No Evidence of a Relation Between 11β-hydroxysteroid Dehydrogenase Type 2 Activity and Salt Sensitivity

Olle Melander, Erik Frandsen, Leif Groop, and U. Lennart Hulthén

**Background:** The ratio of urinary concentrations of tetrahydrocortisol plus allotetrahydrocortisol to tetrahydrocortisone \([\text{THF} + \text{ATHF}] / \text{THE}\) reflects the activity of the enzyme 11β-hydroxysteroid dehydrogenase type 2 (11BHSD2), which converts cortisol to cortisone in the kidney and thereby protects the mineralocorticoid receptor from the mineralocorticoid action of cortisol. The aim of the present study was to investigate whether 11BHSD2 activity is affected by salt intake and if it is related to salt sensitivity.

**Methods:** Concentrations of THF, ATHF, and THE in 24-h urine collections was determined by gas chromatography at baseline, after 1 week on a low salt diet (10 mmol/d), and after another week on a high salt diet (240 mmol/d) in 29 healthy subjects with heredity for hypertension. Salt sensitivity was defined as the difference between mean arterial blood pressure (BP) after the high salt diet and mean arterial BP after the low salt diet.

**Results:** The high salt diet increased \([\text{THF} + \text{ATHF}] / \text{THE}\) by 5.1% ± 9.4% \(P = .009\) when compared to the low salt diet. The salt-induced change of \([\text{THF} + \text{ATHF}] / \text{THE}\) was not related to salt sensitivity. There was no correlation between salt sensitivity and \([\text{THF} + \text{ATHF}] / \text{THE}\) at baseline \((r = -0.18, P = .34)\), whereas salt sensitivity was inversely related to \([\text{THF} + \text{ATHF}] / \text{THE}\) after the low \((r = -0.38, P = .05)\) and after the high \((r = -0.39, P = .04)\) salt diets.

**Conclusions:** We found no support for an association between enhanced salt sensitivity and reduced 11BHSD2 activity.

**Key Words:** Salt sensitivity, 11β-hydroxysteroid dehydrogenase type 2, cortisol and hypertension, primary hypertension.

Primary hypertension is caused by a complex interaction between genetic and environmental factors. A high salt intake is one of its most important environmental risk factors and dietary salt reduction lowers blood pressure (BP). However, the individual BP response to a salt load varies among individuals and has a Gaussian distribution in the population. Patients with primary hypertension are more salt sensitive (eg, they respond with a greater increase in BP after a salt load) compared to normotensive subjects. Subjects classified as salt sensitive display a greater increase in BP over time when compared to subjects classified as salt resistant, and normotensive subjects with a family history of hypertension are more salt sensitive than normotensive subjects without such a family history. Therefore, a high salt sensitivity may be a risk factor for the development of primary hypertension.

Cortisol circulates in the bloodstream in much higher concentrations than aldosterone and the two hormones bind with the same affinity to the mineralocorticoid receptor in vitro. However, the enzyme 11β-hydroxysteroid dehydrogenase type 2 (11BHSD2) normally protects the mineralocorticoid receptor from the mineralocorticoid actions of cortisol by converting cortisol to cortisone, which has a low affinity for the mineralocorticoid receptor, allowing aldosterone to be the major mineralocorticoid in vivo. Loss of function mutations in the human 11BHSD2 gene is the cause of the syndrome of apparent mineralocorticoid excess (AME), a monogenic form of hypertension. The reduced activity of the mutated 11BHSD2 in
AME allows cortisol to stimulate the mineralocorticoid receptor in the kidney, resulting in early onset of hypertension, hypokalemic alkalosis, suppression of the renin-angiotensin-aldosterone system, and an elevated ratio of urinary concentrations of [tetrahydrocortisol (THF) + allo-tetrahydrocortisol (ATHF)]/tetrahydrocortisone (THE).\textsuperscript{10}

The active component of licorice, glycyrrhetinic acid, potently inhibits 11BHS2D2 and therefore, licorice consumption can lead to a similar clinical picture as seen in patients with AME.\textsuperscript{11} Because only 50 to 100 g of licorice per day is enough to cause a significant elevation in BP,\textsuperscript{12} licorice-induced inhibition of 11BHS2D2 may be a more common cause of increased salt sensitivity and hypertension in the population than thought thus far.

A subset of patients with primary hypertension are characterized by prolonged half-life of cortisol and respond to dexamethasone therapy,\textsuperscript{13,14} suggesting that reduced 11BHS2D2 activity could be an important pathophysiological factor in primary hypertension. Accordingly, Lovati et al.\textsuperscript{15} reported that in healthy subjects the (THF + ATHF)/THE ratio, measured during low salt conditions, was directly correlated with degree of salt sensitivity, indicating that high salt sensitivity may be a consequence of reduced 11BHS2D2 activity. Whether salt intake influences 11BHS2D2 activity has not been studied.

The aim of the present study was to examine whether salt intake influences 11BHS2D2 activity and whether salt sensitivity is related to 11BHS2D2 activity at different levels of salt intake in healthy subjects with a positive family history of hypertension. As a measure of 11BHS2D2 activity we used the ratio of cortisol to cortisone metabolites in urine [(THF + ATHF)/THE].

### Methods

The protocol of the study was approved by the ethics committee of Lund University and all participants gave their informed consent. The procedures were in accordance with institutional guidelines.

### Study Population

Twenty-nine unrelated subjects (13 men and 16 women, aged 48.3 ± 6.7 years with a body mass index of 26.9 ± 3.6 kg/m\textsuperscript{2}) with at least one first degree relative with primary hypertension, were studied. The relation between salt sensitivity and natriuretic peptides has been reported previously in these subjects (n = 30),\textsuperscript{16} but urine for analysis of cortisol and cortisone metabolites was lacking for one subject. None of the subjects had hypertension. The baseline characteristics of the subjects are shown in Table 1. None of them received any medication or had ever been on antihypertensive treatment, neither did they have diabetes mellitus, kidney disease, or any other chronic disease. Of the women, all but three were postmenopausal. The premenopausal women were all examined between days 5 to 10 of the menstrual cycle (follicular phase).

### Procedures

All subjects were investigated at baseline, after 1 and 2 weeks. After the baseline investigation, the study subjects were put on a low salt diet (10 mmol of sodium and 70 mmol of potassium per day) for 1 week. During the second week sodium chloride capsules (230 mmol/d) were added to the diet to give a high salt intake of 240 mmol/d. The diet was composed by a dietitian and the daily energy intake was adjusted according to body weight and sex (8400 to 11,760 kJ). It did not contain licorice or any other ingredients known to affect 11BHS2D2 activity. The study subjects received all meals and drinks from a metabolic ward and were not allowed to consume anything else. Blood pressure was measured in the morning in the fasting state in supine position after 30 min of rest at 4-min intervals during 40 min with an automatic oscillometric device (DINMAP 1846 SX, Critikon, Tampa, FL), and the mean value of the 10 measurements was used. Salt sensitivity was defined as the difference between the mean arterial BP (the diastolic BP plus one-third of the pulse

### Table 1. Characteristics of study subjects (n = 29) when 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>135 ± 12</td>
<td>124 ± 10</td>
<td>136 ± 18</td>
<td>&lt;.00001</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>83.7 ± 7.2</td>
<td>77.2 ± 6.9</td>
<td>82.1 ± 7.8</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Mean arterial blood pressure (mm Hg)</td>
<td>101 ± 8.2</td>
<td>92.7 ± 7.3</td>
<td>100 ± 11</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>61.0 ± 6.6</td>
<td>60.7 ± 6.6</td>
<td>57.0 ± 7.0</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>80.5 ± 13</td>
<td>78.5 ± 12</td>
<td>79.2 ± 12</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Serum sodium (mmol/L)</td>
<td>141 ± 1.6</td>
<td>140 ± 1.8</td>
<td>141 ± 1.3</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>Serum potassium (mmol/L)</td>
<td>4.2 ± 0.2</td>
<td>4.1 ± 0.3</td>
<td>4.0 ± 0.2</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>Urine sodium excretion (mmol/24 h)</td>
<td>159 ± 72</td>
<td>10.1 ± 5.7</td>
<td>223 ± 63</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Urine potassium excretion (mmol/24 h)</td>
<td>68.4 ± 27</td>
<td>52.2 ± 17</td>
<td>49.3 ± 15</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma renin activity (µg · L\textsuperscript{-1} · h\textsuperscript{-1})</td>
<td>0.70 ± 0.6</td>
<td>2.06 ± 1.2</td>
<td>0.24 ± 0.24</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Plasma aldosterone (pmol/L)</td>
<td>92.1 ± 40</td>
<td>246 ± 147</td>
<td>54.1 ± 25</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>(THF + ATHF)/THE</td>
<td>0.91 ± 0.24</td>
<td>0.84 ± 0.19</td>
<td>0.90 ± 0.22</td>
<td>&lt;.01</td>
</tr>
</tbody>
</table>

Data are ± SD. P values refer to the change in the variable on the high versus the low salt diet. (THF + ATHF)/THE: ratio of urinary concentration of (tetrahydrocortisol + allo-tetrahydrocortisol)/tetrahydrocortisone.
pressure) after the high and after the low salt diet. After the BP measurements, fasting blood samples were drawn with the patients in the supine position. Urine samples (24 h) were collected before the baseline investigation and at the end of the low and high salt diet periods.

Biochemical Assays

Urinary THF, ATHF, and THE were measured using gas chromatographic (GC) separation of derivatized metabolites. Before derivatization and GC application, urine samples were concentrated by solid phase extraction using Sep-pak C18 cartridges and hydrolyzed using Helix pomatia enzyme preparations. The procedure follows the method described by Shackleton and Whitney. Serum and urine concentrations of sodium and potassium were measured by standard biochemical methods. Plasma renin activity (PRA) and plasma aldosterone concentrations (PAC) were measured with RIA diagnostic kits (Abbot laboratories, Wiesbaden, Germany).

Statistics

Data are expressed as mean ± SD. Because salt sensitivity is normally distributed in the population, we regarded it as a continuous variable. The significance of differences between paired variables was tested with paired t test or Wilcoxon’s signed rank test, where appropriate. The salt-induced intraindividual changes of the (THF + ATHF)/THE ratio were normally distributed and to calculate whether the percent change was significantly different from zero one-sample t test was used. Correlations were determined using Pearson’s correlation coefficient if the residuals were normally distributed, otherwise Spearman’s correlations were used. For all these analyses, an NCSS statistical software (version 6.021, Statistical solutions limited, Cork, Ireland) was used. The power of our study sample to detect the correlation between (THF + ATHF)/THE and salt sensitivity reported by Lovati et al15 was estimated with the SOLO Power Analysis program (Biomedical Data Processing, Los Angeles, CA). With our study sample (n = 29) we had >95% power to detect the correlation between salt sensitivity and the (THF + ATHF)/THE ratio previously described by Lovati et al.15 All P values were calculated from two-sided tests and a level of < .05 was considered statistically significant.

Results

The 24-h urinary sodium excretion during the low and high salt diets indicated good dietary compliance (Table 1). Blood pressure, body weight, and serum sodium concentrations increased, whereas PRA, PAC, heart rate, and serum potassium decreased significantly on the high compared with the low salt diet (Table 1). The mean salt sensitivity was 7.2 mm Hg (95% confidence interval 3.5–10.9). Salt sensitivity was inversely related to PRA at baseline (r = −0.54, P = .002) and after the low salt diet (r = −0.61, P = .0005). After the high salt diet, when PRA was suppressed (Table 1), the inverse relationship between salt sensitivity and PRA was of borderline significance (r = −0.36, P = .06). Salt sensitivity was inversely related to the salt induced change in PRA (PRA after low salt – PRA after high salt) (r = −0.54, P = .002).

The (THF + ATHF)/THE ratio was significantly higher after the high than after the low salt diet (Table 1). The percent change in the (THF + ATHF)/THE ratio when moving from the low to the high salt diet was 5.1% ± 9.4% (P = .009). The salt-induced change in the (THF + ATHF)/THE ratio was not correlated with salt sensitivity (r = −0.26; P = .19).

There was no correlation between salt sensitivity and the (THF + ATHF)/THE ratio at baseline (r = −0.18; P = .34), whereas salt sensitivity was inversely related to the (THF + ATHF)/THE ratio after the low (r = −0.38; P = .05) and after the high (r = −0.39; P = .04) salt diets.

Exclusion of the three premenopausal women from the analyses did not change the results. As before, moving from the low to the high salt diet increased the (THF + ATHF)/THE ratio with 5.1% ± 9.9% (P = .02). The salt-induced change in (THF + ATHF)/THE was not related to salt sensitivity (r = −0.27, P = .21). There was no correlation between salt sensitivity and (THF + ATHF)/THE at baseline (r = −0.17, P = .41), whereas salt sensitivity was inversely related to (THF + ATHF)/THE after the low (r = −0.45, P = .03) and after the high (r = −0.39, P = .05) salt diets.

Discussion

We found that high versus low salt intake induced a slight increase in the (THF + ATHF)/THE ratio suggesting that a high salt intake reduces 11BHSD2 activity. However, the inhibitory effect of salt on 11BHSD2 was relatively small and its importance for salt-induced BP elevation (ie, the degree of salt sensitivity) seems to be minor. In comparison, the moderate dose of 100 g of nonsalted licorice per day, a known inhibitor of 11BHSD2, increases the (THF + ATHF)/THE ratio by about 55% and systolic BP by about 6.5 mm Hg, whereas the mean salt-induced increase in the (THF + ATHF)/THE ratio in the present study was 5.1% and the increase in systolic BP was 12 mm Hg. In addition, there was no significant correlation between the degree of salt sensitivity and the salt-induced change in (THF + ATHF)/THE ratio. Thus, most of the observed BP elevation after increased salt intake has occurred independently of the salt-induced inhibition of 11BHSD2. However, as the range of the salt-induced change in the (THF + ATHF)/THE ratio was quite wide (−19.0 to +20.5%), it cannot be excluded that the level of salt intake affects the relationship between salt sensitivity and 11BHSD2 activity. Recently, Lovati et al15 reported that the (THF + ATHF)/THE ratio, measured under low salt conditions, was directly correlated with the degree of

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salt sensitivity in normotensive German subjects, suggesting that the lower the 11BHS2D activity, the greater is the degree of salt sensitivity. In contrast, we found a weak inverse relationship between salt sensitivity and the (THF + ATHF)/THE ratio both during high and low salt intake. Therefore, our data argues against reduced 11BHS2D activity as an important factor for enhanced salt sensitivity.

The reason for the discrepancy between the two studies is unclear. We had >95% power to detect the effect reported by Lovati et al.15 The dietary protocols used to determine salt sensitivity were comparable with only slight differences in the amount of salt ingested during the low and high salt periods (10 and 240 mmol/d in our study versus 20 and 220 mmol/d). Both studies were performed in healthy whites. The main differences between the two study populations were that our subjects were older, had higher BP, and all had a positive family history of hypertension. These are all factors that are associated with greater degree of salt sensitivity.4,6 Thus, one potential explanation for the divergent results could be that our subjects were more salt sensitive and that the mechanism proposed by Lovati et al15 only operates in the lower range of salt sensitivity. However, this is unlikely as the range of salt sensitivity in our study (–6.5 to +44.2 mm Hg) had a major overlap with that in the previous study. Still, it should be emphasized that the present study included only 29 subjects. Although our data argue against reduced 11BHS2D activity as a common cause of enhanced salt sensitivity, it cannot be excluded that reduced 11BHS2D activity is of importance for salt sensitivity in certain groups of the population.

We defined salt sensitivity as the difference between mean arterial BP after the high and the low salt diet. Other definitions of salt sensitivity, such as the salt-induced change in PRA, have also been suggested. In the present study, salt sensitivity was inversely related to PRA and to the salt-induced change in PRA suggesting that salt-sensitive subjects have low PRA and therefore, may have a reduced capacity to suppress PRA further during salt loaded conditions.

Three of the women in our study were premenopausal and differences in estrogen and progesterone concentrations during the menstrual cycle may affect sodium balance. Although we tried to overcome this potential problem by examining all of these three women during days 5 to 10 of the menstrual cycle (follicular phase), it cannot be excluded that the hormonal status of the women would affect the relation between salt intake, salt sensitivity, and (THF + ATHF)/THE ratio. However, exclusion of the three premenopausal women from the analyses did not affect the results.

Importantly, it has been shown that the ratio between urinary free cortisol (UFF) and urinary free cortisone (UFE) appears to be a more sensitive index of 11BHS2D activity than the conventional (THF + ATHF)/THE ratio.18 It is possible that our results would have been modified if the UFF/UFE ratio had been used instead of the (THF + ATHF)/THE ratio. However, the (THF + ATHF)/THE ratio is increased in all known situations of reduced 11BHS2D activity,10,12 and Lovati et al15 used the (THF + ATHF)/THE ratio in their study. Therefore, despite the limitations of the (THF + ATHF)/THE ratio as a measure of 11BHS2D activity, we believe our finding of an inverse relationship between salt sensitivity and the (THF + ATHF)/THE ratio argues against reduced 11BHS2D activity as an important factor in salt sensitivity. However, it is more uncertain whether the small increase in the (THF + ATHF)/THE ratio on high versus low salt intake really reflects a true reduction in 11BHS2D activity. In any case this change does not seem to be of any physiologic importance as it did not correlate with BP changes.

No study other than the present one and the one by Lovati et al15 has investigated the relation between salt intake, salt sensitivity, and the (THF + ATHF)/THE ratio. However, Litchfield et al19 reported a significant salt-induced increase in UFF as well as inverse relationships between salt sensitivity and UFF, both during low and high salt conditions, in a mixed normotensive and hypertensive population. These results are in accordance with our data, as decreased 11BHS2D activity would be expected to result in increased UFF, and vice-versa.

In conclusion, salt sensitivity was inversely related to the (THF + ATHF)/THE ratio measured under low and high salt conditions. This argues against the hypothesis that reduced 11BHS2D activity contributes to enhanced salt sensitivity.

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**References**


