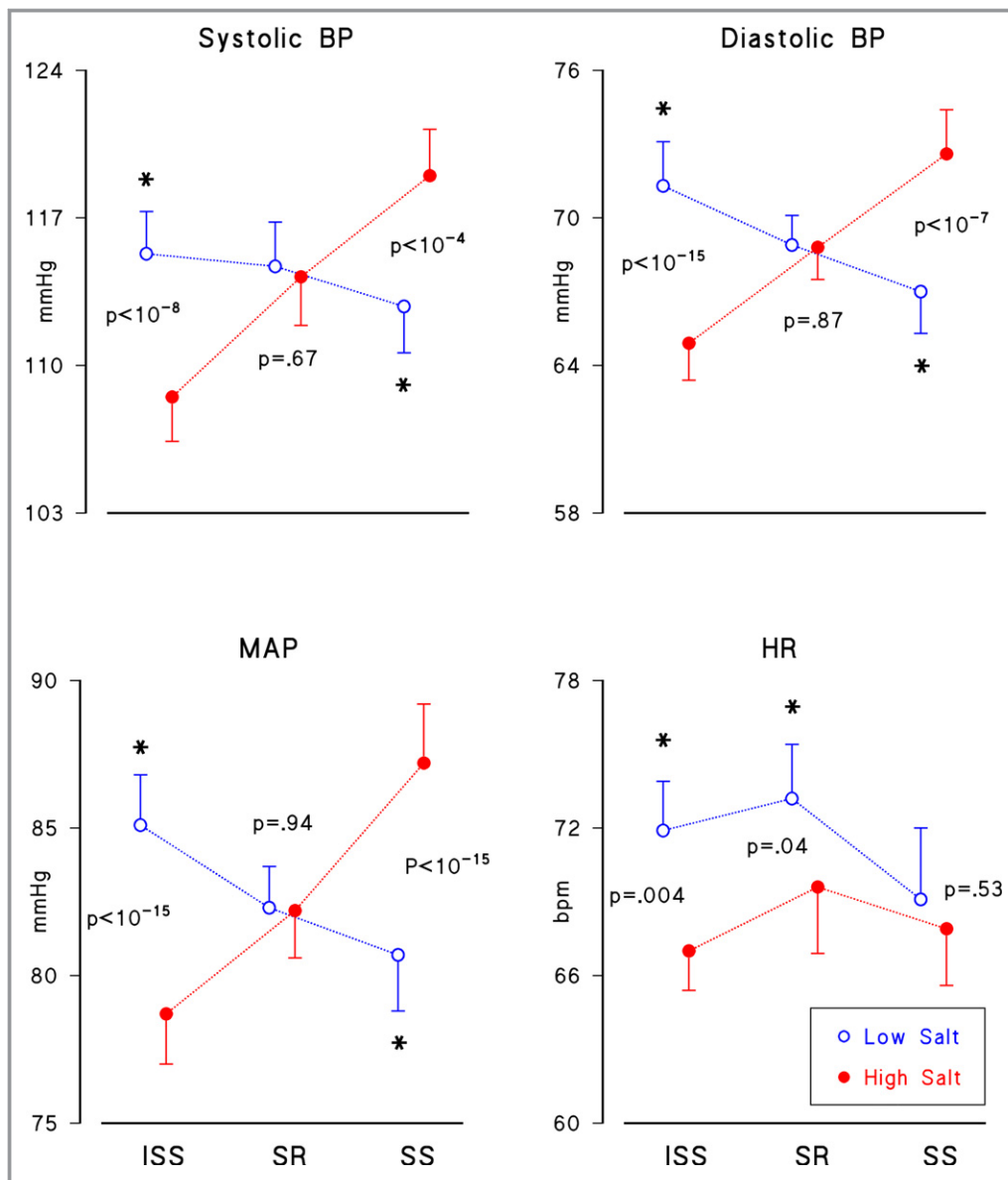


Figure 1. Brachial measures of blood pressure (BP) and heart rate (HR). Mean (SEM) after low-salt (open circles) and high-salt (solid circles) diets in 3 groups of normotensive volunteers, defined on the basis of SS-Index: a sodium-sensitive group, SS, with SS-Index >15 mm Hg/(mmol·day), and 2 sodium-resistant groups: 1 unresponsive to sodium load, SR, with $-15 \leq$ SS-Index \leq 15; and 1 responding to sodium load with a BP decrease, ISS, with SS-index < -15 . ISS indicates inverse sodium sensitive; MAP, mean arterial pressure. The “*” marks significant differences between diets.

68.9±6.2 to 68.8±6.7). The high-salt diet also decreased HR significantly in ISS (from 71.9±10.9 to 67.0±8.6 bpm) and SR (from 73.2±10.9 to 69.6±13.4) groups, but not in the SS group (from 69.1±12.1 to 67.9±9.5).

Figure 2 shows left ventricular ejection time and other hemodynamic parameters from model-flow analysis. The high-salt diet increased left ventricle ejection time in all groups (+10.8, +17.8, and +13.8 ms in ISS, SR, and SS, respectively). However, it induced opposite changes of SVR and arterial compliance in ISS and SS, without affecting SR

($P < 0.001$ for factors interaction). In fact, after the high-salt diet, SVR decreased in ISS (from 1.22±0.34 to 1.07±0.20 mm Hg×s/mL), tended to increase in SS (from 1.00±0.27 to 1.12±0.31) without substantial changes in SR (from 1.03±0.19 to 1.07±0.26), while arterial compliance increased in ISS (from 2.16±0.43 to 2.33±0.45 mL/mm Hg) and decreased in SS (from 2.45±0.56 to 2.31±0.58), also in this case without substantial changes in SR (from 2.35±0.38 to 2.38±0.41). Aortic characteristic impedance decreased in ISS only.

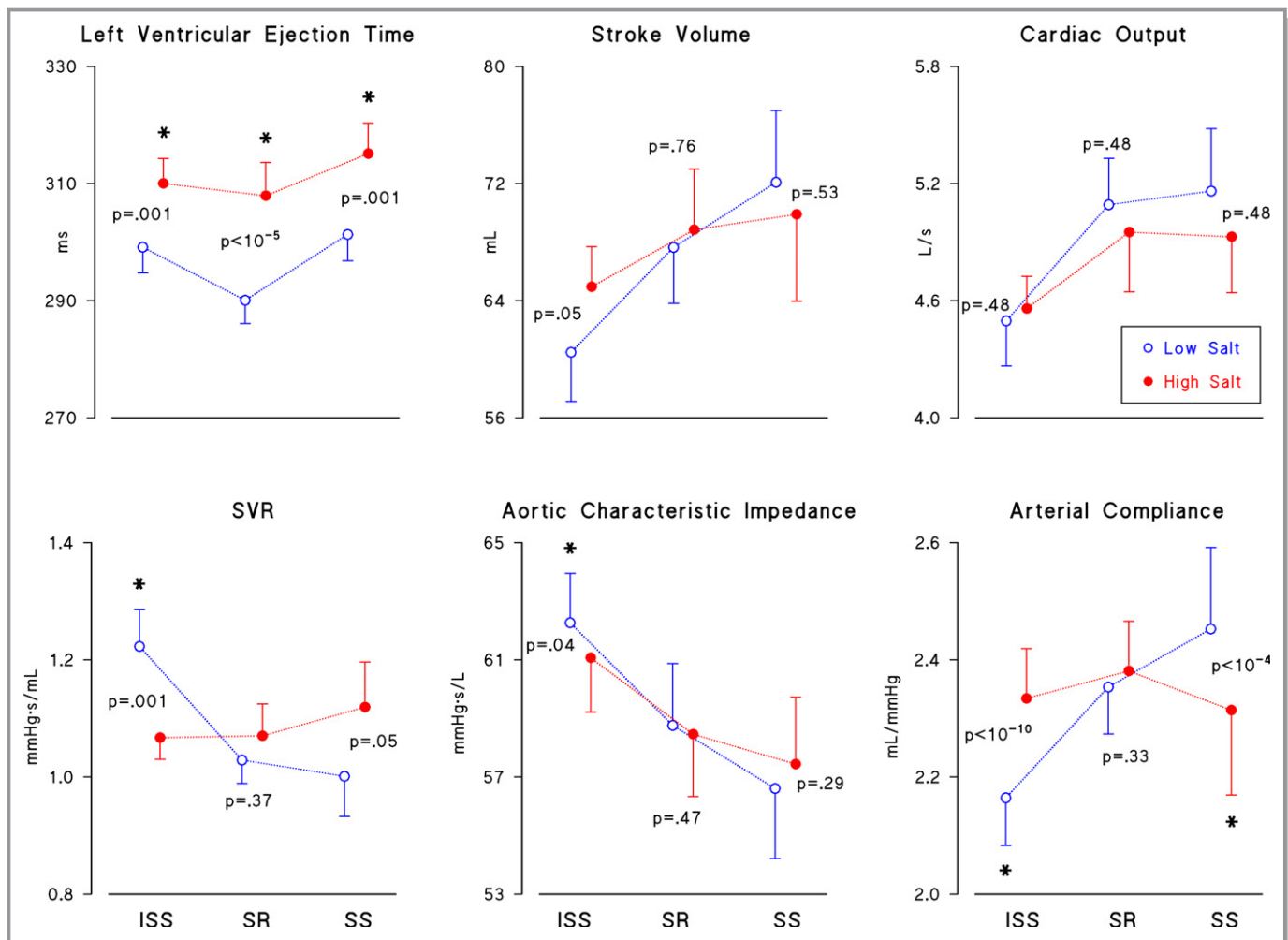


Figure 2. Hemodynamic parameters. Mean (SEM) after low-salt and high-salt diets in inverse sodium-sensitive (ISS), sodium-resistant (SR), and sodium-sensitive (SS) individuals; SVR indicates systemic vascular resistances. The “*” marks significant differences between diets.

Considering HR variability (Figure 3), the high-salt diet decreased spectral and complexity-based indices of cardiac sympatho/vagal balance (PI LF/HF decreased by -21% , -20% , and -16% and α_1 decreased by -6% , -4% , and -7% in ISS, SR, and SS groups, respectively). The high-salt diet also increased time-domain and spectral indices of vagal HR modulation ($+32\%$, $+36\%$, and $+24\%$ for NN50+, $+51\%$, $+54\%$, and $+38\%$ for PI HF, in ISS, SR, and SS groups, respectively). The vagal HR modulation tended to decrease with the severity of sodium sensitivity and the effect of sodium loading appeared less marked in the SS group.

Figure 4 shows that the high-salt diet increased the baroreflex sensitivity in all groups (BRS LF: $+22\%$, $+38\%$, and $+23\%$; BRS HF: $+29\%$, $+48\%$, and $+28\%$ in ISS, SR, and SS groups). The high-salt diet also decreased the LF power of diastolic BP in ISS (-24%), in SR (-52%), and in SS (-24%) groups, with the change reaching the significance threshold in ISS and SR groups only. Sodium loading also decreased the diastolic BP fluctuations of longer term (very-low frequency

power), significantly in the ISS group (-32%), and close to the significance level in the SR group (-26%). By contrast, very-low frequency power of diastolic BP was only marginally influenced by the sodium diets in the SS group (-6%).

The SS-Index also allows describing the severity of sodium sensitivity in a continuous fashion, without introducing predefined classes of salt sensitivity. In this regard, Figure 5 shows mean values of SVR, cardiac output, and MAP by quintiles of SS-Index, separately after the 2 diets. Interestingly, SVR progressively decreased and cardiac output progressively increased from the lower to the upper quintile after the low-salt diet while, after the high-salt diet, SVR appeared uniformly distributed and the increase of cardiac output with SS-Index was much less pronounced. MAP was substantially higher in the 2 upper quintiles after the high-salt diet while, after the low-salt diet, the highest value was associated with the lower quintile. Table 2 reports the Kendall's associations between SS-Index and all the other variables of the study by diets. As expected, BP increased with

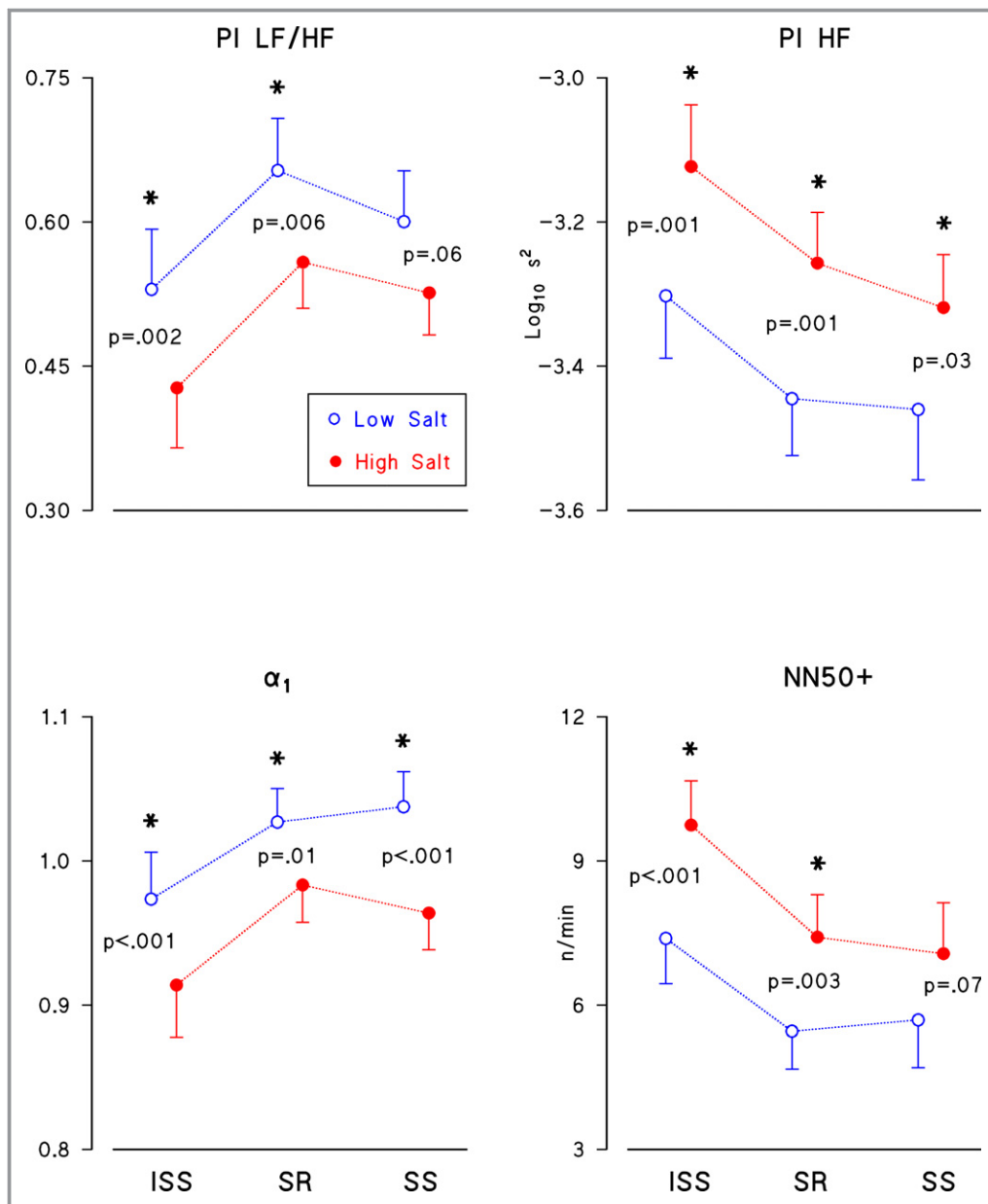


Figure 3. Autonomic indices of HR variability. Left: frequency- and complexity-domain indices of cardiac sympatho/vagal balance (PI LF/HF and α_1); right: frequency- and time-domain indices of vagal modulations of HR (PI HF and NN50+), in inverse sodium-sensitive (ISS), sodium-resistant (SR), and sodium-sensitive (SS) individuals. α_1 indicates short-term scale coefficient of PI; HF indicates high frequency; HR, heart rate; LF, low frequency; NN50+, number of PI increases per minute larger than 50 ms; PI, pulse interval. Spectral indices are represented in logarithmic scale. The “*” marks significant differences between diets.

SS-Index after the high-salt diet. Indices of cardiovascular hemodynamics were also associated with SS-Index but only after the low-salt diet: stroke volume, cardiac output, and arterial compliance increased while SVR and aortic characteristic impedance decreased with SS-Index. As regards HR and BP variability, SS-Index was negatively associated with vagal HR modulation after the high-salt diet: NN50+ decreased significantly with SS-Index, and the same trend,

close to the significance threshold ($P=0.07$), characterized the frequency-domain index of vagal HR modulation, PI HF.

Discussion

This study investigated whether hemodynamic and autonomic responses to salt intake depend on the degree of salt sensitivity, for the first time in a population of white, young,

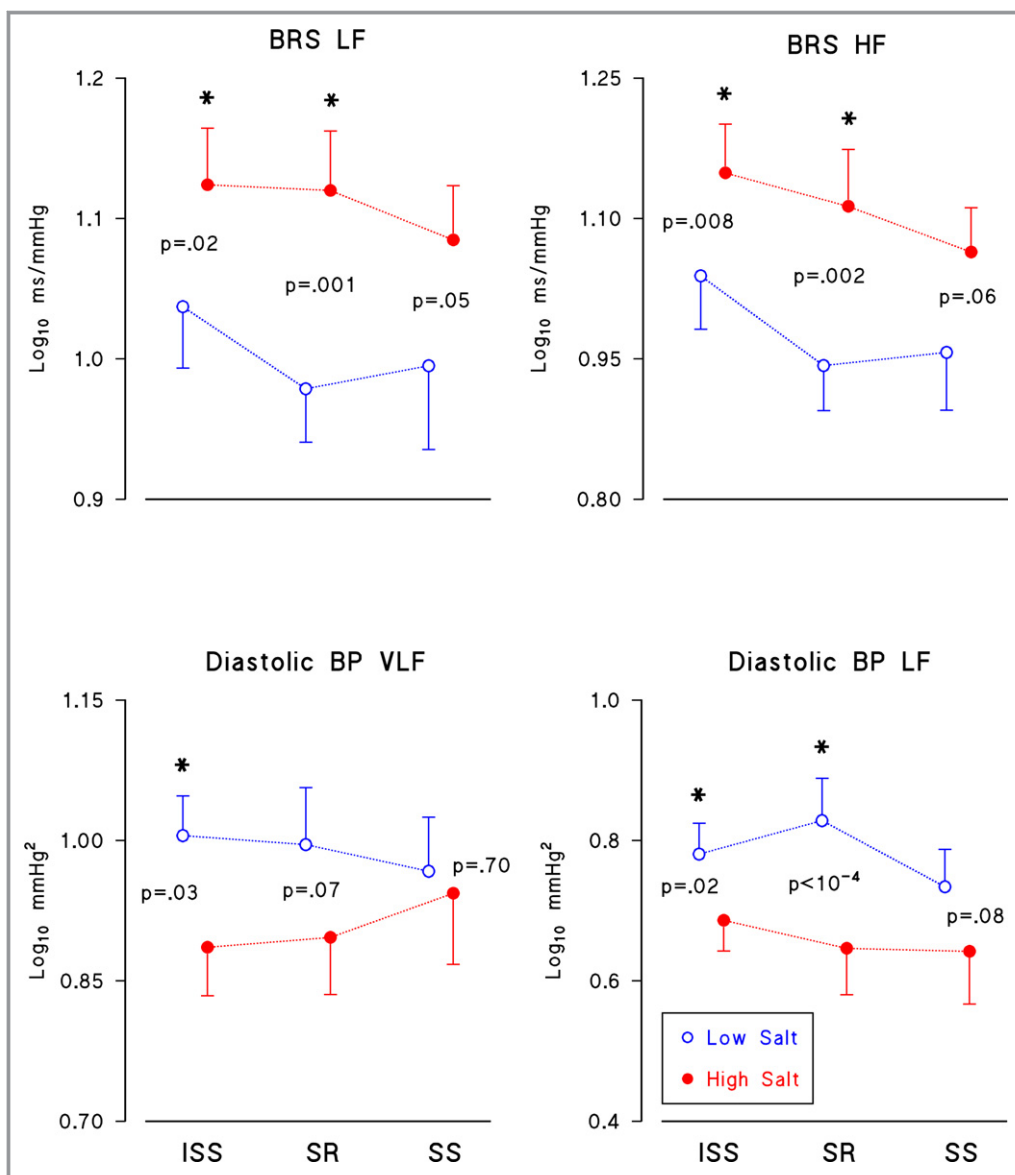


Figure 4. Autonomic indices of BP variability. Indices of baroreflex sensitivity (BRS LF and BRS HF) and of sympathetic modulations of vascular tone (diastolic BP VLF and LF) represented in logarithmic scale, in inverse sodium sensitive (ISS), sodium resistant (SR), and sodium sensitive (SS) individuals. BP indicates blood pressure; HF, high frequency; LF, low frequency; VLF, very-low frequency.

normotensive volunteers and by adopting a classification in 3 rather than in 2 classes: ISS, SR, and SS. In fact, the SS-Index distribution emphasized the oversimplification of the traditional dichotomous approach in sensitive/resistant individuals to sodium loading, because normotensive subjects traditionally classified as sodium resistant do not all respond to sodium loading in the same way.

The BP decrease induced by sodium loading in the ISS group appeared to be a consequence of a dramatic decrease of vascular resistances. By contrast, vascular resistances did not decrease in SR and SS groups. In this regard, SR normotensives seemed more similar to SS than to ISS individuals, and after the low-salt diet peripheral resistances

in both SR and SS groups were about 20% lower than in the ISS group.

Our findings support the notion that the disproportion between cardiac output and vascular resistances in SS subjects is determined by their failure to adequately lower vascular resistances during sodium repletion,²¹ and confirm data collected in a mostly normotensive population of black individuals dichotomously classified in salt-sensitive and salt-resistant groups,⁶ in whom vascular resistances increased in salt-sensitive and decreased in salt-resistant individuals after 5 days of high-salt diet without significant changes in cardiac output. However, by splitting salt-resistant individuals into the ISS and SR subgroups, we provide evidence that only in ISS

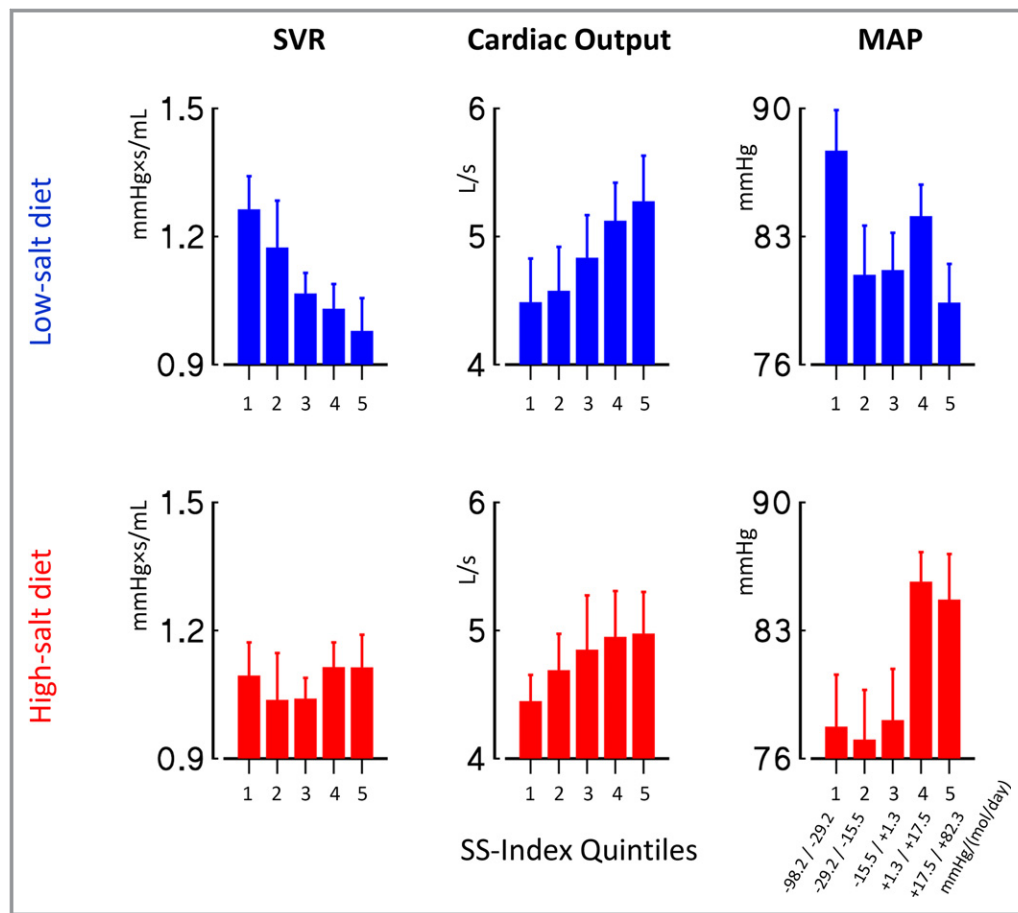


Figure 5. Dependence of SVR, cardiac output, and MAP on SS-Index. Mean and SEM by quintiles of the SS-Index distribution, plotted separately after the low-salt diet (upper panels) and the high-salt diet (lower panels). SS-Index range for each quintile is reported on the lower right panel. MAP indicates mean arterial pressure; SVR, systemic vascular resistances.

normotensives was the unchanged cardiac output after the high-salt diet the result of a significantly lower HR level, which acted as a compensatory factor against a substantially higher stroke volume.

The role of peripheral resistances in mediating salt-induced increases of blood pressure is still a matter of lively debate.^{22,23} The Guyton's theory concluded that salt loading induces hypertension causing transient large increases in cardiac output, whereas systemic vascular resistance initially remains normal or unchanged.^{24,25} A recent review challenged this view, proposing that direct response to sodium load in SS individuals is attributable to an initial inhibitory effect on arteriolar vasodilation that is able to prevent vascular resistance decrease.²⁶ Our observation that the initial hemodynamic pattern after sodium loading is a lack of vasodilation in SS individuals seems to confirm this vasodys-function theory. However, we also showed that during low-salt diet the SS-Index is positively associated with cardiac output and arterial compliance and negatively associated with SVR and characteristic impedance, suggesting a residual positive

salt balance in SS subjects during salt depletion and giving support to Guyton's physiology. We may also hypothesize a role played by altered modulations of the renin–angiotensin–aldosterone system or of the sympathetic nervous system, reported to occur in hypertensive SS individuals.^{3,27} However, studies in normotensive humans revealed that an increased dietary salt intake (5–7 days of high-salt regimen) induces a profound reduction in vascular nitric oxide bioavailability, which limits endothelium-dependent vasodilation.^{28,29} Thus, a reduced bioavailability of nitric oxide after 5 days of high-salt diet might determine the altered response of vascular resistances in SS and SR normotensive subjects. Future studies could validate this speculation by measuring plasma levels of nitric oxide and of interacting cytokines, such as transforming growth factor $\beta 1$,²⁸ in ISS, SR, and SS groups undergoing a high-salt diet.

Our data also indicate effects of the sodium loading/depletion maneuver on the autonomic cardiovascular modulation. First, sodium loading increased vagal modulation of HR (PI HF and NN50+) and the HR BRS (Figure 4), with these changes

Table 2. Correlation Between SS-Index and Hemodynamic or Autonomic Variables (Kendall's τ and Significance P) at the End of Each Diet

	Low-Salt Diet		High-Salt Diet	
	τ	P Value	τ	P Value
Systolic BP	-0.09	0.29	0.24*	0.003
Diastolic BP	-0.14	0.08	0.31*	<0.001
MAP	-0.18	0.02*	0.29*	<0.001
Pulse pressure	-0.08	0.29	0.08	0.30
HR	-0.09	0.27	0.03	0.68
Model flow analysis				
Left ventricular ejection time	-0.02	0.77	0.03	0.76
Stroke volume	0.19*	0.02	0.05	0.51
Cardiac output	0.17*	0.04	0.10	0.23
SVR	-0.21*	0.01	0.03	0.68
Arterial compliance	0.18*	0.03	0.02	0.82
Aortic characteristic impedance	-0.19*	0.02	-0.14	0.10
HR and BP variability analysis				
PI HF	-0.09	0.26	-0.15	0.07
PI LF/HF	0.08	0.33	0.13	0.12
Diastolic BP LF	-0.05	0.53	0.03	0.70
Diastolic BP VLF	-0.06	0.45	-0.05	0.53
BRS HF	-0.025	0.77	-0.04	0.65
BRS LF	-0.07	0.38	-0.12	0.16
NN50+	-0.12	0.15	-0.21*	0.013
α_1	0.10	0.22	0.10	0.24

α_1 indicates short-term scale coefficient of PI; BP, blood pressure; BRS, baroreflex sensitivity; HF, high frequency; HR, heart rate; LF, low frequency; MAP, mean arterial pressure; NN50+, number of PI increases per minute larger than 50 ms; PI, pulse interval; SVR, systemic vascular resistances; VLF, very-low frequency.

*Correlations significant at $P < 0.05$.

appearing less pronounced in SS individuals, and decreased HR significantly, but not in SS normotensives (Figure 1). These findings suggest an increased vagal modulation of HR after a high-salt diet, partially blunted in the SS group.

Second, sodium loading decreased indices reflecting cardiac sympatho/vagal balance (PI LF/HF and α_1) and vasomotor oscillations considered to reflect sympathetic vascular modulation associated with a baroreflex resonance (diastolic BP LF in Figure 4). In line with these findings, it was observed that a low-salt diet increases the muscle sympathetic nerve traffic in hypertensive individuals.³⁰

Third, sodium loading significantly decreased slow vasomotor components of diastolic BP variability (Figure 4), likely generated by sympathetic modulation of SVR, only in the ISS

group, in which the high-salt diet also dramatically decreased SVR. This suggests that the preserved adaptation of vascular resistances to dietary sodium intake in ISS individuals may reflect the preserved ability of the autonomic nervous system to modulate SVR.

The effects of sodium diets we reported in normotensive individuals are similar to changes described in hypertensive patients.^{4,5} Increased BRS, increased cardiac vagal index, and decreased HR and cardiac sympatho/vagal balance have been reported in hypertensive patients after a high-salt diet, with these changes being blunted in those hypertensive patients with the more severe degree of sodium sensitivity. Therefore, our results indicate that the same type of cardiac autonomic alterations characterizing sodium sensitivity in hypertensive patients are already detectable in a population of young, healthy, white normotensive individuals, with possible clinical implications for prevention of the adverse cardiovascular effects of sodium loading as well as of an increased sodium sensitivity.

We also identified associations between SS-Index and several hemodynamic variables, but only after the low-salt diet (Table 2). This was not reported by Schmidlin et al, probably because of the dichotomous classification of sodium sensitivity, or of the lower sample size.⁶ We found that after 5 days of a low-salt diet, individuals with the lowest degree of sodium sensitivity had the highest SVR, the highest aortic impedance, and the lowest arterial compliance. These trends were responsible for a reduction in cardiac afterload with increased sodium sensitivity, and for an increase in the Windkessel effect with increased sodium sensitivity under reduced sodium loading. In this condition of sodium depletion, BP levels did not depend on the SS-Index significantly, as for systolic and diastolic BP, or even decreased with the severity of sodium sensitivity, as for MAP. By contrast, the high-salt diet uncoupled SS-Index from cardiac afterload and Windkessel effect, and concomitantly BP increased significantly with SS-Index.

We also detected a negative association between SS-Index and NN50+, but only after sodium loading (Table 2), indicating a partially blunted vagal control of HR in individuals with the higher degree of sodium sensitivity, when facing a high-salt diet. Interestingly, this hypothesis is supported by previous evidence of a blunted vagal modulation of HR in SS normotensive individuals following their habitual diet.³¹

Our results might also help to clarify recent controversial findings that indicate a U-shaped association between sodium intake and health outcomes.³² In fact, our findings seem to suggest that an ideal healthy sodium diet, valid for all normotensive individuals, might not exist. In a fraction of our volunteers, the diet with a very low sodium content induced hemodynamic adaptations that are generally associated with increased risk of cardiovascular events. Volunteers with

Table 3. SVR (mm Hg×s/mL) at the End of Each Diet in the 3 Sodium Sensitivity Classes (ISS, SR, and SS) by Sex: Mean (SEM)

	ISS	SR	SS
Females (N=41)			
Low-salt	1.28 (0.07)	1.09 (0.04)	1.16 (0.08)
High-salt	1.12 (0.04)	1.16 (0.06)	1.31 (0.08)
Males (N=26)			
Low-salt	1.10 (0.13)	0.95 (0.07)	0.79 (0.06)
High-salt	0.95 (0.06)	0.95 (0.09)	0.87 (0.06)

ISS indicates inverse sodium-sensitive; SR, sodium resistant; SS, sodium sensitive; SVR, systemic vascular resistances.

SS-Index lower than -30 mm Hg/(mol-day) even had higher MAP values after the low-salt diet, as a consequence of a remarkable increase of BP induced by salt restriction (Figure 5). This would imply that a very low-sodium diet, likely beneficial or at least not harmful for most normotensive individuals, should be recommended only if inverse sensitivity to sodium can be excluded. Future epidemiological and prospective studies might clarify whether the ISS individuals described in our study significantly contribute to the observed greater incidence of cardiovascular events in populations exposed to extremely low dietary sodium intakes.

Two study limitations should be considered. First, participants in our study, enrolled among students and young medical doctors of our University Hospital on a voluntary basis, might not be fully representative of a white normotensive population, due to their educational level, lifestyle habits, and sex distribution. In case the BP response to sodium intake might depend on sex, the higher prevalence of females among our participants might have biased our results. Therefore, to evaluate whether the imbalance between males and females might have influenced our results, we have also quantified the SVR response to dietary salt by sex in Table 3. The table suggests that differences between sexes may affect the absolute values of SVR (higher in females than in males), but are unlikely to affect the response to dietary salt, because in both sexes SVR decreased in the ISS group and increased in the SS group.

Second, the salt-sensitivity test, based on high- and low-salt diets, lasted several days. Therefore, we cannot extend our results to individuals classified as ISS, SR, or SS by the sodium loading/depletion maneuver based on saline infusion and diuretics (the so-called “Indiana protocol”)¹⁵ because this maneuver lasts only a few hours and might not elicit the same autonomic and hemodynamic adaptations observed in our study.

In conclusion, our study provides evidence that in SS normotensive subjects, an impaired vasodilatory response to

dietary salt loading can be a major pathogenetic factor in determining the pressor effect of dietary salt intake. This alteration seems to be associated with a dysfunction in autonomic cardiovascular regulation, as suggested by a blunted vagal cardiac modulation and a reduced vascular sympathetic inhibition in SS normotensives during high salt intake.

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Disclosures

None.

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SUPPLEMENTAL MATERIAL

Data S1. Hemodynamic parameters from BP waveform analysis and autonomic indices from HRV and BP variability analysis.

Frequency domain analysis of HRV and BP variability. Beat-by-beat series of systolic BP, diastolic BP and PI were resampled at 5 Hz. The Welch periodogram was estimated over the 2-hour recording by 90%-overlapped Hann data windows of 1638.4 s, and smoothed by a broadband procedure.¹ Power spectra were integrated over a very low frequency (VLF) band, between 0.001 and 0.04 Hz; a low frequency (LF) band, between 0.04 and 0.15 Hz; and a high-frequency (HF) band, between 0.15 and 0.50 Hz. In this way, the HF power of PI (PI HF), the ratio between LF and HF powers of PI (PI LF/HF powers ratio), the LF power of diastolic BP and the VLF power of diastolic BP, were estimated. The sensitivity of baroreflex control of HR was estimated by the transfer function method separately over the LF (BRS LF) and HF (BRS HF) bands. This was done by calculating systolic BP and PI spectra and cross-spectra over 120-s long, 50% overlapped, Hann data windows, considering reliable only spectral lines with squared coherence modulus between systolic BP and PI greater than 0.25.²

Time-domain and complexity-domain analysis of HRV. Increases larger than 50 ms between consecutive PI values were counted over the whole 2-hour recording and expressed as rate of increases per minute (NN50+).³ The self-similar characteristics of PI were assessed by the short-term scale coefficient α_1 through detrended fluctuation analysis, considering blocks of PI data not greater than 12 beats.⁴

BP waveform analysis. Hemodynamic parameters were derived through the Beatscope software,⁵ that analyzed finger BP waveforms beat-by-beat for the whole 2-hour recording. The software was initialized by entering sex, height and body weight of each individual as measured at the end of each diet. The shape of the brachial BP waveform was reconstructed from the finger BP waveform by

applying a proper filtering procedure implemented in Beatscope. The left ventricular ejection time was estimated as time interval between systolic upstroke and diastolic notch on the pulse waveform. Systemic vascular resistances (SVR), aortic characteristic impedance and arterial compliance were estimated by a nonlinear three-element model of the aortic input impedance called Model Flow.⁶ Stroke volume was estimated integrating the flow simulated by the model during systole; cardiac output was calculated multiplying stroke volume and HR. Values associated to valid BP waveforms (i.e., with Beatscope artifact code = 10000000) were averaged over the 2 hours recording at the end of each diet.

Limitations. The “Model Flow” method, originally designed for analyzing invasive aortic BP waveforms, has some limits when applied to noninvasive finger BP, like all noninvasive methods for determining cardiac output.⁷ Stroke volume is calculated as output of a 3-element Windkessel model, with the BP waveform as input, and both characteristic impedance and compliance of the model depend on the level of the measured BP. Therefore, discrepancies between invasive and non-invasive measures of BP may influence the estimation of stroke volume and, consequently, cardiac output.

When invasive BP measures were used, the model flow showed good agreement with the thermodilution technique,^{8, 9} the difference between model-flow and thermodilution measures of cardiac output being 0.70 (1.08) L/min, as mean (SD), in 25 awake patients in sitting position. As expected, the model-flow method applied to the noninvasive finger BP was less precise in estimating absolute values,⁸ nevertheless it tracked precisely relative changes of cardiac output and stroke volume.^{9, 10} Stroke volumes estimated from noninvasive BP by model flow and from Doppler ultrasound measures in the aorta were compared in 6 healthy subjects resting supine, and the percent error between the two methods was 0% (4.2%).¹¹

It should be considered, however, that calibration of the peripheral finger BP with an arm cuff BP measure and application of a proper filtering procedure to reconstruct the central BP waveform from the peripheral BP waveform greatly reduced the discrepancies between model-flow estimates of stroke

volume based on finger BP and on invasive brachial BP¹². In our study brachial BP waveforms were not only reconstructed from the finger BP waveform with the same filtering procedure, but the reconstructed BP waveforms were calibrated with brachial BP measures, in order to greatly reduce discrepancies between finger and brachial BP levels responsible for the errors due to use of a peripheral pulse wave.¹³

Another possible source of errors in the model-flow analysis is that characteristic impedance and compliance also depend on age, sex, weight and height of the subject. The model flow method takes this dependence into account through a statistical fitting of the aorta mechanical properties.⁶ Since in our study the ISS, SR and SS groups had substantially similar age, body mass index and sex distribution, possible inaccuracies in the statistical fitting should have affected similarly the three groups, with limited impact on the quantification of differences among groups.

Data S2. Hemodynamic and autonomic indices in salt-resistant and salt-sensitive groups as classified by traditional criteria.

Traditionally, individuals are classified in only two classes of salt-sensitivity. The dichotomous classification as salt-resistant or salt-sensitive is done on the base of the increase in mean arterial pressure after a high sodium diet compared to the mean arterial pressure level after a low sodium diet. Unlike criteria based on the SS-index, this classification does not take into account the actual intake of dietary salt as quantified by 24-hour urinary sodium excretion rate. Usually, the threshold for classifying an individual as salt-sensitive is an increase in mean arterial pressure $\geq 5\%$ after the high-salt diet.

Table S1 shows hemodynamic and autonomic indices in salt-resistant and salt-sensitive groups classified following this traditional criterion. Including all normotensive individuals that do not increase BP after sodium loading in a single “salt resistant” class, the dramatic fall of SVR after sodium loading that characterizes the ISS group only cannot be detected. By contrast, autonomic changes induced by dietary salt that similarly affect ISS and SR groups appear clearly in the traditionally defined “salt resistant” class.

Data S3. SS-Index distribution in normotensives and definition of sodium-sensitivity groups

The frequency histogram of SS-Index for the 71 normotensive individuals (figure S1) shows a relative maximum at 0 mmHg/(mol/day), with symmetric tails on the left and right contiguous bins, and an absolute maximum at -20 mmHg/(mol/day). This distribution suggests classifying normotensive individuals into three groups: a sodium sensitive (SS) group that increases blood pressure in response to sodium loading; a sodium resistant (SR) group, unresponsive to sodium; and an inverse sodium sensitive (ISS) group that decreases blood pressure in response to sodium loading. The symmetry of the distribution around SS-Index=0 suggests to set the thresholds for separating the SR group from the other two groups at +15 and -15 mmHg/(mol/day). In this way, the histogram peak at -20 mmHg/(mol/day) is associated with the ISS group.

It is worth noting that the ISS phenomenon does not regard only the sodium sensitivity test based on high- and low-salt diets. In fact, sodium loading also induced negative blood pressure responses, lower than -5 mmHg, in about 20% of a normotensive population tested for sodium sensitivity according to the Indiana protocol (saline infusion vs. diuretics) ¹⁴.

Table S1. Hemodynamic and autonomic indices in traditionally defined salt resistant and salt sensitive groups: mean (standard error of the mean).

	Salt Resistant (N=57)			Salt Sensitive (N=10)		
	Low-Salt	High-Salt	p	Low-Salt	High-Salt	p
HR (bpm)	76.6 (9.9)	73.0 (9.5)	**	70.9 (12.2)	70.0 (7.7)	n.s.
Left ventricle ejection time (ms)	296 (21)	309 (24)	**	300 (19)	317 (23)	**
Stroke Volume (mL)	63.5 (18)	65.6 (16.7)	n.s.	78.3 (18.7)	78.1 (26.2)	n.s.
Cardiac Output (L/s)	4.76 (1.22)	4.69 (1.14)	n.s.	5.42 (1.19)	5.29 (1.25)	n.s.
SVR (mmHg \times s /mL)	1.13 (0.29)	1.09 (0.23)	n.s.	0.92 (0.28)	1.04 (0.35)	n.s.
Aortic characteristic impedance (mmHg \times s /L)	60.7 (9.4)	60.1 (9.5)	n.s.	54.1 (9.6)	54.7 (10.3)	n.s.
Arterial Compliance (mL/mmHg)	2.24 (0.4)	2.32 (0.42)	**	2.64 (0.62)	2.50 (0.66)	**
PI LF/HF	4.7 (3.5)	3.7 (2.4)	**	5.0 (2.0)	3.8 (1.0)	n.s.
α_1	1.00 (0.15)	0.95 (0.16)	**	1.06 (0.06)	0.97 (0.06)	**
PI HF (ms ²)	739 (1268)	1072 (1907)	**	536 (557)	603 (346)	n.s.
NN50+ (n/min)	6.3 (4.4)	8.4 (4.6)	**	6.4 (4.6)	7.9 (4.6)	n.s.
BRS LF (ms/mmHg)	11.7 (6.0)	14.8 (7.7)	**	10.4 (4.8)	11.9 (2.8)	n.s.
BRS HF (ms/mmHg)	12.1 (8.7)	16.4 (11.7)	**	9.1 (4.6)	11.3 (3.2)	n.s.
Diastolic BP VLF (mmHg ²)	12.0 (9.2)	9.3 (5.9)	**	10.1 (4.9)	13.0 (12.0)	n.s.
Diastolic BP LF (mmHg ²)	7.3 (5.1)	5.4 (3.2)	**	6.6 (3.1)	6.0 (4.0)	n.s.

Statistical analysis by means profiles, with False Discovery Rate post-hoc comparison; p is the significance of the difference between low-salt and high-salt diets; ** indicates p<0.01; n.s.= not significant (p>0.05). BP=blood pressure; HR= heart rate; SVR=systemic vascular resistances; PI=pulse interval; HF= high frequency; LF=low frequency; VLF= very-low frequency; BRS=baroreflex sensitivity; NN50+=number of PI increases per minute larger than 50 ms; α_1 =short-term scale coefficient of PI.

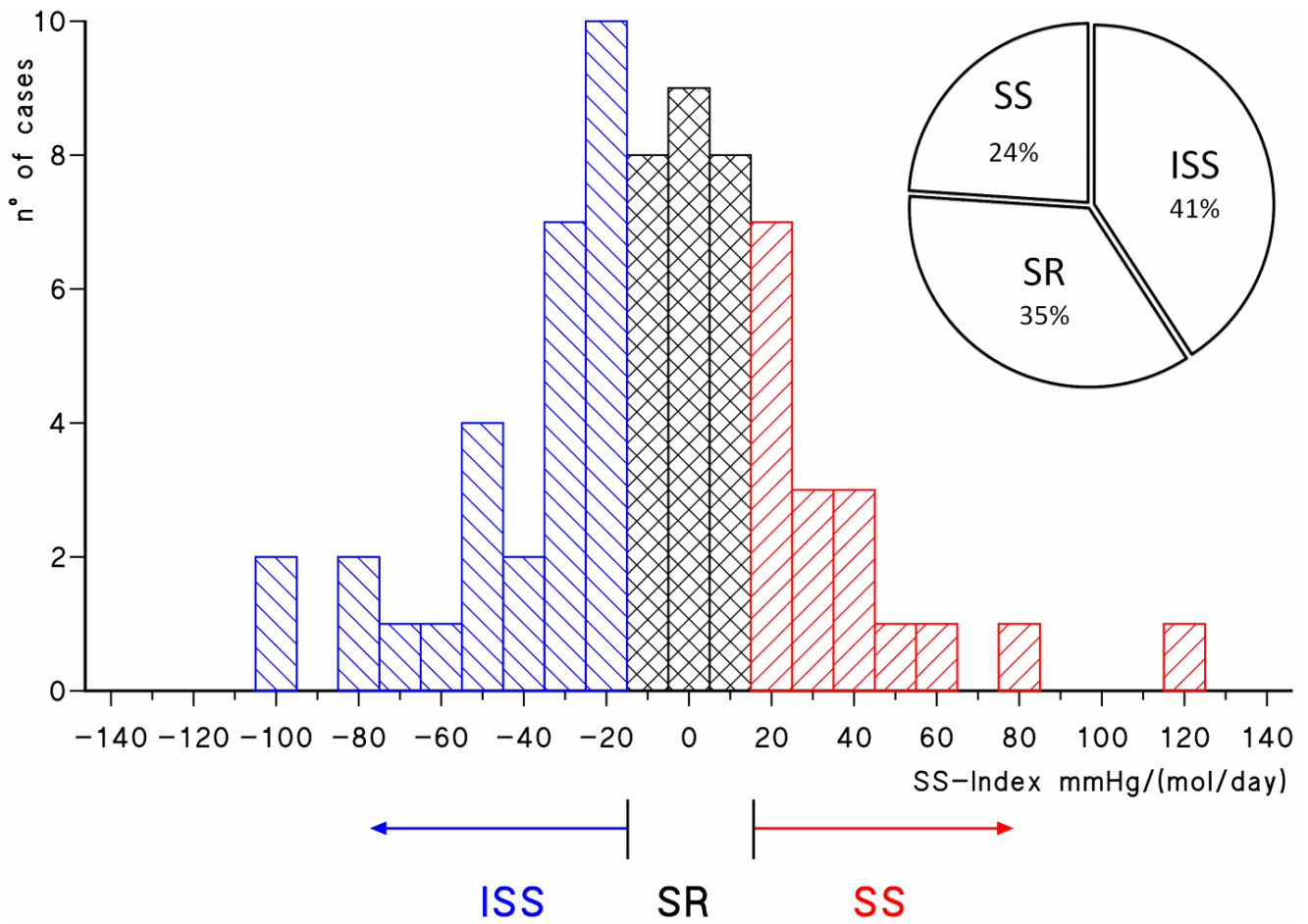


Figure S1: SS-Index distribution over 71 normotensive volunteers. The distribution was decomposed into three groups representing inverse sodium sensitive (ISS), sodium sensitive (SS) and sodium resistant (SR) individuals by setting the thresholds for identifying SR individuals at -15 and +15 mmHg/(mol/day). This choice, although arbitrary, defines an SR distribution symmetrically centred on 0, and associate the distribution peak occurring at a negative SS-Index value, to the ISS group.

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