Urinary cortisol to cortisone metabolites ratio in prednisone-treated and spontaneously hypertensive patients
Oliviero Olivieri, Francesca Pizzolo, Viviana Ravagnani, Lorenzo Moretti, Antonio Carletto, Giovanni Faccini, Francesco Pasini, Simonetta Friso, and Roberto Corrocher

Objective and methods Prednisone and its active metabolite prednisolone, both substrates for 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2), may represent a pharmacological challenge for the enzyme. The aim of the present work was to define the possible role of abnormal cortisol/cortisone handling, as revealed by an urinary tetrahydrocortisol + allotetrahydrocortisol (THFs)/tetrahydrocortisone (THE) ratio between 1.5 and 3.0, by measuring urinary cortisol and cortisone metabolites in: normotensive individuals (n = 100) who received 7–8 mg/day of oral prednisone and were then followed for development of hypertension; essential hypertensive (EH) participants from primary care (n = 103); and EH hypertensive patients referred to the Hypertension Unit (n = 141).

Results About one-third (14 out of 47, 30%) of glucocorticoid-treated patients who developed hypertension showed a THFs/THE ratio >1.5, which was seen in 3% (n = 3) and 14% (n = 19) of primary and tertiary care hypertensive patients, respectively. A THFs/THE ratio >1.5 was associated with a 3.8-fold incremental risk of hypertension after glucocorticoid therapy, regardless of duration and intensity of prednisone therapy.

Introduction Normal activity of 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2), a microsomal enzyme responsible for the conversion of 11-hydroxy glucocorticoids to their 11-keto metabolites, is essential for preventing the activation of mineralocorticoid receptor (MR) by cortisol [1]. Since cortisol and aldosterone have the same affinity for MR and the plasma concentration of the former is much higher than that of the latter, MR activation by aldosterone depends strictly on the inactivation of cortisol into cortisone by 11β-HSD2 [2].

Inappropriate MR stimulation by cortisol is the pathogenetic mechanism responsible for the ‘apparent mineralocorticoid excess’ (AME) syndrome [3], an autosomal recessive form of salt-sensitive hypertension caused by mutations in the HSD11B2 gene leading to substantial or complete loss of enzymatic activity [4]. In spite of its intrinsic interest, classic AME has for a long time been considered a rare condition with a limited impact on clinical practice.

More recently, however, evidence that partially defective 11β-HSD2 activity may play a role in hypertension has changed this view [5]. Since the 1990s, moderate impairment of 11β-HSD2 has been recognized in some patients with essential hypertension (EH) [6–8], and several genetic mutations or polymorphisms with milder effects on 11β-HSD2 activity than those described in classic AME may be present in some EH patients [9–14]. Although the results of these investigations have not been conclusive, and genetic mutations with a definite causal role have not been identified, a milder phenotype of AME (also called AME type II) has been documented in some hypertensive patients and a form of hypertension – indistinguishable from EH – was described in their relatives [15]. In addition, impaired 11β-HSD2 activity has been claimed to be associated with salt sensitivity in healthy individuals [16], but this finding has not been confirmed and still remains the object of controversy [17].

In clinical practice, 11β-HSD2 activity is not currently evaluated in EH patients, probably reflecting the
uncertain pathogenetic role and prevalence of mildly impaired enzyme activity in the hypertensive population.

Traditionally, the profile of urinary steroids has served as the main tool for detecting AME or AME-like phenotype, typically characterized by a relative increase in the excretion of A-ring-reduced metabolites of cortisol (tetrahydrocortisol (THF) + allotetrahydrocortisol (αTHF)), plus the corresponding decrease of the levels of A-ring-reduced metabolites of cortisone (tetrahydrocortisone (THE)) [18,19]. While normal activity of 11β-HSD2 produces similar urinary amounts of THF + αTHF (THFs) and THE, with a resulting THFs/THE ratio ≈1, in patients with impaired activity this ratio increases proportionally to the extent of reduction in enzyme activity. Considering THFs/THE ratio as a quantitative marker, a pathogenetic hypertensive role is consistently associated in vivo with a urinary THFs/THE ratio ≥3, a value corresponding to a 50% reduction of enzyme activity [5]; in contrast, the practical relevance of a less dramatic functional impairment, as witnessed by an urinary THFs/THE ratio higher than normal but <3, remains unclear.

Based on the observation that children with AME challenged with exogenous cortisol develop further sodium retention and marked increase in blood pressure (BP) [20], impairment of 11β-HSD2 enzyme activity is considered as one of the possible mechanisms that may explain the occurrence of glucocorticoid-associated hypertension [21,22]. However, the issue has not been object of specific clinical investigation and the proportion of patients who develop hypertension after chronic glucocorticoid exposure and/or also have increased THFs/THE ratios is not known [22]. Hypertensive patients affected by Cushing’s syndrome have a high (approximately two–four times normal) cortisol to cortisone metabolite ratio because 11β-HSD2 is insufficient to handle the elevated circulating levels of cortisol [23]. It is therefore reasonable to suppose that chronic glucocorticoid therapy may increase BP as well as THFs/THE ratio in a similar manner. Since prednisolone, the active metabolite of prednisone, has been demonstrated to be a better substrate for 11β-HSD2 oxidation than cortisol [24], and it is in turn inactivated to prednisone in the kidney by 11β-HSD2 [24], patients receiving such pharmacological treatment may represent a suitable model to prove the hypothesis of a competitive inhibition mechanism of 11β-HSD2. In addition, the similarities of structure between cortisol/cortisone and prednisolone/prednisone represent intuitive support to this hypothesis.

Based on all these considerations, we planned the present study with the purpose of evaluating the prevalence of individuals with an increased THFs/THE ratio in a group of normotensive individuals who developed hypertension after chronic treatment with a moderate–low dose of prednisone. We also estimated the prevalence of EH patients with a similarly high ratio, selected from primary and tertiary care hypertensive populations. In this way, it was possible to identify patients sharing an identical phenotype (arterial hypertension + an elevated urinary THFs/THE ratio) determined by different factors: an ‘acquired’ factor in the case of glucocorticoid-treated patients, and ‘spontaneous’ factor(s) in the case of ‘essential’ hypertensive patients.

Methods

Patient selection: glucocorticoid-treated patients (group A)

Patients were selected among those referred to the Rheumatology Unit of the University Hospital of Verona for rheumatoid arthritis (RA). We retrospectively analysed all the folders of individuals who received chronic glucocorticoid treatment for this disorder and were still on therapy in December 2004. To be included in the study, patients had to show normal BP (<140/90 mmHg) on the first two ambulatory examinations, sequentially scheduled before starting any (including glucocorticoid) treatment; moreover, since patients were examined on a regular basis, persistently elevated or normal BP had to be confirmed during the subsequent follow-up. To assess whether there is any evidence of a significant BP fluctuation over the whole period of observation, the average of the recorded BP values before glucocorticoid treatment was used as a baseline measure and classification for persistent normotension or glucocorticoid-associated hypertension was confirmed when an increase of systolic BP (SBP)/diastolic BP (DBP) was < or > 11/7.5 mmHg, respectively, that is below or above the standard deviation values of baseline BP average, to avoid the so-called ‘regression on the mean’ phenomenon. This arbitrary cut-off was chosen because recorded BP values of patients were often lower at late than at early measurements during the follow-up period. Actually, all of such patients classified as normotensives also showed BP < 140/90 mmHg at the last examination, while all patients who were classified as hypertensives were taking BP-lowering drugs [angiotensin-converting enzyme (ACE) inhibitors, sartans, diuretics, calcium blockers] at the last examination. Given general agreement on 140/90 mmHg as cut-off BP values in terms of risk, all subsequent considerations imply that patients with prednisone-induced hypertension were at least 140/90 mmHg and had a ≥ 11/7.5 mmHg increase from baseline average BP.

According to a previously approved rheumatological protocol, all patients were treated with oral prednisone at doses ranging from 5 to 15 mg/day. The patients meeting the requirements described above were recalled for biochemical tests and 24-h urine collection. BP-lowering drugs and glucocorticoids were not withdrawn before sampling. When assayed, all patients were in stable remission of disease and were taking the same dose of oral prednisone for at least 3 months (according to the
Table 1  Clinical and biochemical features of glucorticoid-treated patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normotensive patients (n = 53)</th>
<th>Hypertensive patients (n = 47)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>50.8 ± 12.8</td>
<td>56.7 ± 12.3</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>11/42</td>
<td>7/40</td>
<td>NS</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>122.3 ± 10.9</td>
<td>138.5 ± 15.3</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>78.2 ± 7.5</td>
<td>87.20 ± 6.0</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>S-Na⁺ (mmol/l)</td>
<td>141.8 ± 2.5</td>
<td>141.5 ± 2.6</td>
<td>NS</td>
</tr>
<tr>
<td>S-K⁻ (mmol/l)</td>
<td>3.9 ± 0.3</td>
<td>3.77 ± 0.4</td>
<td>NS</td>
</tr>
<tr>
<td>U-Na⁺ (mmol/24 h)</td>
<td>159 ± 58</td>
<td>151 ± 60</td>
<td>NS</td>
</tr>
<tr>
<td>U-K⁻ (mmol/24 h)</td>
<td>56 ± 17</td>
<td>58 ± 20</td>
<td>NS</td>
</tr>
<tr>
<td>S-Creatinine (μmol/l)</td>
<td>67 ± 14</td>
<td>68 ± 15</td>
<td>NS</td>
</tr>
<tr>
<td>S-Cortisol (μg/dl)</td>
<td>12.6 ± 8.7</td>
<td>9.5 ± 6.9</td>
<td>NS</td>
</tr>
<tr>
<td>P- ACTH (pg/ml)</td>
<td>20.1 ± 14.6</td>
<td>15.7 ± 13.0</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>P- Aldosterone (pg/ml)</td>
<td>182 ± 130</td>
<td>229 ± 168</td>
<td>NS</td>
</tr>
<tr>
<td>P- Renin (pg/ml)</td>
<td>14.8 ± 15</td>
<td>96.6 ± 56</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>ARR (μg/ml/Lpg/ml)</td>
<td>21.4 ± 18.0</td>
<td>21.2 ± 20.5</td>
<td>NS</td>
</tr>
<tr>
<td>THF (μg/24 h)</td>
<td>0.85 ± 0.6</td>
<td>0.93 ± 0.8</td>
<td>NS</td>
</tr>
<tr>
<td>e-THF (μg/24 h)</td>
<td>0.41 ± 0.4</td>
<td>0.53 ± 0.6</td>
<td>NS</td>
</tr>
<tr>
<td>THE (μg/24h)</td>
<td>1.70 ± 1.3</td>
<td>1.47 ± 1.3</td>
<td>NS</td>
</tr>
<tr>
<td>THFs/THE ratio</td>
<td>0.92 ± 0.70</td>
<td>1.44 ± 1.1</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>THFs/THE ratio &gt; 1.5</td>
<td>9% (5/53)</td>
<td>30% (14/47)</td>
<td>0.01</td>
</tr>
<tr>
<td>Duration of therapy (months)</td>
<td>27.8 ± 57</td>
<td>31.0 ± 58</td>
<td>NS</td>
</tr>
<tr>
<td>Prednisone dose (mg/day)</td>
<td>7.45 ± 2.9</td>
<td>7.79 ± 3.8</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are means ± SD. ACTH, adrenocorticotrophic hormone; ARR, aldosterone to renin ratio; DBP, diastolic blood pressure; P, plasma; S, serum; SBP, systolic blood pressure; THE, tetrahydrocortisone; THF, tetrahydrocortisol; THFs, tetrahydrocortisol + allotetrahydrocortisol; U, urinary. Statistical significance (P < 0.05) was defined by Student’s t-test, Kruskal–Wallis or χ² test.

Patient selection: primary care (group B)
A detailed description of the selection of patients is provided in reference [25]. Briefly, a sample of 1462 adults aged 35–74 years, randomly selected from the population register of the Bussolengo Health District (northern Italy) and representative of the total population of the district, was examined by the general practitioners who participated in the study. After a detailed explanation and after obtaining written informed consent, patients affected by hypertension (defined according to the criteria specified below) were included in the study and examined by biochemical testing. Of 412 identified hypertensive patients, 103 agreed to give blood and to collect 24-h urine. These patients were similar for demographic and socio-economic features to the hypertensive individuals who refused examination (data not shown), so they should be representative of the entire group. The inclusion criteria were:

1. SBP > 140 mmHg or DBP > 90 mmHg, measured twice in both arms, with the patient in the sitting position for 5 min, providing that he/she had not drunk any coffee or smoked during the previous 30 min; a third measurement (or further measurements, if necessary) was obtained in the case of a difference in BP values > 5 mmHg. To establish a firm diagnosis, high BP also had to be confirmed 2 weeks later (the same procedure was repeated as described above); or
2. current therapy for a previous diagnosis of hypertension.

Since the study was aimed at defining aldosterone to renin ratio [25], drugs were withdrawn for the 4 weeks following inclusion in the study (wash-out period); if necessary, BP reduction was obtained using agents such as verapamil and alpha-blockers [25]. Samples for aldosterone and renin were obtained after at least 2 h in the upright posture and a subsequent period of 10 min in the seated position. No patient had current or past chronic glucocorticoid treatment.

Patient selection: tertiary care (group C)
All patients referred to the Hypertension Unit of the University Hospital of Verona for resistant hypertension or for possible secondary causes of hypertension over the previous 2 years were examined by biochemical testing and 24-h urine cortisol/cortisone metabolite assay. Only patients for whom a diagnosis of EH was made at the end of the work-up were included in the study; patients with secondary forms of hypertension (in particular patients with nephro-parenchymal disease in whom an abnormal THFs/THE ratio might occur) were therefore excluded. Similarly, patients currently treated with glucocorticoids, or with a clinical history of previous glucocorticoid treatment, were excluded. No patient showed liquorice-induced hypertension or was taking liquorice-containing sweets.

A total of 141 EH patients were finally considered. According to our protocol, all patients were on a sodium-controlled diet (NaCl 110–120 mmol/day) for 3 days before blood and urine sampling, and took no hypotensive drugs other than verapamil and/or alpha-blockers over the previous 4 weeks [26]. Samples for aldosterone and renin were obtained after at least 2 h in the upright posture and a subsequent period of 10 min in the seated position.

Biochemical assays
Patient blood samples for hormonal and routine parameters were collected after overnight fasting between 0800 and 0900 h. Plasma aldosterone and direct active renin were measured by commercially available methods (Nichols Diagnostics, San Clemente, California, USA) [27]. Cortisol and plasma adrenocorticotrophic hormone (ACTH) were assayed by routine immunometric methods (Diagnostic Products Corporation, Los Angeles, California, USA); intra- and inter-assay coefficients of variation were 6.2–7.3% for cortisol and 6.7–8.2% for ACTH, respectively. For THFs/THE assay, urine samples were analysed by gas chromatography–mass spectrometry (Hewlett-Packard 6890-5973 Hp mass spectrometer;
Hewlett-Packard, Milan, Italy) as described previously [27]. Intra-assay and inter-assay coefficients of variation were 7.7 and 9.4%, respectively.

**Statistical analysis**
Data analysis was performed using the SPSS 13.0 for Windows (SPSS, Chicago, Illinois, USA). Quantitative values were expressed as means ± standard deviation. Student’s t-test for unpaired observations or the Kruskal–Wallis test was used for normally distributed or skewed variables, respectively. Comparison of proportions was carried out by cross-tabulation, Pearson’s chi-squared and Fisher’s exact test.

To assess the extent to which covariates were associated with the risk of having glucocorticoid-associated hypertension, odds ratios (OR) with 95% confidence intervals (CI) were estimated by logistic-regression analysis.

**Results**
**Glucocorticoid-treated patients (group A)**
In agreement with the prevalence of rheumatoid arthritis, most patients (82%) included in the study were women. After starting glucocorticoid treatment, a total of 47 (47%) patients developed hypertension while the remaining individuals (n = 53, 53%) did not substantially modify their BP during the period of follow-up. As reported in Table 1, these two subgroups of patients matched for most clinical and biochemical features; in particular, duration (27.8 ± 56.6 versus 30.9 ± 58 months) and dosage (7.45 ± 2.9 versus 7.78 ± 3.8 mg/day) of prednisone therapy were not statistically different between subgroups of individuals. However, hypertensive patients were older and had higher renin values than normotensive individuals, probably reflecting concurrent BP-lowering treatment (Table 1).

Hypertensive patients had lower ACTH values and higher urinary cortisol to cortisone metabolites ratio than normotensive individuals (Table 1). Moreover, by evaluating how many individuals showed THFs/THE > 1.5, an altered cortisol/cortisone metabolite ratio was observed in about one-third (30%) of hypertensive patients but in only 9.4% of normotensive participants (Table 1).

To assess the extent to which age, ACTH and raised THFs/THE ratio (i.e. the main covariates differently distributed between the subgroups) were associated with the development of hypertension, OR with 95% CI was estimated by logistic-regression analysis (Table 2). With THFs/THE ratio as a continuous or categorical variable, it proved to be a predictor of development of hypertension; in particular, a THFs/THE ratio > 1.5 was associated with a 3.8-fold increased risk of hypertension after glucocorticoid therapy. In contrast, low ACTH levels were not significantly related to this risk. Adjustment for sex, duration and dose of glucocorticoid therapy did not change the results (data not shown).

The entire group of patients taking glucocorticoids was then divided into two subgroups according to THFs/THE ratio values: patients with an elevated ratio (n = 19, 19% of total group) more often had hypertension (74 versus 41%, P = 0.01), higher urinary excretion of potassium (68 ± 16 versus 54 ± 18 mmol/24h, P < 0.01), lower levels of ACTH (11.1 ± 11.5 versus 19.6 ± 14.1 pg/ml, P = 0.001), lower cortisol (5.4 ± 5.4 versus 12.6 ± 8.0 µg/dl, P = 0.001), and a shorter mean time of steroid therapy (15.2 ± 16.1 versus 32.6 ± 63 months, P < 0.05) than patients with a normal THFs/THE ratio. In contrast, mean prednisone dosage taken by the patients of the two subgroups was similar (7.4 ± 3.2 versus 8.3 ± 3.7 mg/day).

Since five patients showed a raised THFs/THE ratio but normal BP, we analysed possible distinguishing features of these individuals who were resistant to the development of hypertension in comparison with the patients (n = 14) showing similarly increased THFs/THE ratio but high BP. Although the small number of cases represents a statistical limitation, the subgroups were matched for all the features listed in Table 1. In particular there were no differences in serum and urinary electrolytes, serum cortisol, ACTH, THFs/THE ratio, duration and dose of glucocorticoid therapy (data not shown). Of note, four out of five normotensive individuals were fertile females with a regular menstrual cycle.

**Hypertensive patients from primary and tertiary care**
The clinical and biochemical features of hypertensive patients selected from primary (group B) or tertiary care (group C) are reported for comparison in Table 3. As expected, patients referred to the Hypertension Unit were younger and showed more severe hypertension (46% had BP values consistent with stage II of JNC 7 classification [28], despite treatment with verapamil and/or α-adrenergic blockers); they also had increased levels of serum creatinine and plasma aldosterone, and a more frequent positive family history for hypertension than primary-care patients (Table 3). Obesity, diabetes, plasma lipids and smoking were similarly distributed between the two groups (data not shown).
Table 3 Clinical and biochemical features of hypertensive patients selected from primary or tertiary care

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients from primary care ( (n = 103) )</th>
<th>Patients from tertiary care ( (n = 141) )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age ( (\text{years}) )</td>
<td>56.4 ± 3.8</td>
<td>51.8 ± 14.6</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Gender ( (\text{M/F}) )</td>
<td>51/52</td>
<td>75/66</td>
<td>NS</td>
</tr>
<tr>
<td>SBP ( (\text{mmHg}) )</td>
<td>145.8 ± 11.9</td>
<td>161.1 ± 21.5</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>DBP ( (\text{mmHg}) )</td>
<td>89.1 ± 9</td>
<td>99.8 ± 12.8</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Hypertension stage II*</td>
<td>10.7%</td>
<td>46.3%</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>S-Na(^+) ( (\text{mmol/l}) )</td>
<td>141.7 ± 2</td>
<td>141.3 ± 2.7</td>
<td>NS</td>
</tr>
<tr>
<td>S-K(^-) ( (\text{mmol/l}) )</td>
<td>4.59 ± 0.49</td>
<td>3.84 ± 0.4</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>S-Creatinin ( (\mu\text{mol/l}) )</td>
<td>68.44 ± 17.13</td>
<td>80.6 ± 18.5</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>S-Cortisol ( (\mu\text{g/dl}) )</td>
<td>Not available</td>
<td>26 ± 64</td>
<td>–</td>
</tr>
<tr>
<td>P-ACTH ( (\text{pg/ml}) )</td>
<td>Not available</td>
<td>30.7 ± 23.6</td>
<td>–</td>
</tr>
<tr>
<td>P-Aldosterone ( (\mu\text{g/ml}) )</td>
<td>168.9 ± 93</td>
<td>218.2 ± 121.4</td>
<td>0.001</td>
</tr>
<tr>
<td>P-Renin ( (\text{pg/ml}) )</td>
<td>14.3 ± 19.7</td>
<td>15.7 ± 6.2</td>
<td>NS</td>
</tr>
<tr>
<td>ARR ( (\text{pg/mg/ml}) )</td>
<td>30 ± 25</td>
<td>27.3 ± 24.9</td>
<td>NS</td>
</tr>
<tr>
<td>THF ( (\mu\text{g/24 h}) )</td>
<td>1.92 ± 0.99</td>
<td>1.99 ± 1.1</td>
<td>NS</td>
</tr>
<tr>
<td>( \text{u-THF} \ (\mu\text{g/24 h}) )</td>
<td>0.98 ± 0.6</td>
<td>1.44 ± 12</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>THE ( (\mu\text{g/24 h}) )</td>
<td>3.25 ± 1.46</td>
<td>3.32 ± 1.7</td>
<td>NS</td>
</tr>
<tr>
<td>THFs/THE ratio</td>
<td>0.01 ± 0.26</td>
<td>1.06 ± 0.5</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>THFs/THE ratio ≥ 1.5</td>
<td>3 (3%)</td>
<td>19 (14%)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Family history of hypertension</td>
<td>27%</td>
<td>62%</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Data are means ± SD. ACTH, adrenocorticotropic hormone; ARR, aldosterone to renin ratio; DBP, diastolic blood pressure; P, plasma; S, serum; SBP, systolic blood pressure; THE, tetrahydrocortisone; THF, tetrahydrocortisol; THFs, tetrahydrocortisone + allo-tetrahydrocortisol. Statistical significance \( (P < 0.05) \) was defined by Student’s \( t \)-test, Kruskal–Wallis or \( \chi^2 \) test. \*Classification of BP values according to JNC 7 \( (\text{SBP} > 160 \text{mmHg} \text{ or DBP} > 100 \text{mmHg}) \) \([28]\) in patients treated with verapamil and/or \( \beta \)-adrenergic blockers.

On average, \( \alpha \)-THF and, in turn, the THFs/THE ratio were higher in the urine of patients from tertiary care than in individuals selected from primary care (Table 3). Accordingly, a ratio > 1.5 was observed in 19 of 141 (14%) patients examined in the Hypertension Unit but only in three out of 103 (3%) hypertensive individuals selected by the general practitioners.

The total population of hypertensive patients was also analysed in order to find out possible clinical hallmarks characterizing the patients showing an elevated THFs/THE ratio. In the hypertensive population considered as a whole, 10 patients had previously documented brain ischaemic damage, four of them showed an urinary THFs/THE ratio > 1.5, that is 18.1% (4/22) of this subgroup. At echo-Doppler ultrasonography, a significant (>50% lumen reduction) carotid artery stenosis was demonstrated in 11 patients, five of them showed an urinary THFs/THE ratio > 1.5, that is 22.7% (5/22) of this subgroup. Differences in distribution of both these clinical features between high and normal ratio patients were statistically significant \( (P < 0.05) \). In contrast, diabetes was no more frequent, and body mass index and blood glucose concentrations were similar in normal patients and those with a high THFs/THE ratio.

Comparison between all the patients with elevated versus normal THFs/THE ratio

The entire study population (groups A + B + C) was then divided in two subgroups according to THFs/THE ratio values (< or ≥ 1.5, respectively). No differences were observed for most clinical and biochemical features (data not shown) including age (53.8 ± 12 versus 52.5 ± 15 years) and gender (male 53.7% versus 40.3%); however, remarkably, patients with an elevated ratio were characterized by abnormal potassium handling, with lower serum levels (3.8 ± 0.47 versus 4.1 ± 0.5 mmol/l, \( P < 0.05 \)) and higher urinary concentrations (56.1 ± 24.9 versus 47.3 ± 18.7 mmol/l, \( P < 0.05 \)) than those of the other patients. This aspect was consistent with the supposed mineralocorticoid effect induced by an impaired renal 11β-HSD2 activity. Renin (15.5 ± 12.3 vs. 15.9 ± 22.3 pg/ml) and aldosterone (185 ± 155 vs. 199 ± 119 pg/ml) were not different in the two groups of patients. The significantly lower cortisol (12.1 ± 8.5 versus 20.7 ± 54 µg/dl) and higher ACTH (18.7 ± 18.5 versus 26.1 ± 20.9 pg/ml) values, although within the normal range, are consistent with an inhibition of the adrenal–pituitary axis in patients with an elevated ratio.

Discussion

The main purpose of the present work was to establish the possible role of abnormal cortisol/cortisone handling, as shown by urinary THFs/THE ratios ranging from 1.5 to 3.0, in human hypertension. We obtained evidence that a significant number of individuals who received glucocorticoid treatment share a phenotype similar to that previously described in some EH patients [6–8], characterized by both arterial hypertension and a mildly elevated (>1.5) urinary THFs/THE ratio. Moreover, for the first time, we quantified the prevalence of such EH patients in different clinical settings, showing that a definite proportion may be found in both primary and tertiary care. Overall, these results support the view an ‘acquired’ factor (in the case of glucocorticoid-treated patients) or ‘constitutive’ factor(s) (in the case of EH patients) similarly affect a common pathway involved in BP regulation.

It has been supposed that chronic glucocorticoid therapy may challenge the functional capability of 11β-hydroxysteroid dehydrogenases and facilitate the development of hypertension [20,21]. Prednisone and its active metabolite prednisolone are substrates for both 11β-HSD type 1 and 2 [23]; compared with cortisol, prednisone and especially prednisolone are preferentially oxidized by 11β-HSD2 in an in vitro model, providing the pharmacokinetic explanation for their reduced mineralocorticoid activity [24]. Chemical structures of cortisol/cortisone and prednisolone/prednisone are indeed very similar, differing only for the \( \Delta^4 \)-dehydroconfiguration that characterizes prednisolone/prednisone but not the adrenal hormones. Possible inhibitory competition for renal 11β-HSD2 is therefore plausible, although not yet demonstrated in vivo or in a clinical context.

In our population, an unbalanced increase in tetrahydro-metabolites of cortisol versus tetrahydro-metabolites of
cortisone occurred in about 30% of individuals chronically treated with low–moderate doses (on average 7–8 mg/day) of prednisone. Most of these individuals with initially normal BP became hypertensive during treatment, whereas only 5 patients with a THFs/THE ratio >1.5 remained normotensive, so that this condition was associated with a 3.8-fold higher risk of developing hypertension after glucocorticoid therapy. Although dose and duration of such therapy did not affect the overall risk of developing hypertension (Table 1), patients with an elevated THFs/THE ratio had significantly lower ACTH and cortisol values than individuals with a normal ratio, thus suggesting an increased "sensitivity" to the glucocorticoids and a more marked inhibition of the adrenal–pituitary axis despite similar prednisone therapy. Long-term competition of prednisolone with cortisol for β-oxidation by 11β-HSD2 may allow for a prolonged half-life of the residual cortisol not transformed into cortisone, and a more elevated (though normal) concentration of active cortisol available at the pituitary cellular level, thus possibly suggesting that some individuals are more exposed to such a competitive mechanism than others.

Another possible interpretation of these findings could be that of a greater prednisone-related induction of liver 11β-HSD1 isofrom in these patients, with an increased conversion of cortisone into cortisol. For some authors [29,30] THFs/THE ratio represents a ‘global’ indicator of conversion of cortisone into cortisol. For some authors [29,30] THFs/THE ratio had significantly lower ACTH and cortisol values than individuals with a normal ratio, thus suggesting an increased 'sensitivity' to the glucocorticoids and a more marked inhibition of the adrenal–pituitary axis despite similar prednisone therapy. Long-term competition of prednisolone with cortisol for β-oxidation by 11β-HSD2 may allow for a prolonged half-life of the residual cortisol not transformed into cortisone, and a more elevated (though normal) concentration of active cortisol available at the pituitary cellular level, thus possibly suggesting that some individuals are more exposed to such a competitive mechanism than others.

As expected on the basis of previously reported evidence [21,22], our data also show that inhibition of 11β-HSD2 activity is not the only mechanism leading to glucocorticoid-associated hypertension. Correspondingly, impaired 11β-HSD2 activity may not necessarily result in hypertension but other factors may interfere with this mechanism. As previously reported, in our population, a normal THFs/THE ratio was found in more than two-thirds of prednisone-treated hypertensive patients, whereas five women remained normotensive despite an elevated THFs/THE ratio. Of note, four out of these women were rather young and had a regular menstrual cycle, suggesting that female steroid hormones may to some extent offset or block the mechanism leading to hypertension.

The second relevant result of the work is that an increase of urinary THFs/THE ratio ranging between values >1.5 and 3 characterizes some spontaneously hypertensive patients, with a substantially higher prevalence in more severely affected individuals (14%) than in patients with mild hypertension (3%, see Table 3). In 60 healthy normotensive controls, nobody showed an urinary THFs/THE ratio >1.5.

To our knowledge, this is the first work exploring the ratio of cortisol to cortisone metabolites in hypertensive patients from primary care and, in turn, comparing this prevalence with that observed in patients referred to a Hypertension Unit. A clear gradient between these different settings was observed, suggesting that an abnormal urinary ratio of cortisol to cortisone metabolites is preferentially associated with more severe hypertension. This conclusion is consistent with a recent report showing that a mild impairment of 11β-HSD2 activity is associated with left ventricular mass in essential hypertension [33]. Interestingly, an increased THFs/THE ratio (above the mean normal value + 2SD = 1.54) and left ventricular hypertrophy at echocardiography were found in the same percentage of hypertensive patients [33]. In our population of EH patients, though quantitatively limited, the proportion of patients with an elevated THFs/THE ratio who also presented previous cerebrovascular damage (4/22, 18%) or carotid artery stenosis (5/22, 23%) was striking. Overall, these findings may suggest that the phenotype of hypertension plus a high ratio may have a poorer cardiovascular prognosis than EH patients with a normal ratio. This is also consistent with the epidemiological evidence that glucocorticoid exposure (i.e. a prednisolone equivalent ≥7.5 mg/day, a dose quite similar to that taken by our arthritic patients) is associated with an elevated risk of heart failure,
myocardial infarction and stroke [32]. Thus, if caution is usually suggested in glucocorticoid treatment of patients with hypertension, alternative therapeutic options should probably be considered in the case of an associated increase of THFs/THE ratio.

Hypertensive patients with an increased THFs/THE ratio were clinically indistinguishable from the other hypertensive patients. Lower (but still normal) potassium levels and male gender were the only variables characterizing hypertensive individuals with elevated THFs/THE ratio, but obviously both these variables are not sufficiently specific to suspect the problem in practice. Thus, subtly altered cortisol metabolism does not necessarily affect traditional markers of mineralocorticoid hypertension such as renin or aldosterone, although quantitatively more obviously expressed defects such as classic AME do. In other reports, individuals heterozygous for mutations inactivating the HSD11B2 gene, who were studied as first-degree relatives of AME patients, and having modestly increased THFs/THE ratio, were clinically indistinguishable from EH patients, and their renin activity was normal or not suppressed [15,26]. In our RA patients, active renin was not different between individuals with an increased or a normal THFs/THE ratio, but BP-lowering treatment (mainly based on ACE inhibitors and diuretics) could have influenced the result. This is a clear limitation of our study. Moreover, it is also possible that the use of less precise, direct active renin rather than the plasma renin–angiotensin (PRA) assay have influenced this aspect. Some previous literature findings reported a weak association between THFs/THE ratio and recumbent – but not upright – renin activity in hypertensive patients [12,29]. Since we studied upright active renin, it is possible that the relationship between these parameters was missed.

Several other studies suggest that an altered vasoactive response, derived by an increased vasoconstrictor sensitivity to catecholamines and/or endothelin-1, rather than tissue specificity is enzyme, not receptor mediated. Science 1987; 242:583–585.


Acknowledgements

We are grateful to the following general practitioners for their important contribution in the data collection and recruitment of patients in the primary-care setting: Paolo Adami, Carlo Ballarini, Anna Barbera, Gaetano Benati, Bruno Benedetti, Claudio Bertaiola, Tiziano Bonaldi, Annarosa Bovo, Umberto Cacchi, Marco Cherubini, Damiano Chiesa, Massimo Chincarini, Alberto Ciacciarelli, Giorgio Ciampalini, Roberto Ciresa, Giuseppe Coccia, Chiara Cressoni, Giuliana Dal Pozzo, Roberto Esposti, Francesco Faraci, Marina Ferrari, Guido Filippini, Anna Fioretta, Adelheid Fischer, Carlo Andrea Franchini, Guglielmo Frapporti, Federica Galvani, Beatrice Gargiulo, Angelo Garofalo, Walter Idolazzi, Giorgio Lacinia, Moreno Leoncini, Livio Libardi, Serena Losi, Italo Lovato, Silvio Mantovani, Raffaella Marrocchella, Innocenzo Maurelli, Marco Pietro Mazzi, Silvia Menegazi, Ernesto Menini, Alessio Micchi, Mauro Montana, Oswaldo Morbioli, Franco Motta, Antonio Panzino, Adriano Ramanzi, Giandomenico Righetti, Arianna Rizzi, Rudi Santini, Gustavo Sartori, Paolo Scarpolini, Francesco Schiera, Maurizio Sciortino, Alessandro Severi, Lionello Signorati, Cesare Testi, Mariella Varaschin and Matteo Venturi. We are also grateful to Gianstefano Blengio and Denise Signorelli for their rigorous work in selecting individuals from the Demographic Register of Bussolengo, and to Giorgio Parise for his valuable help in collecting blood and urine samples.

This work has been supported by grants from the Ministry of the University and Scientific and Technological Research (O.O.), the Veneto Region Department of Health (O.O.) and Cariverona Foundation (S.F.).

The authors report no conflict of interest.

References


