Effect of Salt on Insulin Sensitivity Differs According to Gender and Degree of Salt Sensitivity

Olle Melander, Leif Groop, U. Lennart Hulthén

Abstract—The aim of the present study was to investigate the effect of salt intake on insulin sensitivity and the relation between salt sensitivity and insulin sensitivity in genetically hypertension-prone individuals. Twenty-eight healthy subjects (13 men and 15 women) with a family history of hypertension were examined at baseline, after 1 week of salt restriction (10 mmol/d), and after 1 week of salt loading (240 mmol/d). Insulin sensitivity was measured with the hyperinsulinemic euglycemic clamp after the low- and high-salt diets. Salt sensitivity was defined as the difference in mean arterial blood pressure between the high-salt and the low-salt diets. There was no significant relationship between insulin sensitivity and salt sensitivity after either of the 2 diets. In the men, salt sensitivity was inversely related to plasma renin activity \( r = -0.61, P = 0.03 \) and plasma aldosterone \( r = -0.74, P = 0.004 \), whereas salt sensitivity in women was directly correlated with the salt-induced increase in body weight \( r = 0.68, P = 0.005 \). In men, the high-salt diet induced a change in glucose disposal that was strongly correlated with the degree of salt sensitivity \( r = 0.83, P = 0.0004 \), plasma renin activity \( r = -0.82, P = 0.0006 \), and plasma aldosterone concentrations \( r = -0.87, P = 0.00009 \) (eg, the greater the salt sensitivity and the lower the activity of the renin-angiotensin-aldosterone system, the greater improvement in insulin sensitivity). No such relationships were observed in women. In conclusion, increased salt sensitivity and decreased activity of the renin-angiotensin-aldosterone system predict improved insulin sensitivity with high-salt intake compared with low-salt intake in men, suggesting an interaction among salt intake, salt sensitivity, the renin-angiotensin-aldosterone system, and insulin action. (Hypertension. 2000;35:827-831.)

Key Words: sodium ■ insulin ■ hypertension, genetic ■ renin ■ aldosterone ■ glucose

Raised blood pressure after high-salt intake is referred to as salt sensitivity, and it is a continuous normally distributed trait that is more common in patients with primary hypertension than in normotensive subjects. A greater blood pressure increase over time has been found in salt-sensitive compared with salt-resistant subjects. Salt sensitivity may therefore be a risk factor for primary hypertension.

A large proportion of patients with primary hypertension are characterized by insulin resistance, which is suggested to be the cause of a cluster of cardiovascular risk factors called “the metabolic syndrome.” In addition to disturbances in glucose metabolism and hypertension, this syndrome includes dyslipidemia, abdominal obesity, and microalbuminuria. Both salt sensitivity and insulin resistance are associated with a positive family history of hypertension and may therefore be part of the inherited predisposition to primary hypertension. Earlier studies have suggested that normotensive and hypertensive salt-sensitive subjects are hyperinsulinemic, insulin resistant, or both compared with salt-resistant subjects, suggesting that salt sensitivity and insulin sensitivity may be related. A change in salt intake may thus have different effects on insulin sensitivity in salt-sensitive compared with salt-resistant individuals. Studies on the effect of salt intake on serum insulin concentrations have provided conflicting results. However, serum insulin level is only a rough measure of insulin sensitivity. The influence of salt on insulin sensitivity has also been assessed through measurement of insulin sensitivity with the hyperinsulinemic euglycemic clamp or the insulin suppression test. High- compared with low-salt intake has been reported either to have no effect or to decrease insulin sensitivity in nondiabetic subjects. However, the effects of salt on insulin sensitivity were not analyzed with regard to the continuous distribution of salt sensitivity. Furthermore, varying cutoff points have been used in different studies to define salt sensitivity. With this notion, there is no consensus on whether a high-salt diet really influences insulin sensitivity. This information, however, may be very important for dietary guidelines for persons at risk for the metabolic syndrome and cardiovascular disease. Furthermore, studies on the interplay among dietary salt, salt sensitivity, the renin-angiotensin-aldosterone system (RAAS), and insulin sensitivity may help to provide new insights into the pathogenesis of primary hypertension. The present study was undertaken to investigate whether high-salt versus low-salt intake influences insulin sensitivity and whether salt sensi-

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tivity and insulin sensitivity are related in genetically hypertension-prone individuals.

**Methods**

**Subjects**

The protocol of the present study was approved by the Ethics Committee of Lund University (Lund, Sweden), and all participants gave their informed consent. The procedures were in accordance with institutional guidelines.

Twenty-eight unrelated subjects (13 men and 15 women, mean age 48.6 ± 6.6 years, body mass index 26.9 ± 3.7 kg/m²) with at least 1 first-degree relative with primary hypertension were recruited from an ongoing collection of families with a high frequency of primary hypertension in the Scania region of southern Sweden. There was no significant difference between men and women with respect to age (46.7 ± 6.0 versus 50.3 ± 6.9 years, P = 0.15) or body mass index (27.4 ± 3.4 versus 26.4 ± 3.9 kg/m², P = 0.52). None of the subjects received any medication or had ever received antihypertensive treatment, nor did they have diabetes mellitus, kidney disease, or any other chronic disease. All except 3 women were postmenopausal. The premenopausal women were examined while in the follicular phase of the menstrual cycle.

**Procedures**

All subjects were investigated at baseline and after 1 and 2 weeks. The procedures were in accordance with institutional guidelines. The subjects in the supine position after 30 minutes of rest at 4-minute intervals during 40 minutes with an automatic oscillometric device (DINAMAP 1846 SX; Critikon), and the mean value of the 10 measurements was used. The difference in mean arterial blood pressure (the diastolic blood pressure plus one third of the pulse pressure) after the high-salt diet compared with that after the low-salt diet was defined as the degree of salt sensitivity. Because salt sensitivity is normally distributed in the population, and cutoff points for dichotomization of the trait are arbitrary and differ among studies, we regarded salt sensitivity as a continuous variable. After the blood pressure measurements, fasting blood samples were drawn with the patients in the supine position. Urine samples (24-hour samples) were collected before the baseline investigation and at the end of the high- and low-salt diet weeks. Insulin sensitivity was measured with a 2-hour hyperinsulinemic euglycemic clamp at the end of the 2 diet periods. Insulin (Actrapid; Novo Nordisk) was infused with an infusion pump (Perfusor Secura FT; Braun) at a rate of 45 mU/m² body surface area per minute. Blood glucose was analyzed every 5 minutes, and a constant infusion of a 20% glucose solution was adjusted to keep blood glucose constant at 5.0 mmol/L. The glucose disposal rate was calculated as the total amount of glucose infused during the second hour of the clamp (in μmol·kg⁻¹·min⁻¹).

**Biochemical Assays**

Serum and urine concentrations of sodium and potassium were measured with standard biochemical methods. The serum insulin concentrations were measured with a specific ELISA (DAKO), and free fatty acids were measured with an enzymatic colorimetric method (Wako Chemicals). Plasma renin activity (PRA) and plasma aldosterone concentrations (PAC) were measured with RIA diagnostic kits (Abbott Laboratories), and urinary catecholamines were measured with a fluorimetric method.

**Statistical Analysis**

An NCSS statistical software (version 6.0.21; Statistical Solutions Limited) was used for the statistical analyses. Data are expressed as mean ± SD. Differences between groupwise and paired variables were compared with the use of unpaired and paired t tests or with Mann-Whitney and Wilcoxon’s paired rank tests, where appropriate.

**Clinical Characteristics of Study Subjects on Different Salt Diets**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>Low-Salt Diet</th>
<th>High-Salt Diet</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>134 ± 13</td>
<td>123 ± 10</td>
<td>136 ± 18</td>
<td>0.0002</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>83.6 ± 7.5</td>
<td>77.1 ± 7.1</td>
<td>82.0 ± 8.0</td>
<td>0.001</td>
</tr>
<tr>
<td>Mean arterial blood pressure, mm Hg</td>
<td>100.5 ± 8.6</td>
<td>92.5 ± 7.5</td>
<td>99.9 ± 10.8</td>
<td>0.0001</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>60.2 ± 6.0</td>
<td>60.0 ± 5.6</td>
<td>55.8 ± 5.4</td>
<td>0.00009</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>80.5 ± 13.2</td>
<td>78.5 ± 12.5</td>
<td>79.4 ± 12.1</td>
<td>0.0002</td>
</tr>
<tr>
<td>Serum albumin, g/L</td>
<td>40.8 ± 2.3</td>
<td>41.9 ± 2.5</td>
<td>39.0 ± 2.0</td>
<td>0.00005</td>
</tr>
<tr>
<td>Serum sodium, mmol/L</td>
<td>141 ± 2</td>
<td>140 ± 2</td>
<td>141 ± 1</td>
<td>0.006</td>
</tr>
<tr>
<td>Serum potassium, mmol/L</td>
<td>4.2 ± 0.2</td>
<td>4.1 ± 0.2</td>
<td>4.0 ± 0.2</td>
<td>0.02</td>
</tr>
<tr>
<td>Urine sodium excretion, mmol/24 h</td>
<td>161 ± 72</td>
<td>9.9 ± 4.7</td>
<td>227 ± 59</td>
<td>0.000004</td>
</tr>
<tr>
<td>Urine potassium excretion, mmol/24 h</td>
<td>69.4 ± 27.3</td>
<td>52.6 ± 16.6</td>
<td>49.7 ± 14.6</td>
<td>0.41</td>
</tr>
<tr>
<td>PRA, μg·L⁻¹·h⁻¹</td>
<td>0.7 ± 0.6</td>
<td>2.3 ± 1.4</td>
<td>0.2 ± 0.2</td>
<td>0.000004</td>
</tr>
<tr>
<td>PAC, pmol/L</td>
<td>89 ± 40</td>
<td>257 ± 156</td>
<td>51 ± 25</td>
<td>0.000004</td>
</tr>
<tr>
<td>Urine norepinephrine excretion, nmol/24 h</td>
<td>246 ± 103</td>
<td>275 ± 106</td>
<td>168 ± 57</td>
<td>0.000006</td>
</tr>
<tr>
<td>Urine epinephrine excretion, nmol/24 h</td>
<td>26.6 ± 19.6</td>
<td>29.4 ± 19.1</td>
<td>24.6 ± 15.9</td>
<td>0.10</td>
</tr>
<tr>
<td>Glucose disposal rate, μmol·kg⁻¹·min⁻¹</td>
<td>...</td>
<td>42.4 ± 11.6</td>
<td>44.6 ± 12.7</td>
<td>0.10</td>
</tr>
<tr>
<td>Fasting serum insulin, pmol/L</td>
<td>30.6 ± 18.6</td>
<td>30.0 ± 16.6</td>
<td>18.9 ± 14.6</td>
<td>0.002</td>
</tr>
<tr>
<td>Fasting blood glucose, mmol/L</td>
<td>4.8 ± 0.8</td>
<td>4.8 ± 0.3</td>
<td>4.6 ± 0.3</td>
<td>0.0008</td>
</tr>
<tr>
<td>Serum free fatty acids, mmol/L</td>
<td>0.50 ± 0.20</td>
<td>0.52 ± 0.15</td>
<td>0.51 ± 0.17</td>
<td>0.83</td>
</tr>
</tbody>
</table>

Data are means ± SD, n = 28. P values refer to differences between low- and high-salt diets.
Relationships among salt sensitivity, PRA, PAC, and metabolic variables were determined with Pearson’s correlation coefficient if the residuals were normally distributed and with Spearman’s correlations otherwise. All probability values were calculated with 2-sided tests, and a level of <0.05 was considered statistically significant.

Results

The 24-hour urinary sodium excretions on the low- and high-salt diets showed good dietary compliance (Table). Blood pressure, body weight, and serum sodium concentrations increased, whereas PRA, PAC, heart rate, urinary norepinephrine excretion, and serum albumin and potassium concentrations decreased on the high-salt diet compared with the low-salt diet (Table). Mean arterial blood pressure at baseline did not differ significantly from that after the high-salt diet (P=0.71) (Table). At the baseline examination, 2 men (diastolic blood pressure range 93 to 97 mm Hg) and 3 women (diastolic blood pressure range 91 to 100 mm Hg) had a diastolic blood pressure of >90 mm Hg. After the high-salt diet, 2 women (diastolic blood pressure range 91 to 102 mm Hg) and 1 man (diastolic blood pressure 91 mm Hg) had a diastolic blood pressure of >90 mm Hg, whereas no subject’s diastolic blood pressure exceeded 90 mm Hg after the low-salt diet.

Body weight at baseline was related to insulin sensitivity only after the low-salt diet (r = −0.49, P=0.008), whereas body mass index at baseline was inversely related to insulin sensitivity after both the low- (r = −0.62, P=0.0005) and the high- (r = −0.51, P=0.006) salt diets. Neither body weight, body mass index, nor mean blood pressure at baseline was related to salt sensitivity or to the salt-induced change in insulin sensitivity (data not shown). Fasting insulin and blood glucose concentrations decreased after the high-salt diet compared with the low-salt diet, but the glucose disposal rate was not significantly changed in the group as a whole (Table). The glucose disposal rate was not related to the degree of salt sensitivity after either the low- (r = 0.009, P=0.96) or the high- (r = 0.21, P=0.27) salt diet.

In men, the high-salt intake induced a change in glucose disposal rate that was closely correlated with the degree of salt sensitivity (r = 0.83, P=0.0004) (ie, the more salt sensitive the men were, the more improved was insulin sensitivity, whereas all of the salt-resistant men [those in whom mean arterial blood pressure decreased from the low- to the high-salt diet] had an impaired insulin sensitivity; Figure 1A). In the women, no such relationship was seen (r = 0.18, P=0.53). The change in glucose disposal rate induced by the high-salt diet in men was strongly inversely related to the baseline PRA (r = −0.82, P=0.0006) (Figure 1B) and PAC (r = −0.87, P=0.0009) (Figure 1C) in the men, whereas this was not seen in women (r = −0.14, P=0.62; r = 0.09, P=0.76, respectively). In men, salt sensitivity correlated inversely with PRA at baseline (r = −0.61, P=0.03), with PAC after the low-salt diet (r = −0.88, P=0.00006), and with the reduction in PRA from low- to high-salt intake (r = −0.86, P=0.0002). No such correlations were seen in the women (r = −0.35, P=0.20; r = −0.33, P=0.24; r = −0.27, P=0.33; respectively). Consequently, in the men, salt sensitivity was inversely related to PAC at baseline (r = −0.74, P=0.004), to PAC after low-salt intake (r = −0.63, P=0.02), and to the reduction in PAC from low- to high-salt intake (r = −0.59, P=0.03). Again, no such relations could be found in the women (r = 0.24, P=0.38; r = −0.06, P=0.83; r = −0.01, P=0.97; respectively). In the women, salt sensitivity was directly correlated with the salt-induced increase in body weight (r = 0.68, P=0.005). This relationship was not observed in the men (r = 0.23, P=0.44). The salt-induced increase in body weight was not significantly related to the change in insulin sensitivity from low- to high-salt intake in either gender (r = 0.30, P=0.28 for women; r = 0.48, P=0.09 for men). The difference in urinary norepinephrine excretion after high-salt intake compared with low-salt intake (Table) was not related to the difference in insulin sensitivity between the same time points in either men (r = 0.09, P=0.77) or women (r = −0.32, P=0.24).

Discussion

In contrast to the results of some previous studies,11–13 we found no significant relationship between insulin sensitivity and salt sensitivity. However, a close relationship between
the effect of salt on insulin sensitivity and the degree of salt sensitivity was observed in men (Figure 1A). Both salt sensitivity and the salt-induced change in insulin sensitivity (Figures 1B and 1C) were closely related to RAAS activity in the men but not in the women. This suggests that a high-salt intake may have beneficial effects on insulin sensitivity in salt-sensitive men but no effect or unfavorable effects in salt-resistant men. The varying effect of salt on insulin sensitivity in men may be coupled to differences in the activity of RAAS. All subjects received the 2 diets in the same order. However, because insulin sensitivity did not change in similar directions in the group of men as a whole but instead differed according to the degree of salt sensitivity and RAAS activity, this finding is unlikely to be an order effect. Of note, the salt-induced change in insulin sensitivity refers to the change in insulin sensitivity from the end of the low-salt diet to the end of the high-salt diet and not to baseline values.

In contrast to the men, salt sensitivity in the women was directly related to body weight gain after salt loading and not to the activity of RAAS. This suggests that the mechanisms leading to salt sensitivity are different in men and women and may explain why the relationship between salt-induced changes in insulin sensitivity and salt sensitivity was restricted to men. No differences existed in age or body mass index between men and women. Furthermore, because the majority of women were postmenopausal, differences in estrogen status are unlikely to explain the gender differences.

The finding of raised urinary norepinephrine excretion or plasma norepinephrine concentrations after salt restriction compared with after salt loading has been described in several earlier studies, suggesting activation of the sympathetic nervous system by the relative hypovolemia induced with salt restriction.

Previous studies that investigated the effect of salt on insulin sensitivity measured with the hyperinsulinemic euglycemic clamp or the insulin suppression test in nondiabetics found either no effect or an impairment of insulin sensitivity after 4 to 7 days of salt loading compared with salt restriction. However, these reports did not relate the change in insulin sensitivity to the continuous distribution of salt sensitivity. In 1 study with mainly salt-resistant men (normotensive with no family history of hypertension), impaired insulin sensitivity was seen after high-salt intake. This is in accordance with the results in our male subjects, although we did not observe any relation between the change in free fatty acids and change in insulin sensitivity after the different salt diets (r=0.16, P=0.61), as reported in the previous study. Three studies used different cutoff levels for salt sensitivity and compared the effect of salt on insulin sensitivity between salt-sensitive and salt-resistant subjects. In 2 studies, no change in insulin sensitivity was seen whereas in a recent study by Fuenmayor et al, insulin sensitivity was impaired after high-salt intake in salt-sensitive subjects. This is clearly at variance with our findings; there may be several possible explanations for this discrepancy. Earlier studies that dichotomized salt sensitivity used different cutoff levels and the definition of the trait is therefore rather arbitrary. Instead, we chose to regard salt sensitivity as a continuous variable. This has the advantage of no assumptions and also allowed us to use the quantitative information. Genetic and environmental differences between the populations studied may also influence the results, as well as differences between the salt doses used and the methods used to measure insulin sensitivity. Our study also suggests gender-specific effects of salt loading on insulin sensitivity, and the gender distribution was not reported in the study by Fuenmayor et al.

The association between salt sensitivity and suppression of RAAS activity is described in several earlier studies. We also observed a close relationship between the salt-induced change in insulin sensitivity in men and PRA (Figure, 1B) and PAC (Figure, 1C) at baseline. These data suggest that different baseline activities of RAAS contribute to the varying effects of salt intake on insulin sensitivity in salt-sensitive and salt-resistant men. Evidence is emerging suggesting a cross-talk between RAAS and the insulin signaling system. Angiotensin II (Ang II) has gluconeogenic and glycogenolytic properties in hepatocytes and it inhibits insulin signaling in both heart muscle and aortic smooth muscle. These effects could be blocked by Ang II receptor antagonists. Both salt itself and RAAS activity have been shown to influence the expression of the Ang II receptor type 1 (AT1); high-salt and low-RAAS activity upregulate and low-salt and high-RAAS activity downregulate AT1 expression. The suppression of RAAS at baseline that we observed in salt-sensitive men was gradually alleviated with increasing salt resistance. Salt-sensitive men may therefore overexpress AT1, whereas salt-resistant men may show relatively low AT1 expression at baseline. Salt restriction activates RAAS in all subjects, leading to increased Ang II levels. This increase could theoretically lead to exaggerated effects in the salt-sensitive men due to AT1 overexpression at baseline, possibly including insulin resistance. In contrast, the suppression of RAAS and Ang II after salt loading may remove this inhibitory effect of Ang II on insulin sensitivity, which is observed as an improvement in insulin sensitivity in salt-sensitive men when changing from a low- to a high-salt intake. However, this hypothesis includes the assumption that downregulation of AT1 after 1 week of salt restriction is not able to fully compensate for the postulated AT1 overexpression at baseline in salt-sensitive men. Indeed, changes in salt concentrations have been shown to influence AT1 expression after 24 hours in vitro. On the other hand, the in vivo changes in AT1 expression in humans after changes in salt balance may be slower than those in vitro and could also be attenuated by other systems. Importantly, the low basal PRA and PAC values in salt-sensitive men reflect chronic RAAS suppression and AT1 overexpression. It could therefore be speculated that AT1 expression in salt-sensitive men is resistant to acute changes in salt intake and RAAS activity. In salt-resistant men, insulin sensitivity would be relatively resistant to changes in RAAS activity because they may have a lower expression of AT1 at baseline because of the high activity of RAAS.

In conclusion, the effect of salt intake on insulin sensitivity depends on the degree of salt sensitivity in genetically hypertension-prone men. With high- versus low-salt intake,
insulin sensitivity gradually improved with the increased degree of salt intake and decreased RAAS activity. This suggests an interaction among salt intake, salt sensitivity, RAAS, and insulin action in men.

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References
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