Salt-sensitivity classification in normotensive adults

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ABSTRACT

The objectives of this study were to assess the reliability, sensitivity and specificity of salt-sensitivity classification in normotensive adults and to determine the predictive power of four clinical indices for salt-sensitivity. A total of 66 healthy, normotensive, free-living adults were administered 11-day salt-sensitivity diagnostic dietary salt challenges on two occasions to permit assessment of classification test–retest reliability. An oral glucose tolerance test, an acute saline loading test, gustatory testing and determination of salivary flow and sodium concentration were carried out to assess (by correlation analysis) their predictive power for salt-sensitivity. Following these procedures, 21 participants followed a reduced-sodium diet for 4 months, during which blood pressure was monitored monthly to allow evaluation of salt-sensitivity classification sensitivity and specificity. Regression was used to develop a predictive model for salt-sensitivity. Salt-sensitivity classification was not highly reliable (κ-value = 0.38), sensitive (0.73) or specific (0.60). No single index was highly predictive of classification status, but a model composed of five indices accounted for 92% of the variance in blood pressure response to acute salt challenge. The dietary salt challenge procedure used here for salt-sensitivity classification of normotensive adults had low test–retest reliability. While a battery of easily measured attributes may facilitate rapid salt-sensitivity classification, such a diagnosis provides only limited insight regarding blood pressure responsiveness to chronic dietary salt restriction in normotensive adults.

INTRODUCTION

It is widely recognized that hypertension has multiple aetiologies. High dietary sodium intake has been implicated as one contributing factor in 20–60% of the U.S. population, termed salt-sensitive (SS) [1–3]. The blood pressure of SS individuals rises and falls with increased and decreased sodium consumption respectively, whereas salt-insensitive (SI) individuals show little or no change in blood pressure or a reversed response pattern. While current dietary guidelines call for moderation of salt use by the general population [4], maximum benefit (i.e. delay or prevention of hypertension onset in normotensives or greater blood pressure reductions in hypertensives) would be expected in SS individuals. Because adoption of such a dietary change is difficult [5–8] and moderate-to-severe salt restriction can lead to elevated blood pressure in some normotensive individuals [9,10], there have been calls to develop methods to identify SS individuals [11–13] so that educational resources can be targeted to this group. The aims of the present work were to assess the reliability, sensitivity and specificity of a

Key words: salt-sensitivity, human, taste, blood pressure, hypertension.
Abbreviations: AUC, area under the curve; BMI, body mass index; MAP, mean arterial blood pressure; OGTT, oral glucose tolerance test; SI, salt-insensitive; SS, salt-sensitive.
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widely used diagnostic procedure and to explore the predictive power of several potential new indices. The work was conducted with normotensive individuals to facilitate development of preventive interventions.

Salt-sensitivity classification inconsistencies may be due to instability of the trait or shortcomings of diagnostic methodologies. Highlighting methodological issues, Pavek and Pavek [13] assessed reliability in mildly hypertensive patients by comparing the consistency of response across different methods of blood pressure assessment (i.e. auscultatory, oscillometric in the office and ambulatory oscillometric). Only eight of twelve SS patients and six of twelve SI patients were classified consistently by all three methods. The route of salt loading may also influence responses. Weinberger and Fineberg [14] used an acute challenge protocol involving intravenous saline loading followed by furosemide-induced depletion in hypertensive patients and, upon repeated assessments, found that only 18 of 28 patients responded consistently. Zoccali et al. [15] reported a kappa value of only 0.24 for essential hypertensive patients undergoing repeat dietary challenges, but others using a dietary challenge protocol have reported better test–retest reliability [16–18]. Using normotensive adults, Sharma et al. [17] reported a kappa value of 0.87, and Overlack et al. [18] noted that 28 of 31 normotensive adults tested consistently. However, Sharma et al. [17] reported that the actual blood pressure responses to the two challenges were not significantly correlated, whereas they were ($r = 0.71$) in the study of Overlack et al. [18].

A difference between these studies that could account for the outcome discrepancy is the more stringent classification criteria used by Overlack et al. [18] [mean arterial blood pressure (MAP) change of $> 5$ mmHg compared with $3$ mmHg]. Neither group provided sufficient information regarding the age, sex, family history of hypertension or weight of the subjects tested, all of which may influence responsiveness in normotensive adults [17–20]. The present study sought to replicate and verify the reliability of the methodology used by Overlack et al. [18] in a well defined sample of normotensive adults. The intravenous challenge procedure was not deemed appropriate, because it appears to be insensitive at identifying salt-sensitivity in normotensive individuals under 60 years of age [14] and yields inconsistent responses with the more nutritionally relevant dietary challenge approach [21].

The utility of salt-sensitivity classification can only be established by demonstrating that it predicts blood pressure levels in individuals chronically adhering to diets low or high in sodium. Preliminary evidence indicates that SS normotensive individuals are more likely to become hypertensive over time when consuming a typical North American diet [22]. To our knowledge, no data are available on the utility of salt-sensitivity classification as a predictor of the blood pressure response to a chronic decrease in sodium intake in normotensive adults. A heterogeneous decrease has been reported among normotensives [3,9], but never linked to salt-sensitivity status documented by the response to an acute salt challenge. A second aim of the present work was to determine the predictive power of salt-sensitivity classification for responses to moderate dietary sodium restriction.

While development of a valid and reliable index of salt-sensitivity would be a boon to basic and clinical studies, application in a large-scale screening effort will require that the classification method be rapid, safe, non-invasive and inexpensive. Published methods generally would not be appropriate, as they require strict adherence to diets that are extremely high and/or low in sodium (typically for over 2 weeks) or hospital admittance for several days in the case of intravenous challenge. A third aim of the present work was to identify indices associated with salt-sensitivity status that would be suitable for screening efforts. In addition to an array of routinely assessed clinical characteristics (e.g. age, sex, body weight and composition, blood pressure, sodium intake and family history of hypertension), reliably classified SS and SI participants were tested for salivary flow and composition, susceptibility to suppression of salt taste by amiloride, acute diuretic response to a saline load and glucose tolerance, to ascertain the predictive power of these indices.

**METHODS**

**General protocol**

Healthy, normotensive adults were recruited by public advertisement. Research was carried out in accordance with the Declaration of Helsinki (1989) of the World Medical Association, and was approved by the Ethics Committee of Purdue University and the University of Pennsylvania. Informed consent was obtained from all subjects. Eligibility was established during two sessions (held 1 week apart) where health and dietary questionnaires were completed, and height, weight, body composition and blood pressure were measured. Eligibility criteria included: no chronic or acute health disorder, no use of medications, aged 18–70 years, no special diet, no history of high or low blood pressure, stable body weight (no change $> 3$ kg over the previous 3 months), stable activity level, ratings of concern about dietary sodium intake, heart disease and hypertension of 5 or less on a 9-point category scale ($9 = $ extreme), control of over 50% of food purchasing and preparation, and blood pressure consistently $< 140/90$ mmHg. Recruitment was especially targeted to individuals at elevated risk for salt-sensitivity, such as African–Americans, the obese and those with a positive family history of hypertension, to ensure inclusion of an
adequate sample of SS participants. The study protocol is outlined in Figure 1. Females began the study on the first day of menses so that salt-challenge and diuresis tests would not occur just prior to or during menses. Slight variations in timing were required so that females did not collect urine on days of menses or to accommodate unanticipated scheduling conflicts with individual participants.

**Subjects**

Table 1 contains selected characteristics of the study participants. A total of 66 individuals were recruited. Of these, 21 were determined to be non-compliant with the study protocol, based on analysis of urine sodium values during salt-challenge trials or failure to attend scheduled study sessions. Furthermore, 15 individuals met all study conditions, but were not classified consistently following the replicate challenge trials (Incon in Table 1). Of the remainder, 17 individuals consistently tested as SS and 13 consistently tested as SI. There were no statistically significant differences between the individuals not following the protocol, those testing inconsistently and those classified as SS or SI.

**Blood pressure**

Systolic (phase I) and diastolic (phase V) blood pressure values were determined with a random-zero sphygmomanometer (Hawksley; W. A. Braun Co., Copiague, NY, U.S.A.) and an appropriately sized cuff after subjects had been standing, sitting and supine for at least 5 min. MAP was determined as the diastolic pressure plus one-third of the pulse pressure. Blood pressure readings were taken in duplicate on four occasions over a 2-week period prior to recruitment. To be eligible, values had to agree within 4 mmHg of each other. Duplicate readings were also taken at all subsequent time points.

**Body weight and composition**

Body weight was measured by clinical scale (Scale-Tronix, Inc., Wheaton, IL, U.S.A.) and height by stadiometer (Holtain Ltd., Crymych, Dyfed, U.K.). Body composition was measured by bioelectrical impedance analysis (Model 101 Analyzer; RJL Systems, Detroit, MI, U.S.A.).

**Diuresis test**

The procedure of Gebruers et al. [23] was used to measure urine osmolality following an oral saline load. Subjects arrived at 09.00 hours, having fasted since 22.00 hours the previous evening. They remained supine, except when voiding urine at 20-min intervals, until three urine collections of comparable volume (±5%) were obtained. After a stable baseline was established, 1 litre of 0.9% (w/v) NaCl was ingested over a 10 min period. The load was at room temperature and contained seasonings.
Immediately afterwards, a 1 mM citric acid, 0 mM KCl or 0.5 mM amiloride was placed over the same tongue site. The saltiness, sweetness, sourness and bitterness of the stimulus was rated on a 200 mm visual analogue scale. Another piece of filter paper soaked in amiloride was then reapplied to the tongue for 1 min, followed by stimulation with another taste solution. The order of stimulus presentation was randomized. Citric acid, KCl and sodium gluconate were included as specificity controls, since amiloride does not reduce suprathreshold sensitivity to the first two [27,28] and a greater differential response was anticipated to sodium gluconate [29].

Dietary compliance
Urinary sodium excretion was the principal index of dietary compliance during the challenge and reduced-salt-diet periods. Participants were provided with 2-litre wide-mouth plastic containers containing 2 g of boric acid (a preservative) for each 24-h collection. The samples were returned to the laboratory within 3 days of collection. The total volume was determined and a 100 ml aliquot was frozen for later analysis. Samples were analysed for Na and K by flame photometry and for creatinine by a colorimetric method (Sigma diagnostic kit no. 555).

Stress tests
The State form of Spielberger’s State–Trait Anxiety Inventory [24] and the Daily Stress Inventory [25] were used to assess participant stress levels at the end of each phase of the salt-challenge tests.

Sensory tests
Amiloride suppression of salt taste was evaluated using a modification of the methods of Schiffman et al. [26] and McCutcheon [27]. Filter paper (2.3 cm diam.) soaked with 0 mM, 0.1 mM or 0.5 mM amiloride was placed over the tongue tip for two consecutive 2.5 min periods. Immediately afterwards, a 1.27 cm-diam. piece of filter paper soaked in a solution of 0.2 mM or 0.7 M NaCl, 0.1 mM citric acid, 0.1 mM KCl or 0.1 mM sodium gluconate was placed over the same tongue site. The saltiness, sweetness, sourness and bitterness of the stimulus was rated on a 200 mm visual analogue scale. Another piece of filter paper soaked in amiloride was then reapplied to the tongue for 1 min, followed by stimulation with another taste solution. The order of stimulus presentation was randomized. Citric acid, KCl and sodium gluconate were included as specificity controls, since amiloride does not reduce suprathreshold sensitivity to the first two [27,28] and a greater differential response was anticipated to sodium gluconate [29].

Salivary flow and sodium concentration
Resting saliva was collected by the method of Navazesh and Christensen [30]. Participants rinsed their mouths with deionized, distilled water three times. They swallowed to empty their mouth, tilted their heads forward over a funnel fitted to a pre-weighed test tube and drooled into the tube for 1 min. The participant then expectorated all remaining saliva into the vial. This sample was discarded and a similar collection trial was conducted for 3 min. Stimulated saliva was collected next using the same procedure, except that subjects chewed an unflavoured gum base at a rate of 80 chews per min (timed with a metronome) and expectorated at the end of each 1 min. Again, the first sample was discarded and the subsequent 3-min sample was saved. These samples were assayed for flow rate by dividing the total volume collected by the collection time to derive rate in g/min, and for sodium level by flame photometry.

Oral glucose tolerance test (OGTT)
A fasting blood sample was obtained for measurement of plasma glucose and insulin levels immediately before a
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75 g glucose load. Additional samples were obtained at 0.5, 1 and 2 h after the load. Glucose was analysed by the glucose oxidase technique with a Glucostat Analyzer (YSI; model 27). Plasma insulin was determined with a solid-phase radioimmunooassay, 'Coat-a-Count', manufactured by Diagnostic Products Corp. (Los Angeles, CA, U.S.A.). The area under the insulin curve was determined by the trapezoidal rule.

**Salt challenge**

Participants were prescribed a diet containing about 40 mmol of sodium for 4 days. This was followed immediately by a return to each subject's customary diet supplemented with 172 mmol of sodium for 7 days. The extra sodium was provided as slow-sodium tablets (CIBA Laboratories, Horsham, West Sussex, U.K.). To minimize gastric complications, the dose was 5 g of NaCl on day 1, 7.5 g on day 2 and 10 g on days 3–7. A MAP rise of at least 5 mmHg from the end of the low-salt-diet period and could be classified. Table 2 contains the classification results derived from blood pressure readings obtained in the supine, standing and sitting positions with a change of MAP of 5 mmHg (10% MAP change also shown for sitting) between the ends of the low- and high-salt periods. To be considered compliant with the salt challenges, a minimal difference of 100 mmol of urinary sodium between the ends of the low- and high-salt periods was required. Mean (S.E.M.) 24 h urinary sodium excretion values during the two low- and two high-salt challenge periods for the SS subjects were: low, 55.8 ± 12.8 mmol/day (median 42 mmol/day); high, 379.9 ± 40.7 mmol/day (median 332 mmol/day); low, 82.3 ± 20.8 mmol/day (median 52 mmol/day); high, 312.4 ± 32.4 mmol/day (median 312 mmol/day). Values for the SI subjects were: low, 70.7 ± 11.2 mmol/day (median 63 mmol/day); high, 488.5 ± 55.5 mmol/day (median 434 mmol/day); low, 66.3 ± 15.9 mmol/day (median = 47 mmol/day); high, 500.8 ± 66.2 mmol/day (median 469 mmol/day).

Based upon the κ-statistic, the most reliable, and only statistically significant, method involved measurement of blood pressure in the sitting position and using a 5 mmHg change of MAP. To determine whether better reliability would be achieved by salt challenges that were both lower and higher than customary intake, κ was computed for individuals following salt challenges that were more than 25% below and above baseline consumption. Using a criterion of a 5 mmHg change of blood pressure, reliability was comparable with that based on a minimal 100 mmol/day difference in urinary sodium excretion (κ = 0.32; P = 0.046), although only 37 of the 45 compliant participants met this challenge criterion. Correlations between measurements were weak, with only 54% of subjects classified consistently based upon sitting and supine blood pressure readings. Comparisons between sitting and standing or supine and standing yielded consistent classifications for only 48% and 56% of compliant individuals.

Sitting blood pressures at the end of the two low-salt periods were significantly correlated in both the SS (r = 0.76, P < 0.001) and the SI (r = 0.82, P < 0.001) subjects. This was also true of blood pressures following the two salt-loading periods for both the SS (r = 0.67, P = 0.003) and the SI (r = 0.86, P < 0.001) subjects.

The measured blood pressure response to the reduced sodium diet (mean of duplicate readings at months 3 and 4) was used as the ‘gold standard’ for salt-sensitivity, and permitted calculation of the sensitivity (detection of true positives) and specificity (detection of true negatives) of salt-sensitivity classification based upon the acute salt challenges. Using the criteria of a sitting MAP change of > 5 mmHg and a urinary sodium excretion difference of > 100 mmol, sensitivity was 0.73 and specificity was 0.60. Thus the false-positive rate was 40% and false-negative rate was 27%. The change in blood pressure during salt challenge was not significantly correlated with
Table 2  Salt-sensitivity classification results based upon blood pressure readings obtained in the supine, standing and sitting positions based upon a change of MAP of 5 mmHg between the ends of the low- and high-salt periods

The final column contains results based upon a 10% change in MAP.

<table>
<thead>
<tr>
<th>Classification</th>
<th>MAP change of 5 mmHg</th>
<th>MAP change of 10%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Supine</td>
<td>Standing</td>
</tr>
<tr>
<td>Reliable SS</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>Inconsistent</td>
<td>21 (46.4%)</td>
<td>20 (44.4%)</td>
</tr>
<tr>
<td>Reliable SI</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Missing</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>(\kappa)</td>
<td>0.092 ((P = 0.482))</td>
<td>0.105 ((P = 0.463))</td>
</tr>
</tbody>
</table>

Figure 2  Resting and stimulated salivary flow rate and salivary sodium concentration

the change in blood pressure observed during any month of the 4-month reduced-sodium diet period in either group.

Salivary assessment

The salivary flow rates and sodium concentrations in the unstimulated and stimulated saliva of SS and SI individuals are presented in Figure 2. Oral stimulation led to significantly higher salivary flow rates in both the SS \((t = 6.57, P < 0.001)\) and the SI \((t = 4.61, P < 0.001)\) subjects. However, the stimulated flow rate was significantly higher in the SI than in the SS group \((t = 2.48, P = 0.021)\). The salivary sodium concentration did not differ between resting and stimulated samples for the SS subjects, but did for the SI group \((t = 4.93, P = 0.01)\). The ratio of sodium concentration (mM Na/l) to flow rate (g/min) in stimulated samples was higher in the SI group \((12.0 \pm 2.26)\) than in the SS group \([6.13 \pm 1.19 \,(t = 2.10, P = 0.046)]\).

OGTT

No significant differences between groups were observed for the insulin area under the curve (AUC) values (SS, 268.1 ± 29.8; SI, 220.6 ± 17.1 ng·h⁻¹·ml⁻¹), or the baseline, 30, 60 or 120 min insulin concentrations (SS, 0.71 ± 0.17, 2.74 ± 0.32, 2.99 ± 0.35 and 2.25 ± 0.36 ng/ml respectively; SI, 0.76 ± 0.19, 3.13 ± 0.26, 2.40 ± 0.24 and 1.80 ± 0.22 ng/ml respectively). There were also no significant differences in glucose concentrations (SS, 84.8 ± 2.5, 119.3 ± 6.1, 106.8 ± 0.92 and 87.9 ± 8.0 mg/dl respectively; SI, 85.3 ± 1.7, 124.8 ± 4.2, 100.5 ± 5.2 and 87.8 ± 4.5 mg/dl respectively) or glucose/insulin ratios (SS, 149.0 ± 15.8, 63.8 ± 19.4, 40.3 ± 5.8 and 48.1 ± 4.8 respectively; SI, 151.1 ± 16.2, 43.5 ± 3.9, 48.7 ± 6.8 and 57.8 ± 8.1 respectively) at these time points.
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Figure 3 Urine osmolality following ingestion of 1 litre of 0.9% NaCl in SS (■) and SI (□) individuals

Figure 4 Individual taste intensity rating changes from baseline

Taste intensity rating changes were measured for NaCl (20 mM, 70 mM), KCl (10 mM, 50 mM), 140 mM sodium gluconate (NaG) and 2 mM citric acid (CA) following pretreatment with 0.5 mM amiloride in SS (■) and SI (□) individuals.

Diuresis test

Data from the diuresis test are presented in Figure 3. Repeated administration of this test demonstrated that it was highly reliable; correlations at each of the time points ranged from 0.48 to 0.68 (all P < 0.001). There were no significant SS–SI group differences in urine osmolality at any sampling time, percentage changes from baseline or AUC values. However, the SS group had a significantly smaller decrease in urine osmolality 20 min post-loading than did the SI group [F(1,15) = 7.72, P = 0.015].

Amiloride suppression test

Exemplary results of the amiloride suppression test involving adaptation to 0.5 mM amiloride are shown in Figure 4. There was marked individual variability in changes of perceived intensity ratings for the test stimuli. There were no significant group differences. Responses across the different amiloride adaptation concentrations were generally reliable. Except for the association between ratings of 70 mM NaCl after adaptation to 1 mM amiloride (r = 0.28), all other responses to NaCl were significantly related (r = 0.44–0.54; all P < 0.03).

Dietary compliance

Twelve SS and nine SI individuals volunteered to adhere to a reduced-sodium diet for 4 months. The SS participants were 32.8 ± 3.8 years old and had a mean BMI of 25.3 ± 1.5 kg/m²; ten were male, four were African-American and eight were Caucasian. Eight had a positive family history of hypertension. The SI participants were 29.8 ± 2.4 years old and had a mean body mass index of 21.5 ± 0.7 kg/m²; seven were male, eight were Caucasian and one was Asian. Four had a positive family history of hypertension. Figure 5 shows their monthly urinary sodium excretion values and MAP changes from baseline during this time. Both groups achieved a significant and sustained reduction in sodium intake, as measured by urinary excretion relative to baseline [F(4,64) = 9.72, P < 0.001]. Values were significantly different from baseline for each of the diet months, but not between months. There was no significant difference in sodium excretion between the groups. MAP was consistently reduced in the SS subjects, although not significantly so relative to pre-diet values. Within the SS group, there was 80% power to detect a change in MAP representing 30% of the variance in this variable at the 5% probability level. Nine of the twelve SS individuals had a MAP reduction by the end of the dietary period. The SI subjects had smaller and less consistent changes in MAP, with means oscillating between small reductions and increases. Three of the nine SI individuals had a reduction in MAP at the end of the dietary period. Urinary sodium excretion was not significantly correlated with blood pressure response in either group.

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Multiple regression

Multiple regression was used to derive a model to predict the mean MAP change over the two salt-challenge trials. The demographic and dietary attributes entered into the initial model were age, race, sex, family history of hypertension, body weight, percentage body fat, waist/hip ratio, alcohol use, cigarette use, baseline urinary sodium excretion and baseline MAP. Percentage body fat was the only significant predictor of MAP change ($r = 0.521$). However, addition of indices from the proposed predictor variables (i.e. insulin AUC value, salivary sodium/stimulated salivary flow ratio, diuresis to acute sodium loading and reduced salt taste intensity after adaptation to 0.5 mM amiloride) led to a strongly predictive model. With stepwise addition, it included AUC ($r = 0.524$), age ($r = 0.714$), salivary ratio ($r = 0.827$), amiloride suppression ($r = 0.901$) and percentage body fat ($r = 0.960$). Thus this model accounted for about 92% of the variance in MAP response to the acute salt challenges.

**DISCUSSION**

The present findings raise questions about the reliability of salt-sensitivity classification. We were unable to confirm the strong test–retest response consistency observed by Overlack et al. [18] using comparable methodology. This diagnostic approach had previously yielded the most reliable results in terms of salt-sensitivity criterion attainment (28/31 cases) and magnitude of blood pressure shift ($r = 0.71$). We found that sitting MAPs were significantly correlated across trials ($r = 0.67–0.86$), but consistent blood pressure responses were observed in only 30 out of 45 participants.

The basis for the discrepancy is not clear. Characteristics of the subset of subjects undergoing reliability testing by Overlack et al. [18] were not reported. Various attributes (e.g. age [18,32], sex [20], body weight [3], ethnicity [32–35] and family history of hypertension [18,19,36,37] have been linked with salt-sensitivity, but their influence on classification reliability has not been explored previously. The present data did not reveal an association between age, gender, ethnicity, body weight, BMI, waist/hip ratio or family history of hypertension and reliability of the blood pressure response to an acute dietary salt challenge. However, our sample was not randomly selected and may not have provided an accurate estimate of the potential influence of these characteristics on test responsiveness.

The lower reliability of our results could also be due to the shorter duration of the low-salt challenge (4 compared with 7 days). However, Sullivan [22] reported that blood pressure decreases in response to this manipulation were stable after 3–4 days. The lower absolute level of sodium in the low-salt challenge may also be a factor. Dustan et al. [38] observed stronger and more consistent decreases in blood pressure due to sodium depletion than elevations in blood pressure due to sodium supplementation, an observation also apparent from intravenous challenge test data [3]. The protocols that reported the best reliability used diets containing 20 mmol of Na (day) [16–18], whereas our subjects ingested a mean of 63.9 mmol/day on the first trial and 73.6 mmol/day on the second. Zoccali et al. [15] measured blood pressure in subjects on their customary diet and on a 40 mmol/day sodium diet, and obtained poor reliability. Thus a decrease to 20 mmol/day may be required to achieve a maximal response.

We, Overlack et al. [18] and others have classified subjects by contrasting blood pressure values taken on the final days of divergent salt challenges. If random variability across these days exceeded the criterion change, mis-classification would occur. Averaging multiple blood pressure determinations at each time point would theoretically minimize this risk. However, the variance in our duplicate readings was comparable with the mean variance of the 12 readings reported by Overlack et al. [18], and thus does not account for our different results. Evidence that 24 h ambulatory blood

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![Figure 5](image-url)  
**Figure 5** Urinary sodium excretion and changes in MAP in subjects consuming a reduced-sodium diet

Left panel: urinary sodium excretion (mean of two 24 h collections) during baseline and at monthly intervals during adherence to a reduced-sodium diet for 4 months in SS (■) and SI (○) individuals. Right panel: change from baseline of MAP in SS (■) and SI (○) individuals at monthly intervals during adherence to a reduced-sodium diet for 4 months.
pressure monitoring does little to improve reliability [39] suggests this is not the weak link in classification.

It should also be noted that our high-salt challenge exceeded that used by most other workers, yet did not result in more reliable classification. Overlack et al. [18] also provided all food to participants during the salt challenge period, and obtained better compliance than we achieved.

In an attempt to elucidate the optimal conditions for reliable classification, we also explored the influence of obtaining blood pressure readings in the supine, sitting and standing positions, using different criteria for the nature of the salt challenge, and two cut-off levels for the blood pressure response. Sharma et al. [17] used supine readings, and reported less consistent blood pressure responses than Overlack et al. [18], who obtained sitting values. Our data support the superiority of sitting values over both supine and standing readings in terms of reliability. The observation of enhanced reliability with more moderate criteria for blood pressure was also observed in the present study. A MAP change of 10% produced a less consistent response than an absolute change of 5 mmHg. Requiring challenges to be both > 25% lower and > 25% higher than customary sodium consumption did not improve upon results based on an absolute difference exceeding 100 mmol/day.

To be useful, a diagnostic tool must also be sensitive (i.e., able to detect true-positive cases) and specific (i.e., able to detect true-negative cases). To our knowledge, no previous study has assessed these facets of the diagnostic approach, in part because they lacked a required 'gold standard'. Sullivan [22] reported that the incidence of hypertension was higher in seven SS compared with 24 SI individuals in a follow-up study in which they consumed their customary diet, but they did not report more specific findings. Weinberger et al. [3] reported that normotensive adults could lower their blood pressure by reducing sodium consumption, but did not associate this with salt-sensitivity. Following diagnosis, 21 of the reliably classified participants in our study followed a reduced-sodium diet for 4 months. Using their measured change in blood pressure in response to this diet as a true index of their salt-sensitivity, the SS classification procedure had a sensitivity of 73% and specificity of 60%.

Thus 27% of participants whose blood pressure did decline on the reduced-sodium diet were not identified, and 40% of individuals whose blood pressure did not decline were classified as SS. Such high false-positive and -negative rates indicate that salt-sensitivity classification will be of limited value in targeting preventative lifestyle interventions. A critical review of the literature has led to a similar conclusion by others [40]. However, it should be noted that, in moderating dietary sodium consumption, the intake of other nutrients (e.g., potassium, calcium, magnesium) capable of mitigating the effects of this intervention may have occurred, obscuring an association. Based on diet record analyses this did not occur in the present study, but such records are an imperfect measure of true intake.

The proposed new indices for salt-sensitivity classification did not provide the expected level of discriminatory power. Skrabal et al. [19] reported lower salivary sodium levels in normotensive SS subjects compared with SI subjects, and proposed that this index of sodium retention reflected a characteristic of salt-sensitivity that would hold diagnostic value. We observed no significant differences in resting or stimulated salivary sodium concentrations, although the ratio of sodium concentration to flow rate in stimulated samples was higher in the SI than in the SS group. This difference is consistent with enhanced sodium resorption by the SS subjects, but was not of sufficient consistency to provide a strong independent basis for salt-sensitivity classification in our normotensive sample.

Salt-sensitivity, glucose intolerance and insulin resistance commonly co-occur in hypertensive individuals, and there is evidence for an association in normotensives [41,42]. Sharma et al. [43] reported higher plasma glucose and insulin levels in normotensive adults during an OGTT. Thus it was hypothesized that assessment of glucose tolerance could provide a rapid index of salt-sensitivity risk. However, our OGTTs revealed no significant differences between SS and SI individuals for any measure of fasting or stimulated glucose or insulin concentrations. This discrepancy may be attributable to differences in methodology. Sharma et al. [43] tested individuals during low- and high-salt challenges. Differences were only apparent during the high-salt period.

Because of our interest in identifying a rapid screening index, we tested individuals on their customary diets. It may be that the stress of a high-salt diet is required to reveal an association. However, other work has raised questions about the association between glucose tolerance, insulin resistance and salt-sensitivity [44–46]. Thus current evidence does not indicate that fasting glucose or insulin levels or responses to an OGTT in normotensive individuals on their customary diet are independently predictive of salt-sensitivity.

SS hypertensive patients excrete a more hypertonic urine acutely after isotonic intravenous saline challenge than do SI patients [47,48]. Studies in spontaneously hypertensive and normotensive rats indicate that oral sodium loading elicits a larger natriuretic response than does intravenous challenge [49]. Thus it was hypothesized that an oral saline challenge would provide a useful clinical index of salt-sensitivity. Our data indicate that SS individuals consistently excreted urine of higher osmolality during the 2 h after an oral isotonic saline challenge, but the magnitude of the increase was small and statistically significant only at the 20 min time point. This discrepancy in response to our oral compared with the previously used intravenous challenge procedure may

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be due to the acute modulatory effect that orosensory stimulation has on natriuresis and diuresis. In humans, oral exposure to physiological saline elicits little renal response compared with effects noted with hypotonic or hypertonic stimulation [50]. Because of the high response variability, the procedure used here does not appear to hold promise as an independent index for identification of salt-sensitivity.

Studies on inbred Dahl/Rapp SS rats suggest that they are less sensitive to inhibition of taste by amiloride than are SI rats [51]. Thus it was hypothesized that the SS subjects would show reduced suppression of salt taste after topical amiloride application. However, no SS–SI group differences were observed. Individual taste responsiveness was reliable, but not related to salt-sensitivity status.

In the absence of a single reliable proxy measure of salt-sensitivity, multiple regression has been used to derive predictive models. Overlack et al. [18] reported correlations of 0.409, 0.498 and 0.556 for models comprising age alone, age plus body weight, and age, body weight and family history of hypertension respectively. This was not based on individuals with demonstrated reliable salt-sensitivity status. The best model derived from our baseline demographic and dietary data only included percentage body fat, and accounted for only 27% of the variance in blood pressure change averaged over the two salt-challenge trials. The failure to include age and family history in our model may stem from the facts that our sample was younger (salt-sensitivity in-

In summary, this work raises questions about the reliability, sensitivity and specificity of dietary salt challenges for salt-sensitivity classification in normotensive adults. Given the high false-positive and -negative classification rates, the approach is presently inappropriate for guiding preventative or therapeutic inter-

ventions. Additional methodological studies will be needed to establish the veracity of the classification. Should a strong association between salt-sensitivity classification and long-term blood pressure status be established, it may be possible to identify a battery of rapid screening indices for the trait.

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