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

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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 over activation, and reduced suppression of the renin-angiotensin system may play a role in this relation. Am J Clin Nutr 2010;92:77–82.

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-
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 μIU/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 these hypertensive subjects.
was also a correlation between age and SSI (all subjects were analyzed). However, age was inversely correlated with the GIR when compared with the salt-resistant group, signifying 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 4).

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 5).

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 impaired lower UNa at any GIR value ($P < 0.01$; Figure 4).

### Table 1
Clinical characteristics of salt-sensitive (SS) and salt-resistant (SR) patients on a normal-salt diet

<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/m²)</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 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.
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 (\( \leq 5 \, \text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \)), u-NE decreased in only one of the salt-sensitive subjects. Also, at a GIR > 5 \( \text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \), 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. 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 (\( \leq 5 \, \text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \)), u-NE decreased in only one of the salt-sensitive subjects. Also, at a GIR > 5 \( \text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \), 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.

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with Pearson’s correlation coefficient. Statistical significance was estimated with 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 (≤5 mg · kg⁻¹ · 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.

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