Salt sensitivity is associated with insulin resistance, sympathetic overactivity, and decreased suppression of circulating renin activity in lean patients with essential hypertension1–3

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ABSTRACT

Background: The mechanisms by which a derangement of glucose metabolism causes high blood pressure are not fully understood.

Objectives: This study aimed to clarify the relation between salt sensitivity of blood pressure and insulin resistance, which are important subcharacteristics of hypertension and impaired glucose metabolism, respectively. Effects on the renin-angiotensin and sympathetic nervous systems were also studied.

Design: The state of glucose metabolism was assessed by a hyperinsulinemic euglycemic glucose clamp technique and a 75-g oral-glucose-tolerance test in 24 essential hypertensive patients who were lean and without diabetes or chronic kidney disease. The subjects were classified as salt-sensitive or salt-resistant on the basis of the difference (Δ mean blood pressure ≥5%) between 24-h ambulatory blood pressure monitoring results on the seventh day of low-salt (34 mmol/d) and high-salt (252 mmol/d) diets. Urine and blood samples were collected for analyses.

Results: There was a robust inverse relation between the glucose infusion rate (GIR) and the salt sensitivity index. The GIR correlated directly with the change in urinary sodium excretion and was inversely related to the change in hematocrit when the salt diet was changed from low to high, which is indicative of salt and fluid retention in salt-sensitive subjects. The GIR also showed an inverse correlation compared with the changes in urinary norepinephrine excretion, plasma renin activity, and plasma aldosterone concentration.

Conclusions: Salt sensitivity of blood pressure is strongly associated with insulin resistance in lean, essential hypertensive patients. Hyperinsulinemia, sympathetic overactivation, and reduced suppression of the renin-angiotensin system may play a role in this relation.


INTRODUCTION

Derangement of glucose metabolism and hypertension are 2 of the hallmarks of the metabolic syndrome. Although association between these variables has been suspected, the mechanisms leading to this relation are not fully understood (1). Because both hypertension and impaired glucose metabolism are heterogeneous conditions, focusing on specific subcharacteristics of each, namely the salt sensitivity and insulin resistance, may yield more information to delineate and unravel this problem.

Salt-sensitive essential hypertension is a subset of hypertension characterized by a significant blood pressure response to change in dietary salt intake (2). Salt sensitivity of blood pressure is associated with increased risk of cardiovascular events independent of blood pressure (3). Also, insulin resistance and/or compensatory hyperinsulinemia are associated with ischemic heart disease and mortality independent of obesity or diabetes (4).

As described above, salt sensitivity and insulin resistance may be the underlying defects predisposing individuals to the development of full-blown metabolic syndrome. However, a direct correlation between salt sensitivity and insulin resistance has not been consistently found (5, 6). This may be due to the differences in subject population, such as age or presence of obesity, or assay methods (7). In this study, salt sensitivity was assessed by taking 24-h blood pressure measurements during the low- and high-salt diet, and insulin resistance was measured as insulin-mediated glucose uptake with the hyperinsulinemic euglycemic glucose clamp technique, which is considered the best available technique for this state (8).

Possible involvement of the sympathetic nervous system was also studied. Julius (9) suggested that increased sympathetic activity may be the underlying factor of hypertension and metabolic derangements, leading to insulin resistance through various mechanisms, including reduced glucose extraction in skeletal muscle because of vasoconstriction. On the other hand, insulin, besides affecting glucose metabolism, has an antinatriuretic effect by activating the sympathetic nervous system (10, 11), augmenting angiotensin II–mediated aldosterone pro-

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duction (12), and directly promoting renal tubular sodium reabsorption (13). Compensatory hyperinsulinemia is proposed to lead to sodium retention and subsequently hypertension because these salt-retaining effects of insulin are intact in insulin-resistant individuals (14, 15).

In this study, we aimed to clarify the relation between salt sensitivity and insulin resistance, along with the involvement of the sympathetic nervous and renin-angiotensin systems in subjects with uncomplicated essential hypertension.

SUBJECTS AND METHODS
Subjects
Twenty-four lean subjects with untreated essential hypertension and without diabetes or chronic kidney disease who were consecutively admitted to the study hospital met the inclusion criteria, gave consent to participate in the study (12 men and 12 women, 39–67 y old), and were enrolled.

Secondary hypertension was ruled out by laboratory and endocrine testing. Inclusion criteria were a body mass index (BMI; in kg/m²) <30, a normal glucose tolerance [fasting plasma glucose ≤6.1 mmol/L; plasma glucose 2 h after a 75-g oral-glucose-tolerance test (OGTT) ≤7.7 mmol/L], absence of albuminuria, and a creatinine clearance ≥70 mL/min. The salt-loading protocol was performed from June 1991 to November 1996. All participants provided written informed consent, and the study was approved by the Fukushima Medical University Ethics Committee (Fukushima, Japan).

Study protocol
After admission to the study hospital, all subjects were placed on a normal-salt diet (151 mmol/d) for ≥7 d. The subjects were then given a low-salt diet (34 mmol/d) for 7 d immediately followed by a high-salt diet (255 mmol/d) for 7 d. The subjects remained in the hospital throughout the course of the study. A euglycemic glucose clamp technique and 75-g OGTT were performed during the normal-salt diet period. On the last day of each period, blood pressure was monitored every 30 min for 24 h with an automatic oscillometric device (ABPM-630; Nippon Colin, Tokyo, Japan). Twenty-four-hour urinary sodium excretion was measured on the first and the second day of the high-salt period to assess sodium retention. Blood samples were obtained on the last day of each diet period to determine hematocrit concentrations, electrolyte concentrations, plasma renin activity (PRA), and plasma aldosterone concentration (PAC). PRA and PAC were measured by using a radioimmunoassay kit from Abbott Japan (Tokyo, Japan). Urinary norepinephrine was measured according to the HPLC method in 24-h urine samples collected on the last day of each study period.

Assessment of salt sensitivity
The assessment of salt sensitivity of blood pressure is difficult because of the lack of universal consensus on definition (16, 17). For this study, we performed dietary intervention (18–21) following the protocol described above. Mean blood pressure (MBP) values collected during ambulatory blood pressure monitoring in the low- and high-salt diets were used to calculate the salt sensitivity index (SSI). The SSI is the difference between MBP in low- to high-salt diets divided by the MBP during the low-salt diet. Subjects with an SSI ≥5% were considered salt-sensitive (SS), whereas subjects with an SSI <5% were deemed salt-resistant (SR).

Insulin infusion study
Sensitivity to insulin-mediated glucose uptake was assessed by hyperinsulinemic euglycemic glucose clamp technique according to the method described by DeFronzo et al (22). Exogenous insulin was infused at a rate of 1.12 μU·kg⁻¹·min⁻¹ to maintain hyperinsulinemia [serum immunoreactive insulin (IRI): 78–186 μU/mL] and the infusion rate of the 15% glucose solution was adjusted based on frequent measurements of plasma glucose concentration in order to clamp the plasma glucose concentration between 4.4 and 5.8 mmol/L (80–105 mg/dL). The glucose infusion rate (GIR) during the last 30 min of the 2-h study was used as an index of insulin resistance.

OGTT
A 75-g OGTT was carried out on all subjects after an overnight fast. Plasma glucose and insulin concentrations were measured at 0, 30, 60, 90, and 120 min after oral glucose ingestion, and the area under the curve of insulin was calculated during the 120-min study period. Blood glucose concentrations were measured by using an enzyme-electrode method (ARKRAY Inc, Kyoto, Japan), and immunoreactive insulin was determined by one-step sandwich enzyme immunoassay (Abbott Japan).

Statistical analyses
All statistical analyses were performed with SPSS version 12.0 software (SPSS Inc, Chicago, IL). All results are shown as means ± SEs. Two groups were compared by using chi-square and Fisher’s exact tests for categorical variables and the Mann–Whitney test for continuous variables. We used repeated analysis of variance measures before Tukey’s test for in-group comparisons of >2 data sets. Correlations between continuous variables were examined by Pearson’s correlation coefficient, and the significance was estimated by linear regression analysis adjusted for age.

RESULTS
Baseline characteristics of the essential-hypertensive subjects are shown in Table 1. Of the 24 hypertensive subjects, 10 were found to be salt-sensitive, whereas 14 were classified as salt-resistant. Although the age was slightly but significantly higher in the salt-sensitive group than in the salt-resistant group, there were no significant differences in BMI, MBP, creatinine clearance, and serum electrolytes. With the normal-salt diet, there were no significant differences in serum electrolytes and urinary sodium between salt-sensitive and salt-resistant hypertensive subjects.

Euglycemic glucose clamp in hypertensive subjects revealed a lower GIR in the salt-sensitive group than in the salt-resistant group (Figure 1: 5.1 ± 0.7 mg·kg⁻¹·min⁻¹ compared with 6.7 ± 0.4 mg·kg⁻¹·min⁻¹, P < 0.01), indicating the presence of relative insulin resistance in salt-sensitive patients. The SSI was inversely related to the GIR in hypertensive subjects.
All subjects were analyzed (\( n = 24 \)). However, age was inversely correlated with the GIR when the salt-sensitive group experienced lower extracellular fluid volume dynamics. The salt-sensitive group first 2 d of high-salt diet were used as indicators of sodium and relative sodium retention with salt sensitivity. There was no significant difference in UNa between the 2 groups after day 3, indicating that a state of sodium balance has been achieved in both groups. When the UNa on day 1 was plotted as a function of the GIR, a positive relation in both groups was observed, but the salt-sensitive subjects had a lower UNa relative to salt-resistant subjects at any GIR value (\( P < 0.01 \); Figure 2).

Hematocrit concentration was measured on day 7 of each diet as an index of change in extracellular fluid volume. The decrease in hematocrit concentration that occurred with the change from low- to high-salt diet was greater in the salt-sensitive than salt-resistant subjects. In addition, whereas the decrease in hematocrit concentration was inversely related to the GIR in both groups, the decrease in hematocrit concentration was always higher in the salt-sensitive subjects than salt-resistant subjects for any given GIR value (\( P < 0.01 \); Figure 3).

On the normal-salt diet, the salt-sensitive group had significantly lower PRA and PAC than the salt-resistant hypertensive group, consistent with the observation that salt sensitivity is

### Table 1

<table>
<thead>
<tr>
<th>Variables</th>
<th>SR (( n = 14 ))</th>
<th>SS (( n = 10 ))</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>50.6 ± 4.7</td>
<td>56.1 ± 5.8</td>
<td>0.039</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>23.3 ± 2.5</td>
<td>23.8 ± 3.7</td>
<td>NS</td>
</tr>
<tr>
<td>MBP (mm Hg)</td>
<td>110.1 ± 5.8</td>
<td>110.3 ± 8.4</td>
<td>NS</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>39.4 ± 1.0</td>
<td>39.3 ± 1.1</td>
<td>NS</td>
</tr>
<tr>
<td>Ccr (L/d)</td>
<td>109 ± 10</td>
<td>108 ± 11</td>
<td>NS</td>
</tr>
<tr>
<td>s-Na (mEq/L)</td>
<td>141 ± 1</td>
<td>142 ± 2</td>
<td>NS</td>
</tr>
<tr>
<td>s-K (mEq/L)</td>
<td>4.2 ± 0.2</td>
<td>4.3 ± 0.3</td>
<td>NS</td>
</tr>
<tr>
<td>s-Cl (mEq/L)</td>
<td>104 ± 1</td>
<td>105 ± 2</td>
<td>NS</td>
</tr>
<tr>
<td>u-Na (mEq/d)</td>
<td>115 ± 6</td>
<td>113 ± 8</td>
<td>NS</td>
</tr>
</tbody>
</table>

\(^{1}\) All values are means ± SEMs. MBP, mean blood pressure; Ccr, creatinine clearance; u-Na, urinary sodium; s-Cl, serum chloride; s-Na, serum sodium; s-K, serum potassium. Statistical significance was estimated with the Mann-Whitney test.

(Figure 2; \( r = -0.78, P < 0.01 \)). Age was not correlated with the GIR in the salt-resistant (\( n = 14; r = -0.44, \text{NS} \)) or salt-sensitive group (\( n = 10; r = -0.062, \text{NS} \)) when analyzed separately. However, age was inversely correlated with the GIR when all subjects were analyzed (\( n = 24; r = -0.46, P < 0.05 \)). There was also a correlation between age and SSI (\( n = 24; r = 0.54, P < 0.01 \)), which is consistent with previous reports (23, 24). However, age did not affect the relation between the SSI and GIR.

The OGTT was performed to test the insulin secretion and blood glucose response in the hypertensive subjects. Compared with the salt-resistant group, the salt-sensitive subjects showed higher serum immunoreactive insulin (IRI) concentrations at 60, 90, and 120 min after glucose ingestion (\( P < 0.01; \) Figure 2). The peak value of IRI occurred at the 30-min mark during the OGTT and returned to baseline in salt-resistant subjects, whereas the IRI concentrations in salt-sensitive subjects achieved a delayed peak at 60 min and remained higher than baseline 120 min after glucose ingestion.

The changes in urinary sodium excretion (\( \Delta \)UNa) during the first 2 d of high-salt diet were used as indicators of sodium and extracellular fluid volume dynamics. The salt-sensitive group experienced lower \( \Delta \)UNa on both day 1 and day 2 of the high-salt period compared with the salt-resistant group, signifying

**Figure 1.** Mean ±SEM glucose infusion rate in salt-resistant (SR) subjects with essential hypertension (\( n = 14 \), white bar) and salt-sensitive (SS) subjects with essential hypertension (\( n = 10 \), black bar). Statistical significance was estimated with the Mann-Whitney test.

**Figure 2.** Relation between the salt sensitivity index and glucose infusion rate in salt-resistant (SR) subjects (\( n = 14 \), white circles) and salt-sensitive (SS) subjects (\( n = 10 \), black circles) with essential hypertension. Statistical significance was estimated with Pearson’s correlation coefficient.

**Figure 3.** Mean ±SEM changes in serum immunoreactive insulin (IRI) after an oral administration of glucose (75 g) in salt-resistant (SR) subjects (\( n = 14 \), white circles) and salt-sensitive (SS) subjects (\( n = 10 \), black circles) with essential hypertension. **\( P < 0.01 \) for SS subjects compared with SR subjects at the same time point (Mann-Whitney test). **\( P < 0.001 \) and *\( P < 0.01 \) for comparisons with 0-min values of the same group (repeated-measures ANOVA and Tukey’s test with \( P < 0.001 \) for interaction).
associated with low-renin hypertension. PRA and PAC were also measured in subjects on the low- and high-salt diets (Table 2). As expected, both PRA and PAC decreased when the diet was changed to high-salt. However, the change from normal- to high-salt diet in PRA and PAC was lesser in salt-sensitive than in salt-resistant subjects (Figure 6). The change in PRA and PAC with the change in salt intake did not show a significant correlation with the GIR (data not shown, P > 0.1).

Urinary norepinephrine (u-NE) decreased less markedly in salt-sensitive subjects than in salt-resistant subjects when salt intake changed from low to high. The change in u-NE was inversely related with the GIR in both salt-sensitive and salt-resistant subjects (Figure 7) (n = 24; r = -0.55, P < 0.01). However, at the lower GIR (<5 mg · kg⁻¹ · min⁻¹), u-NE decreased in only one of the salt-sensitive subjects. Also, at a GIR >5 mg · kg⁻¹ · min⁻¹, only one salt-sensitive subject had a decrease in u-NE which was interspersed with the values observed in the salt-resistant subjects (Figure 7). These data could indicate a greater state of sympathetic activity in the salt-sensitive subjects than in salt-resistant subjects.

DISCUSSION

The results of this study demonstrate that there is a direct relation between insulin resistance as measured by hyperinsulinemic euglycemic glucose clamp technique and salt sensitivity measured by 24-h ambulatory blood pressure monitoring of low- and high-salt diets in lean, essential-hypertensive subjects without diabetes or chronic kidney disease. The salt-sensitive subjects had a significantly lower GIR than the salt-resistant subjects.

The increase in insulin concentrations after oral glucose administration was greater and remained higher than baseline in the salt-sensitive subjects relative to the salt-resistant subjects, although the fasting plasma glucose and insulin concentrations were not significantly different between the salt-sensitive and
salt-resistant subjects. This is consistent with the general consensus that hepatic insulin resistance and fasting hyperinsulinemia are not characteristic of lean subjects because fasting hyperinsulinemia as a result of hepatic insulin resistance is characteristic of obesity (25, 26). It is possible that the postprandial hyperinsulinemia in lean, salt-sensitive, hypertensive subjects contributes to the elevation of blood pressure through the renal salt-retaining effect of insulin.

One of the issues surrounding insulin resistance and salt sensitivity of blood pressure is the question of whether insulin resistance leads to salt sensitivity or vice versa. Although this question cannot be addressed by the current study design, this issue is important in interpreting the results. Fuenmayor et al (27) observed that salt sensitivity was associated with insulin resistance, and a high-salt diet exacerbated insulin resistance only in salt-sensitive subjects. Our current report is limited by the fact that GIR was not assessed in all the different diets. However, by extrapolating the results of Fuenmayor et al (27), we note the pattern of the relation between salt sensitivity of blood pressure and insulin resistance may be similar, regardless of the diet, as long as all subjects are placed on the same diet; and in this case, the interpretation of our results does not change.

The mechanism by which insulin resistance may cause salt-sensitive hypertension remains to be fully elucidated. In this study, the amount of change in urinary sodium as a function of GIR was always lower in the salt-sensitive than in the salt-resistant subjects, whereas the converse was true for the amount of change in hematocrit concentrations with the increase in sodium intake. The SSI was also higher in the salt-sensitive subjects as compared with the salt-resistant subjects. These data suggest that high sodium intake may lead to greater fluid retention and volume expansion in salt-sensitive, insulin-resistant subjects than in the salt-resistant, insulin-sensitive subjects. However, neither cardiac output nor peripheral resistance was measured in this study, and therefore, the measurable effect of this mechanism on blood pressure could not be determined. Fujita et al (28) showed that a high-salt diet caused a greater retention of sodium and an increase in cardiac output in salt-sensitive subjects than in salt-resistant subjects, but they did not find a significant difference in the change in hematocrit concentrations between them. In this study, we found a greater decrease in hematocrit concentrations in salt-sensitive compared with salt-resistant subjects when the diet was changed from low to high sodium. Although we did not assess cardiac output, it seems that the elevation in blood pressure in salt-sensitive subjects on a high-salt diet is at least partially, through volume overload, caused by sodium and water retention. The ability of insulin to increase renal sodium transport persists in states of insulin resistance (14, 15).

Another observation from this study was the differential regulation of the systemic renin-angiotensin aldosterone system in salt-sensitive hypertensive patients. There have been many studies to show that angiotensin II or the activation of renin-angiotensin system induces insulin resistance (29–31). In this study, PRA and PAC were significantly lower in the salt-sensitive than in the salt-resistant subjects on the baseline diet, which is consistent with other studies (19, 32). Manifestation of low renin in salt-sensitive, insulin-resistant subjects may seem contrary to the deleterious effects of the renin-angiotensin system on insulin sensitivity. However, the suppression of the systemic renin-angiotensin system may be a result of a salt-retaining tendency due to an active “local” renin-angiotensin system (33) or deranged dopaminergic-mediated natriuresis (34). In this latter case, the blunted reduction in plasma renin activity on high-salt diet may exacerbate the glucose dysregulation in salt-sensitive patients.

Sympathetic overactivity has been associated with hypertension (35), salt sensitivity (28), and increased insulin concentrations (36). Insulin may directly stimulate the sympathetic nervous system causing an increase in plasma norepinephrine concentrations (37–39). In our study, we attempted to assess the sympathetic nervous activity by measuring urinary norepinephrine. Although considered less reliable than other methods, the assessment of the measurements of urinary norepinephrine may provide a reasonable estimate of renal sympathetic overflow. Attenuated suppression of plasma norepinephrine in the high-salt period among salt-sensitive hypertensive patients has been reported (40). In the current study, there was an attenuated suppression of urinary norepinephrine excretion in salt-sensitive hypertensive subjects on the high-salt diet compared with the salt-resistant hypertensive subjects. Moreover, the majority of salt-sensitive subjects with lower GIRs ($\leq 5 \text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) had no decrease in urinary norepinephrine when placed on the high-salt diet. These findings suggest the presence of sympathetic overactivity in salt-sensitive subjects.

In conclusion, our study shows that salt sensitivity is associated with insulin-resistance, sympathetic hyperactivity, and low plasma renin activity in lean, essential hypertensive patients. However, their causal relations could not be identified in this study.

We thank Hirohide Yokokawa for assistance with the statistical analyses. The authors’ responsibilities were as follows—MSY: drafted the manuscript; JY and MY: assisted in data collection; MO: collected the patient data; TW, RAF, and PAJ: critically reviewed the paper; and HS: performed statistical analyses and supervised the study. The Salt Science Research Foundation did not have any influence on the design, implementation, analysis, or interpretation of the research. The authors declared there were no conflicts of interest associated with this study.
REFERENCES


Erratum


On page 1735, the last sentence of the first paragraph of the section entitled “Classification of subjects according to vitamin B-12 status and folate fractions” contains a detection limit. The published detection limit for the assay is 0.18 nmol/L, not 0.027 nmol/L. The incorrect limit was also given in the footnotes to Table 1 and Table 4. There were 7 eligible subjects with measured serum folic acid concentrations between 0 and 0.18 nmol/L who were included in the group with detectable unmetabolized folic acid. In addition, in Table 4, the multivariate model was not specified for the row labeled “Mean cell volume.” The results in that row were controlled for sex, age, race-ethnicity, current smoking, current alcohol intake, BMI, self-reported cancer history, and serum concentrations of ferritin, cystatin C, and C-reactive protein.


Erratum


In the print version of this article, the term “selenium-methylselenocysteine” appears in error. The correct term, as provided by the authors, is “Se-methylselenocysteine.” This error occurs in Table 1 on page 1486S, in the legend to Figure 1 on page 1487S, and in text on page 1485S. The correct term appears in the online version.


Erratum


In the print version of this article, 2 lines appear for each group represented in Figure 7; however, only one line should appear for each group. The correct version of Figure 7 appears below. The online version of this figure is correct.

FIGURE 7. Relation between glucose infusion rate and change in urinary norepinephrine (Δu-NE) from the low- to high-salt diet in salt-resistant (SR) subjects (n = 14, open circles) and salt-sensitive (SS) subjects (n = 10, filled circles) with essential hypertension. Statistical significance was estimated with Pearson’s correlation coefficient.