Increased Urinary Free Cortisol
A Potential Intermediate Phenotype of Essential Hypertension

W. Reid Litchfield, Steven C. Hunt, Xavier Jeunemaitre, Naomi D.L. Fisher, Paul N. Hopkins, Roger R. Williams, Pierre Corvol, Gordon H. Williams

Abstract—We evaluated urinary cortisol excretion as a potential intermediate phenotype of essential hypertension in 153 white patients with essential hypertension and 18 normotensive white control subjects. Analyses were controlled for dietary sodium and gender to adjust for potential confounding effects of these variables on cortisol excretion. Urinary cortisol excretion measured on both high- and low-salt diets was significantly related to hypertension by repeated measures ANCOVA (P=.02). Additional determinants of urinary free cortisol included dietary sodium intake and gender; cortisol excretion was significantly higher in men (P=.0006) and during a high-sodium diet (P=.0001). Maximum likelihood analysis showed urinary cortisol to have a bimodal distribution on both 200-mmol (P<.01) and 10-mmol (P<.002) sodium diets in hypertensive subjects. On the low-salt diet, the mean urinary cortisol in normotensive subjects (108.7±44.7 nmol/d) was similar to the mean of hypertensive subjects in the low mode (127.2±43.0 nmol/d). The high mode comprised 31.2% of the hypertensive population and had a mean urinary cortisol of 224.3±93.8 nmol/d. Subjects with the highest urinary free cortisol showed the least sensitivity of blood pressure to dietary sodium loading (P<.05). These data suggest that there is an association between salt-resistant hypertension and high urine cortisol levels. This association may have a genetic basis. (Hypertension. 1998;31:569-574.)

Key Words: cortisol ■ genetics ■ phenotype, intermediate ■ sodium ■ bimodality

Essential hypertension is a heterogeneous syndrome in which several pathogenetic mechanisms result in an increase in blood pressure. One approach that has been used to reduce this heterogeneity is to group patients according to similar pathophysiological characteristics (eg, salt-sensitive and salt-resistant subsets). For example, three genetic forms of salt-sensitive hypertension have been identified: glucocorticoid-remediable aldosteronism,1,2 hypertension in patients carrying the M235T allele of the angiotensinogen gene,3,4 and Liddle’s syndrome.5,6 Thus, substantial progress has been made in identifying potential genetic factors involved in salt-sensitive hypertension. In contrast, no studies have reported genetic factors that could be responsible for salt-resistant hypertension.

A recent potential addition to the list of intermediate phenotypes in hypertension is the abnormal regulation or metabolism of cortisol. The first study to suggest this was the Four Corners study report to the genetics of hypertension remains uncertain.

The primary objective of the present study was to test the hypothesis that cortisol regulation was abnormal in a hypertensive population. If documented, these findings would provide the first evidence of cortisol levels being an intermediate phenotype in hypertension. To circumvent the difficulties of using plasma cortisol as the measure of the activity of the ACTH-cortisol axis, we used urinary cortisol excretion, which integrates the secretion of cortisol over a 24-hour period. Cortisol excretion rates were determined on both a high- and low-sodium diet for two reasons: to assess the salt sensitivity of the blood pressure in these subjects and to pursue scattered unconfirmed reports that cortisol metabolism may be influenced by sodium intake.7–11 Finally, data were analyzed for any gender effect because a gender effect on cortisol excretion has been inconsistently reported.10,12–15

Methods

The hypertensive patients were recruited from three communities: Boston, Mass; Salt Lake City, Utah; and Paris, France. Subjects were

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not presel ected, except for their willingness to participate in the study. Hypertension was defined as a history of hypertension with a diastolic blood pressure $\geq 90$ mm Hg or on one antihypertensive agent, or the need for two or more antihypertensive medications at the time of the screening visit. A group of normotensive individuals served as control subjects. Secondary forms of hypertension were excluded by history and physical examination and when indicated, by biochemical testing. All antihypertensive medications were discontinued at least 2 weeks before the study. Exclusion criteria included diabetes mellitus, obesity (BMI $> 33$ for men and $> 31$ for women), renal insufficiency, or other significant medical problems.

The patients were enrolled at the Clinical Research Centers of the Brigham and Women’s Hospital in Boston, Mass; the University of Utah in Salt Lake City, Utah; and Hospital Brousais in Paris, France. The study was reviewed and approved by the institutional review boards of each center, and all patients gave informed written consent before enrollment.

Each subject consumed both high-sodium (200-mmol/d) and low-sodium (10-mmol/d) diets, which were prepared in the metabolic kitchens of each of the study sites and then consumed in the outpatient setting. Dietary compliance was ensured by the measurement of urinary sodium and creatinine in a 24-hour urine collection after at least 3 days on the high-salt diet and 7 days on the low-salt diet. The high-salt diet was performed first and was immediately followed by the low-salt diet for 7 days. We obtained 24-hour urine collections in 153 consecutive white patients with essential hypertension and in 18 normotensive white subjects who met the above criteria.

### Techniques

Patients were admitted on the last day of each diet to enable standardization of blood pressure measurement. Blood pressure was measured with a Dinamap blood pressure monitor (Critikon Inc) with the patient in the supine position after overnight recumbency and in the fasting state. Blood pressure was reported as the mean of three separate measurements taken at least 5 minutes apart.

Urine was stored without preservatives or additives at $-20^\circ$C until assay. Serum was separated from venous blood and stored at $-20^\circ$C until assay. UFC, serum cortisol, and urine aldosterone (free aldosterone plus aldosterone glucuronide) were measured with commercial radioimmunoassay kits (Incstar Corp). Sodium and potassium in both serum and urine were measured by direct potentiometry with an ion-selective electrode (NOVA analyzer I, Nova Biochemical). Creatinine was measured in both serum and urine on a Beckman creatinine analyzer (model II).

### Statistics

Data were analyzed with repeated measures ANCOVA by the General Linear Models procedure of SAS statistical software (SAS Institute Inc). The repeated measures dependent variable was UFC measured on a low- and high-sodium diet for each individual. The independent variables included age, hypertension status, gender, and the interaction terms of gender and hypertension status. Bimodality analysis was performed with maximum likelihood analysis. Data are reported as mean±SD unless otherwise indicated.

### Results

#### Clinical Characteristics

Because preliminary data suggested that gender modified UFC, patient characteristics are presented separately for men and women (Table 1). Hypertensive and normotensive subjects differed with respect to age and blood pressure. These patients were not different with respect to urinary sodium and potassium levels on either high- or low-salt diets. Serum cortisol was similar in hypertensive and normotensive men and women on both diets. Compared with normotensive patients, hypertensive patients had higher BMI, but this did not reach statistically significant levels.

After hypertensive and normotensive subjects were combined, there was a significant effect of dietary sodium intake on UFC levels. The difference between high- and low-salt UFC was $59.6$ mmol/d ($P<.01$). Gender also significantly modified urinary cortisol levels (Fig 1). The influence of dietary sodium...

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**TABLE 1. Patient Characteristics for 85 Men and 68 Women With Essential Hypertension and 11 Normotensive Men and 7 Normotensive Women**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normotensive Men (n=11)</th>
<th>Hypertensive Men (n=85)</th>
<th>Normotensive Women (n=7)</th>
<th>Hypertensive Women (n=68)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>28.0±7.7</td>
<td>47.7±7.6*</td>
<td>24.7±3.1</td>
<td>48.3±7.4*</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23.3±3.1</td>
<td>27.4±4.0</td>
<td>24.8±2.8</td>
<td>26.3±3.9</td>
</tr>
<tr>
<td>High-salt SBP</td>
<td>115.0±18.0</td>
<td>149.9±18.7*</td>
<td>108.9±11.6</td>
<td>157.2±19.4*</td>
</tr>
<tr>
<td>High-salt DBP</td>
<td>66.4±12.0</td>
<td>90.9±12.6*</td>
<td>66.5±8.6</td>
<td>95.9±11.8*</td>
</tr>
<tr>
<td>Low-salt SBP</td>
<td>112.5±8.9</td>
<td>138.0±19.3*</td>
<td>107.1±5.0</td>
<td>140.4±16.2†</td>
</tr>
<tr>
<td>Low-salt DBP</td>
<td>65.4±3.8</td>
<td>83.2±11.6*</td>
<td>66.8±5.1</td>
<td>89.1±11.9‡</td>
</tr>
<tr>
<td>High-salt UNa, mmol/d</td>
<td>233.4±70.3</td>
<td>214.5±70.0</td>
<td>182.9±38.8</td>
<td>188.5±64.5</td>
</tr>
<tr>
<td>Low-salt UNa, mmol/d</td>
<td>9.4±5.6</td>
<td>16.2±8.8</td>
<td>8.5±5.8</td>
<td>12.8±7.3</td>
</tr>
<tr>
<td>High-salt UK, mmol/d</td>
<td>64.0±27.4</td>
<td>71.6±19.4</td>
<td>50.4±23.2</td>
<td>59.4±19.1</td>
</tr>
<tr>
<td>Low-salt UK, mmol/d</td>
<td>72.4±19.1</td>
<td>74.5±20.5</td>
<td>74.4±17.6</td>
<td>66.8±16.6</td>
</tr>
<tr>
<td>High-salt plasma cortisol, nmol/L</td>
<td>301±44</td>
<td>301±116</td>
<td>237±94</td>
<td>265±110</td>
</tr>
<tr>
<td>Low-salt plasma cortisol, nmol/L</td>
<td>326±83</td>
<td>334±121</td>
<td>287±61</td>
<td>303±99</td>
</tr>
</tbody>
</table>

SBP indicates systolic blood pressure; DBP, diastolic blood pressure; UNa, urinary sodium; and UK, urinary potassium. Low salt=10 mmol Na/d and high salt=200 mmol Na/d. Values are expressed as mean±SD.

* $P<.001$, † $P<.01$, and ‡ $P<.05$ vs normotensive patients on the same diet.
was equally manifest in women and men. Finally, hypertension also influenced the absolute levels of UFC; however, this effect was observed predominantly in the men (Fig 2). There was no significant relation between urinary cortisol and BMI. Urinary aldosterone was not correlated with blood pressure status or gender. As anticipated, there was a significant correlation between urinary aldosterone and dietary sodium \( (P = .03) \), as well as an inverse relation with age (decreased aldosterone excretion rate with increasing age, \( P = .03 \)).

To determine the relative contributions of these various factors to the level of UFC, ANCOVA models were developed for both high- and low-salt UFC measurements (Table 2). With these models, the effect of high- and low-sodium diets on UFC as related to gender and blood pressure was determined. The level of UFC was significantly greater in men \( (P = .009 \text{ and } .0006 \text{ on high- and low-salt diets, respectively}) \). Hypertensive patients also had higher free cortisol levels, which were significant for the low-salt diet \( (P = .017 \text{ on high- and low-salt diets, respectively}) \). The relation between urinary cortisol and blood pressure was strongest on the low-sodium diet. Finally, there was a strong correlation between high- and low-salt UFC \( (r = .50, P < .0001 \text{; Fig 3}) \).

Based on the results from the univariate model, a model that uses repeated measures ANCOVA in a multivariate model was developed. This model showed that gender, hypertension status, and dietary sodium were significantly related to UFC. UFC was significantly higher in men \( (P = .0006 \text{), hypertensive subjects (}P = .020\text{), and those on a high-sodium diet (}P = .0001\text{)} \). The interactions between gender and hypertension status, gender and dietary sodium, and hypertension status and dietary sodium were not statistically significant \( (P = .73, P = .60, \text{and } P = .86, \text{respectively}) \). These results suggest that each variable was having an independent effect on the UFC.

**Bimodality of UFC**

Maximum likelihood analysis of the urinary cortisol data, with statistical adjustment for gender, was performed for hyperten-

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**TABLE 2. Effect of Covariates on Urinary Free Cortisol**

<table>
<thead>
<tr>
<th>Variable</th>
<th>High-Salt Diet</th>
<th>Low-Salt Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>.009</td>
<td>.0006</td>
</tr>
<tr>
<td>Blood pressure status</td>
<td>.081</td>
<td>.017</td>
</tr>
</tbody>
</table>

\( P \)-values for univariate multiple regression analysis of gender and blood pressure status by dietary sodium intake. High-salt diet=200 mmol/d sodium; low-salt diet=10 mmol/d sodium.
Salt Sensitivity of Blood Pressure

Because the sensitivity of blood pressure to salt intake can be influenced by age, blood pressure changes with salt restriction were adjusted for age and sex and then correlated with UFC levels. There was a statistically significant negative correlation between the change in systolic blood pressure and both high-salt \((r=-0.19, \ P=.019)\) and low-salt \((r=-0.24, \ P=.0027)\) UFC levels. Similar results were seen for the changes in diastolic blood pressure: high salt, \(r=-0.16, \ P=.047\) and low salt, \(r=-0.22, \ P=.0056\). These data suggest that the higher the UFC on either a low- or high-salt diet, the smaller the blood pressure change when dietary sodium intake is modified. However, there is no correlation between the change in UFC by dietary manipulation and dietary sodium-induced changes in either systolic or diastolic blood pressure.

In an additional analysis, we analyzed age- and sex-adjusted changes in systolic and diastolic blood pressures after sodium restriction in the hypertensive subjects. Hypertensive subjects were stratified into subgroups on the basis of their UFC and compared by Student’s t test. When the low-salt UFC was considered, a significant decrease in systolic \((P=.024)\) and diastolic \((P=.044)\) blood pressure was observed in patients in the low-mode compared with that observed in high-mode patients. A similar but nonsignificant trend was noted for the high-salt UFC for systolic \((P=.058)\) and diastolic \((P=.11)\) blood pressures. Hypertensive subjects who were part of the high mode for UFC on a low-salt diet were relatively salt resistant compared with hypertensive subjects who fell into the low mode for low-salt UFC. The blood pressure response to salt restriction of these high-mode hypertensive patients was not significantly different from that of normotensive subjects, who showed almost no reduction in diastolic blood pressure after salt restriction (Fig 5).

Discussion

The present study was designed to determine whether abnormalities in the ACTH-cortisol axis were present in individuals with essential hypertension. According to the Four Corners study of normotensive subjects, if such an abnormality is present, it is likely to be genetically determined. The results of this study provide support for this hypothesis. This study documents that the level of UFC is predicted by blood pressure status, with hypertensive subjects having higher UFC levels than subjects with normal blood pressure \((P=.02)\). In addition, UFC levels were bimodally distributed in the hypertensive population, with the clearest separation observed on a low-sodium diet. Normotensive subjects on both a low- and high-salt diet had UFC levels that were equivalent to the low mode of the hypertensive population. Therefore, in the hypertensive population there is a subgroup with elevated UFC levels. Furthermore, this subgroup of the essential hypertensive population appears to be relatively salt resistant. Thus, these data provide the first suggestion of a potentially
genetically determined subgroup of the salt-resistant hypertensive subset. In addition, this subgroup may comprise as much as 30% of the total hypertensive population, a sizable fraction of the salt-resistant subset.

The second major observation in the present data was the substantial influence of dietary sodium intake and gender on UFC levels, whether the subjects were normotensive or hypertensive. Repeated measures ANCOVA showed UFC to be significantly related to gender ($P=0.006$) and dietary sodium intake ($P=0.001$). As a consequence, UFC levels were substantially higher on the high-sodium diet and in male subjects. These results may have implications in relation to using UFC to screen for Cushing’s syndrome in patients with hypertension. Although few of the UFC levels were substantially elevated over the normal range in our patient population, there were some values that would have been clearly considered consistent with mild Cushing’s syndrome if one did not take into account the dietary sodium intake or sex of the subject. Thus, in screening for Cushing’s syndrome in the hypertensive population, care must be taken in assigning diagnosis on the basis of a randomly collected urinary cortisol without reference to dietary sodium intake and gender.

Although there is a precedent for urinary cortisol being higher in hypertensive patients than in normal subjects,17 most previous investigators18,19 failed to detect a difference in UFC between normotensive and hypertensive subjects. The present study provides a likely answer for why this is so. No previous study controlled sodium intake or assessed the effect of gender. Both could substantially obscure any differences, because the impact of diet and gender was greater than the difference observed between normotensive and hypertensive patients. In addition, it is not a generalized increase in UFC levels in the hypertensive patients that produced the statistically significant difference. Instead, a minority subset with a higher-than-normal level of urinary cortisol was responsible for the observed differences. Thus, if previous studies contained only a few of this minority subset, there would have been no observed difference in UFC, even if dietary sodium and gender had been controlled for. Although normotensive and hypertensive patients differed significantly with respect to age and BMI, our analyses showed no relation between UFC and these variables, indicating that the observed difference in cortisol is not easily explained on this basis.

Is an elevated UFC an intermediate phenotype for a subset of the hypertensive population? It is premature to answer “yes.” However, a number of findings in the present study, as well as the study by Watt et al.9 suggest that elevated urinary cortisol may be genetically determined. Our study documented significant differences between hypertensive and normotensive patients for this variable. It also showed urinary cortisol to be bimodally distributed in the hypertensive population. Watt et al9 found evidence of elevated cortisol levels in the normotensive offspring of hypertensive subjects. In total, these findings suggest that elevated urinary cortisol is a strong candidate as a new intermediate phenotype of hypertension. Additional studies assessing family history and determining whether cortisol cosegregates with blood pressure in families will be required to firmly establish this hypothesis.

There are a number of mechanisms that could account for the observed elevation in UFC in this hypertensive subset. For example, a relative deficiency of 11-$\beta$-hydroxysteroid dehydrogenase increases the cortisol:cortisone ratio and enables cortisol to activate the mineralocorticoid receptor. Alternatively, cross-reactivity of cortisone with the cortisol assay could falsely elevate urinary cortisol levels. However, the assay used in this study is documented to have very little cross-reactivity between cortisol and cortisone (<1% cross-reactivity of cortisone with the assay). This fact, plus the absence of salt-sensitive hypertension in these subjects (which would be expected in patients with 11-$\beta$-hydroxysteroid dehydrogenase deficiency), makes these mechanisms unlikely explanations for our observations.

An additional possibility could be a change in cortisol metabolism conveyed through changes in the glucocorticoid receptor. This possibility is supported by the Four Corners study.9 In this scenario, a higher level of cortisol would be required to suppress ACTH release and therefore modify adrenal steroidogenesis. Direct data relevant to this point are not available in the present data set or from the published literature. Although we can safely exclude cortisone as a potential contaminating cross-reacting substance confounding our results, we have not excluded other substances that could cross-react with the cortisol antibody. However, there are no data to suggest such compounds.

Finally, there is the intriguing possibility that the elevated urinary cortisol levels in these patients with relative salt-resistant hypertension is actually a reflection of an altered stress response. There are several lines of evidence suggesting that some hypertensive patients have heightened activity of the adrenergic nervous system. Elevated cortisol levels are part of the output from a generalized stress response that includes activation of the adrenergic nervous system, increase in blood pressure, and an increase in pulse rate. Few published data are available to determine whether individuals with an activated adrenergic system are salt sensitive versus salt resistant. However, a recent study by Brown et al30 using tracer-labeled compartmental analysis of norepinephrine kinetics suggests that increased sympathetic nervous system activity is associated with a decreased sensitivity of blood pressure to dietary sodium intake. These findings are consistent with the report in the present study and the overall hypothesis that the individuals included in the higher mode of UFC may have an increased stress response with increased activity of the adrenergic nervous system. Additional studies will be needed to accept or reject this hypothesis.

In conclusion, we identified a subset of the hypertensive population with increased free cortisol excretion. This subset may compose as much as 30% of the hypertensive population. Our data suggest that if dietary sodium intake is controlled and gender is considered, UFC may serve as an effective intermediate phenotype for a genetic form of hypertension. In contrast to previously defined forms of genetic hypertension, all of which have blood pressure sensitive to salt intake, this subset appears to be relatively salt resistant. Whether this abnormality is associated with alterations in the activity of the adrenergic nervous system, in stress response, and/or in the glucocorticoid receptor remains to be determined. However, it is likely that...
with better characterization of this potential new intermediate phenotype of hypertension, a better understanding of the way in which glucocorticoids may be involved in the pathogenesis of elevated blood pressure will be reached.

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References
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