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ORIGINAL ARTICLE

11β-hydroxysteroid dehydrogenase type-2 and type-1 (11β-HSD2 and 11β-HSD1) and 5β-reductase activities in the pathogenia of essential hypertension

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Abstract Cortisol availability is modulated by several enzymes: 11β-HSD2, which transforms cortisol (F) to cortisone (E) and 11β-HSD1 which predominantly converts inactive E to active F. Additionally, the A-ring reductases (5α- and 5β-reductase) inactivate cortisol (together with 3α-HSD) to tetrahydrometabolites: 5αTHF, 5βTHF, and THE. The aim was to assess 11β-HSD2, 11β-HSD1, and 5β-reductase activity in hypertensive patients. Free urinary F, E, THF, and THE were measured by HPLC–MS/MS in 102 essential hypertensive patients and 18 normotensive controls. 11β-HSD2 enzyme activity was estimated by the F/E ratio, the activity of 11β-HSD1 in compare to 11β-HSD2 was inferred by the (5αTHF + 5βTHF)/THE ratio and 5β-reductase activity assessed using the E/THE ratio. Activity was considered altered when respective ratios exceeded the maximum value observed in the normotensive controls. A 15.7% of patients presented high F/E ratio suggesting a deficit of 11β-HSD2 activity. Of the remaining 86 hypertensive patients, two possessed high (5αTHF + 5βTHF)/THE ratios and 12.8% had high E/THE ratios. We observed a high percentage of alterations in cortisol metabolism at pre-receptor level in hypertensive patients, previously misclassified as essential. 11β-HSD2 and 5β-reductase decreased activity and imbalance of 11β-HSDs should be considered in the future management of hypertensive patients.

Keywords Essential hypertension · 11β-HSD2 · 11β-HSD1 · 5β-reductase

Introduction

Cortisol homeostasis is important in controlling blood pressure. Cortisol deficiency is seen in patients with Addison's syndrome leading to hypotension, while excess cortisol is observed in Cushing's syndrome and determined by the onset of hypertension [1]. Recently our group reported that in essential hypertensive patients, the

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excretion of urinary free cortisol was greater than in normotensive subjects [2].

The tissue-specific responses to cortisol may be influenced as much by local pre-receptor metabolism as by circulating concentration. Cortisol is metabolized by several enzymes. In the kidney and colon, 11β-hydroxy steroid dehydrogenase type 2 (11β-HSD2) inactivates cortisol to cortisone and thereby protecting the mineralocorticoid receptor from illicit cortisol activation [3, 4]. In the liver and adipose tissue, cortisol is regenerated from cortisone by 11β-hydroxy steroid dehydrogenase type 1 (11β-HSD1) which catalyzes predominantly the reverse reductive reaction [5, 6]. Moreover, in the liver, the A-ring reductases (5α and 5β reductase) inactivate cortisol and cortisone (in conjunction with 3α-hydroxy steroid dehydrogenase) to its tetrahydrometabolites: 5α tetrahydrocortisol (5αTHF), 5β tetrahydrocortisol (5βTHF), and tetrahydrocortisone (THE) [7]. Thus, cortisol availability to bind and activate the glucocorticoid and mineralocorticoid receptors may be modified by several enzymes depending on pre-receptor hormone metabolism.

Congenital or acquired defects in 11 β-HSD2 result in cortisol-dependent mineralocorticoid excess. An autosomal recessive congenital deficit was first described by Ulick and New in 1979 and was coined with the term of Apparent Mineralocorticoid Excess (AME) [8]. Its clinical presentation includes: low birth weight, failure to thrive, short stature, severe hypertension, hypokalemia, suppressed plasma renin activity (PRA), and undetectable aldosterone. At the present time, the diagnosis is based on urinary cortisol metabolites which show an increased cortisol/cortisone ratio [9]. In addition, in some patients, the excretion of 5α-cortisol metabolites exceeds that of 5β-cortisol metabolites resulting in a high urinary 5αTHF/THF ratio, which suggests an additional defect in 5β-reductase activity [10].

Other studies have demonstrated a relation between urinary steroids and blood pressure, such as: the observation that urinary levels of glucocorticoids and mineralocorticoids have a strong familial pattern [11]; the finding that partial deficiency of 11β-HSD2 in patients with essential hypertension can be amplified by a high sodium intake [12] and the new concept of fetal programming by high exposure to maternal glucocorticoids that promotes high blood pressure and cardiovascular disease in adulthood [13, 14]. Furthermore, it is currently accepted that the pharmacological inhibition of the 11β-HSD2 activity with licorice or carbenoxolone leads to hypertension, increased urine cortisol excretion and high urine F/E ratio [15–19].

At the beginning AME was considered a rare disease; however, there is growing evidence that partially defective 11β-HSD2 activity, by mutations or polymorphisms, may play a role in triggering hypertension [6, 20–23]. Furthermore, polymorphic markers have been reported, relating to the onset and severity of hypertension. Our group has recently described, in accordance with in vitro studies, a negative correlation between the length of the CA repeat and the levels of cortisol/cortisone, aldosterone, and PRA [24, 25].

The activity of the 11β-HSD1 is bidirectional, however in vivo, its predominant activity is as a reductase, transforming cortisone to cortisol in the presence of NADPH. This enzyme is also expressed in other tissues, such as adrenal, ovary, decidua, blood vessel walls, and dendritic cells among others [5, 26–29]. Emerging reports have suggested an important role in regulating the amount of local cortisol at tissue level and its over-expression has been linked to the onset of hypertension, through the increase in the hepatic synthesis of angiotensinogen [5, 29–32].

The pathogenic role of 11β-HSD1 in the development of hypertension and metabolic disorders has been demonstrated in transgenic mice models with selective overexpression of 11β-HSD1 in adipose tissue and liver [33, 34]. With these new data, it has been proposed that the aberrant expression of 11β-HSD isozymes is involved in the pathogenesis of diverse human diseases including hypertension, insulin resistance, and obesity [35].

The contribution of the 11β-HSD2 enzyme to cortisol metabolism can be estimated by calculating the F/E ratio [1] and the activity of 11β-HSD1 enzyme may be inferred by the relationship between the urinary metabolites tetrahydrocortisol (5αTHF + 5βTHF)/tetrahydrocortisone (THE) in the presence of normal F/E ratio [5, 6, 36, 37]. A high (5αTHF + 5βTHF)/THE ratio indicates an imbalance in 11β-HSDs activities in favor of type 1 as compared to type 2 [38]. Furthermore, deficiencies in the activity of 5α/5β-reductase and the 3α-HSD, that may also contribute to an altered cortisol metabolism, can be assessed through the transformation from F to 5αTHF + 5βTHF and from E to THE [39].

The aim of this study was to assess whether the activities of 11β-HSD2, 11β-HSD1, and 5β-reductase are altered in essential hypertensive patients, which may thus contribute to their pathogenesis.

Patients and methods

Subjects

The protocol followed in this study was approved by the Ethical Committee of the Faculty of Medicine, Pontificia Universidad Católica de Chile and was explained to all participants who gave their written informed consent, according to the guidelines of the Declaration of Helsinki.

We studied 102 essential hypertensive patients selected from a Chilean governmental primary care center. Blood pressure (BP) records were reviewed and those patients

with diastolic BP >90 mmHg and systolic BP >140 mmHg, on at least two occasions on separate days, were considered as hypertensive. Patients with renal disease, diabetes mellitus, hepatic failure, cardiac failure, clinical Cushing's disease, and primary aldosteronism were excluded. As controls, we selected 18 normotensive subjects with BP <140/90 mmHg, determined on at least two different occasions, with no history of diabetes, renal failure, hypothyroidism, exogenous glucocorticoid use, and history of hypertension in first degree relatives. All subjects consumed a normal diet with no attempt taken to control sodium intake and had normal aldosterone and plasma renin activity (PRA). In patients who were using antihypertensives, drugs affecting the renin angiotensin system such as beta-blockers, angiotensin-converting enzyme (ACE) inhibitors, angiotensin II receptor blockers, diuretics, and spironolactone, a washout was performed for at least 15 days.

Methods

Blood and 24-h urine samples were obtained between 9 and 10 h, after a 12-h fast. The patients were left sitting at rest for 10 min before drawing blood samples to measure: sodium, potassium, PRA, and aldosterone (SA) using the methods previously described [2]. In urine samples, collected without preservatives, we measured simultaneously: cortisol (F), cortisone (E), and their free tetrahydrometabolites: THFs (5α-tetrahydrocortisol + 5β-tetrahydrocortisol) and THE (Tetrahydrocortisone) by high performance liquid chromatography associated mass spectrometry—mass (HPLC–MS/MS).

Measurement of F, E, 5αTHF, 5βTHF, and THE in the urine

Five calibrators containing 7.5–120 nmol/l of F, E, αTHF, βTHF, and THE (Steraloids, Newport, USA) were prepared in 40% methanol/water. Forty microliters of internal standard (6 alpha-methyl prednisolone, 3.44 μmol/l in 40% methanol/water) were added to 1.0 ml urine samples previously centrifuged and to each concentration of the calibration curve (1.0 ml each one). Then the steroids from samples and calibration solutions were extracted on columns of solid phase extraction according to the protocol described by Turpeinen et al. [40].

Analysis by HPLC–MS/MS

Five microliters of the extracted (samples and calibrators) were injected on a HPLC (Agilent model 1200 with autosampler) equipped with an API 3200 QTRAP triple quadruple mass spectrometer (Applied Biosystem, Foster City, CA, USA). The liquid chromatography was performed using the protocol previously described by Turpeinen et al. [40]. The concentrations were analyzed in negative-ion mode with the following transitions: Cortisol *m/z* 361.0 to *m/z* 331.0; cortisone: *m/z* 359.0 to *m/z* 329.0; 5αTHF and 5βTHF: *m/z* 365.3 to *m/z* 335.0; THE: *m/z* 363.1 to *m/z* 332.9, and 6 alpha-methyl prednisolone *m/z* 373.0 to *m/z* 343.0. The results were analyzed using a MDS Sciex Analyst software version 1.4.2 in a Windows environment.

The above chromatographic conditions allowed simultaneously quantification of F, E, THE, and THFs but did not allow for the separation of the isomers αTHF and βTHF. Therefore, the concentration of THF is the sum of 5αTHF and 5βTHF and is referred to as THFs. The retention times were (X ± SD) cortisone: 3.87 ± 0.21 min; cortisol: 4.34 ± 0.16 min; THFs: 5.48 ± 0.07 min; THE: 5.66 ± 0.05 min, and 6 alpha-methyl prednisolone: 5.2 ± 0.2 min. A representative chromatogram is shown in Fig. 1. The area under the curve was calculated for each of the peaks obtained. The ratio of the area between the peak for each concentration of steroids tested to the peak obtained by the internal standard was calculated. Finally, we constructed a calibration curve and the linearity of each standard curve was confirmed by plotting the ratio of each steroid against their concentrations (Fig. 2). The coefficients of variation intra- and inter-assay were below 20% for each of the four steroids analyzed.

To validate these analyses, the concentrations of urinary free cortisol from 102 hypertensive patients were compared with those obtained by RIA in automated equipment (IMMULITE 2000, Siemens Healthcare Diagnostics Inc., Germany). We found a correlation between both methods with Pearson coefficient ($r = 0.7477$, $P < 0.0001$) and with an equation for this relationship: $y = 0.4425x–0.9727$ where x = cortisol by RIA and y = cortisol by HPLC–MS/MS (Fig. 3). Even though there was a linear regression, cortisol concentrations obtained by HPLC–MS/MS were lower than those obtained by RIA as previously described by others [41–43]. Additionally, comparison of these two methods by Bland–Altman analysis [44] showed a confidence interval for the average of the differences with an upper limit of 53.8 (CI 95% 49.32; 58.32) and a lower limit of 1.4 (CI 95% −3.13; 5.87). The difference values between both methods tend to increase when the average increases. These differences may be explained by the HPLC–MS/MS being more specific than the RIA assay, since the former by in large eliminates the problem of cross reactivity.

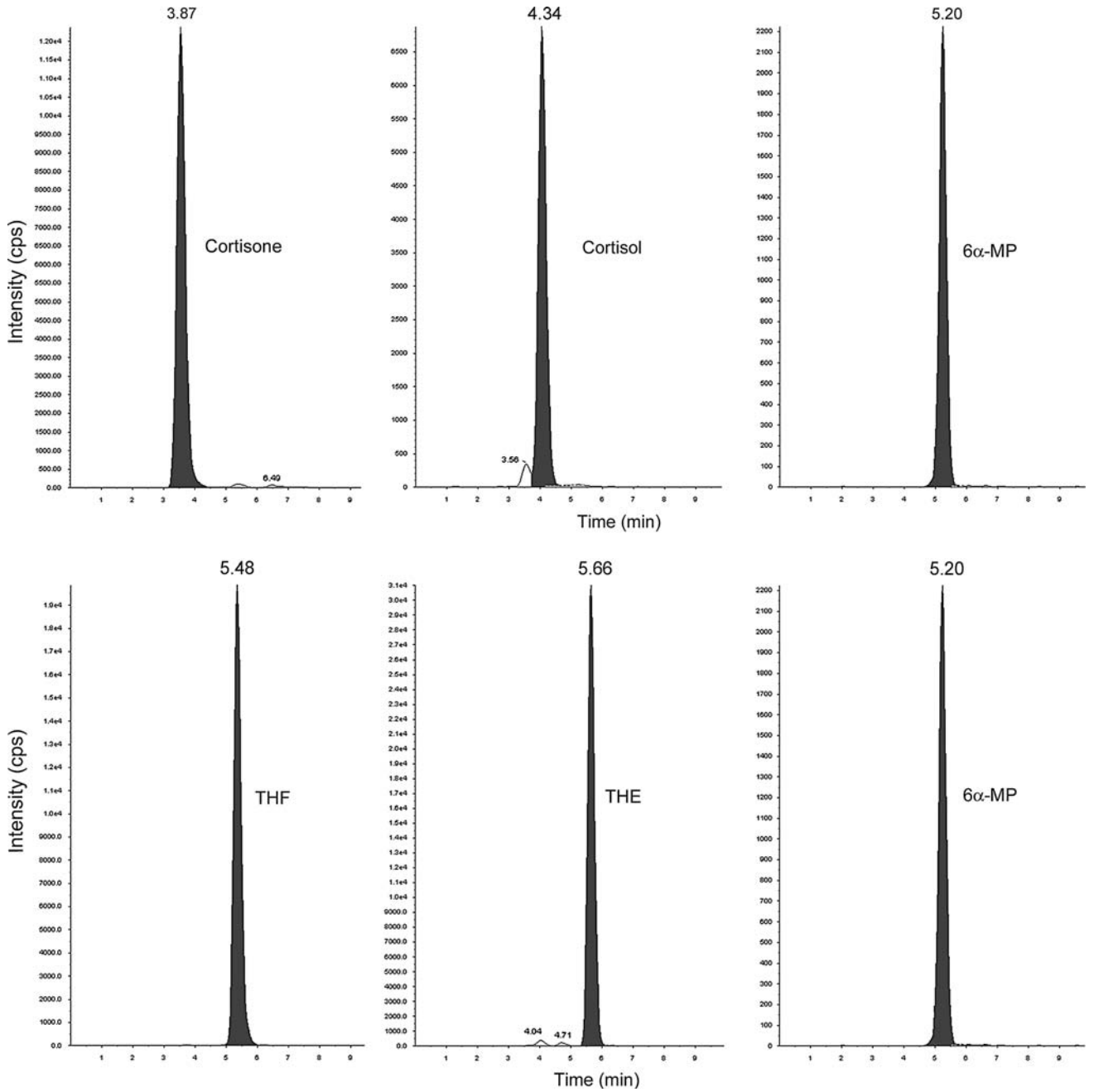

Fig. 1 Representative chromatogram of cortisol, cortisone, tetrahydrocortisol (THF), tetrahydrocortisone (THE) and 6 alpha-methyl prednisolone (6α-MP). THF represents 5αTHF + 5βTHF

Data analysis

The results were expressed as median values (inter-quartile range, (Q1–Q3)). The activity of the enzyme 11β-HSD2 was assessed by measuring the F/E ratio and the activity of 11β-HSD1 was inferred by the THFs/THE ratio. The activity of the enzyme 5β-reductase was estimated by measuring the E/THE ratio. Since the F, E, THFs, and THE concentrations showed normal distribution; the activities of these enzymes were considered altered when their respective ratios exceeded the mean plus 2 standard deviation respect to the normotensive control group. Hypertensive subjects were divided into two groups: those who had high F/E ratio and those who had normal F/E ratio.

The comparison between subject groups was performed using the Kruskal–Wallis and Dunn's multiple comparisons test (program GraphPad Prism, version 4.0). To analyze the relationship between cortisol and cortisone and between THFs and THE, lineal models were estimated. In

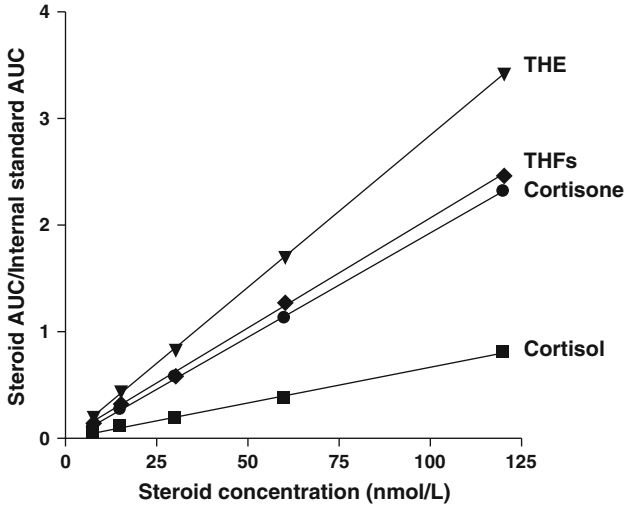

Fig. 2 Calibration curve of cortisol, cortisone, THFs and THE. Data represent the mean of three independent determinations. *AUC* Area under the curve. The equations of the lines are: $y = 0.0067\,x - 0.0037$, $r = 0.9991$, $P < 0.0001$ for cortisol; $y = 0.0196\ x - 0.030$, $r = 0.9998$, $p < 0.0001$ to cortisone, and $x = 0.0207x + 0.0038$, $r = 0.9995$, $P < 0.0001$ for THFs; $y = 0.0286\ x - 0.0096$, $r = 0.9999$, $P < 0.0001$ for THE

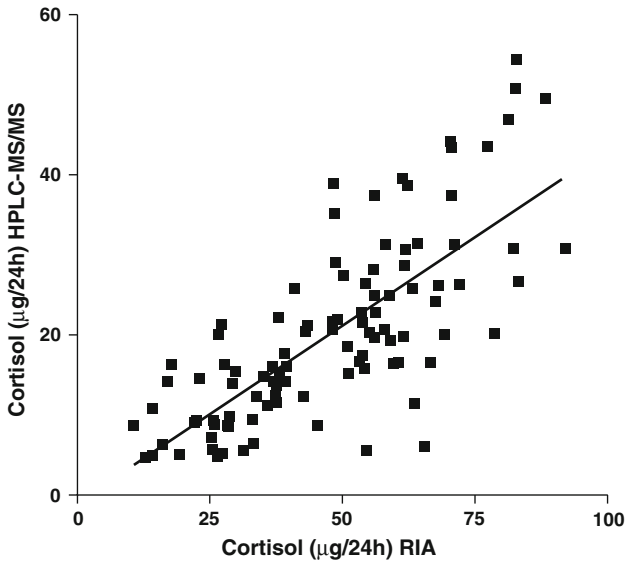

Fig. 3 Correlation between results of urinary free cortisol measurement ($n = 102$ hypertensive patients) obtained by RIA and HPLC–MS/MS methods. The equations of the lines are described in the text

these models, the variable group of subject and the interaction of the group with the independent continuous variable (cortisol, THFs) were included. The comparison of the equations of the regression lines was made by using the likelihood test (Program SPSS ® version 16.0.2). Differences were considered statistically significant at $P < 0.05$.

Results

Activities of 11β-HSD isoenzymes

The activity of 11β-HSD2 estimated by calculating the ratio F/E in urine showed that of the 102 essential hypertensive patients studied, 16 patients (15.7%) showed an increase in the ratio F/E, which was due to an increase in the excretion of cortisol and not to a decrease in the excretion of cortisone (Table 1). The relationship between urinary cortisol and cortisone concentrations (Fig. 4) fitted different regression lines in hypertensive patients with high F/E ratio and with normal F/E ratio. The equations of the lines were: $E = 1.22$ F-2.298 ($r^2 = 0.9173$, $P < 0.0001$) for patients with a high F/E ratio and $E = 1.22$ F $+ 26.69$ ($r^2 = 0.4228$, $P < 0.0001$) for patients with a normal F/E ratio. The equations of these lines have the same slope but differ in the intercept with the y axis indicating that the subjects correspond to different populations ($P < 0.05$). Thus, for the same amount of cortisol excreted in urine, the group with high F/E ratio possesses a similar amount of cortisone. In contrast, hypertensive patients with a normal F/E ratio possess approximately twice the concentration of cortisone. In this sub-group of 16 hypertensive patients, the PRA was lower than the normotensive group (Table 1) but no correlation was found with the ratio F/E ($r = 0.2890$, pNS). No association was found between the ratio F/E and plasma potassium levels ($r = 0.3592$, p NS), serum aldosterone concentration (SA) ($r = -0.2798$, pNS) and the product SA $\times$ ARP ($r = 0.040$, pNS). In this subgroup of hypertensive subjects only one patient had a high THFs/THE ratio. In the group of hypertensive patients with a normal F/E ratio, the excretion of cortisol and cortisone was about 30% higher than in normotensive group allowing them to maintain the normal ratio (Table 1).

The activity of 11β-HSD1, inferred by calculating the THFs/THE ratio, in the remaining 86 essential hypertensive patients showed two patients with a high THFs/THE ratio, indicating an imbalance in 11β-HSDs activity in favor to 11β-HSD1 compared to 11β-HSD2. The normotensive control group showed similar excretion of THFs but lower excretion of THE than the values published by Choi et al. [45] and as a consequence the median THFs/THE ratio is 2.2. Although this ratio is higher than normally reported for total THFs/THE, it is within the reference range previously described for free THFs/THE ratio [36, 46]. The relationship between urinary THFs and THE concentrations (Fig. 5) fitted similar regression lines in hypertensive patients with high F/E ratio and with normal F/E ratio. All the hypertensive patients adjusted to a common regression line, of which

Table 1 Clinical and biochemical characteristics of essential hypertensive patients and normotensive controls classified according to 11β-HSD2 enzyme activity

	Hypertensives		Normotensives
	High F/E ratio	Normal F/E ratio	Controls
n (M/W)	16 (3/13)	86 (16/70)	18 (2/16)
Age (years)	50.5 [44.0–61.5]	55.5 [46.5–61.0]	49 [46.0–51.0]
BMI (kg/m^2)	28.5 [27.2–31.4]	28.7 [26.6–32.1]	27.1[25.0–31.2]
Blood pressure			
Sistolic (mmHg)	140 [137.5–155.5][a]	146.5 [133.0–160.0][a]	115.5 [109.0–123.0]
Diastolic (mmHg)	90 [83.5–98.3][a]	90 [78.0–99.0][a]	73.5 [70.0–80.5]
Na^+ pl (mEq/l)	141.0 [139.0–142.5]	141.0 [139.0–142.0]	141.5 [139.0–142.0]
K^+ pl (mEq/l)	4.3 [3.8–4.7]	4.1 [3.9–4.5]	4.3 [4.0–4.5]
Na^+/K^+	32.8 [29.3–36.9]	34.4 [30.9–36.3]	33.4 [31.7–36.5]
Na + ur (mEq/24 h)	217.5 [169.0–270.5]	190 [149.0–240.5]	160 [113.0–218.0]
SA (ng/dl)	5.4 [3.2–7.7]	5.5 [3.0–7.4]	5.5 [3.2–8.5]
PRA (ng/ml*h)	0.8 [0.3–1.2][a]	0.6 [0.3–1.0][a]	1.4 [1.0–1.9]
SA*PRA product	3.2 [2.0–8.1]	2.9 [1.7–6.1][a]	5.6 [4.1–16.7]
Cortisol (μg/24 h)	30.1 [20.8–43.7][a]	16.4 [9.5–23.4][b]	10.5 [6.1–18.7]
Cortisone (μg/24 h)	40.6 [22.1–45.5]	46.7 [34.7–60.2][a,b]	28.2 [19.5–48.6]
F/E ratio	0.9 [0.8–0.9][a]	0.4 [0.3–0.5][b]	0.4 [0.2–0.5]
THFs (μg/24 h)	30.8 [16.1–48.9]	27.9 [20.7–39.4]	32.6 [20.2–45.2]
THE (μg/24 h)	11.8 [8.5–16.4]	13.1 [9.8–19.5]	14.9 [12.4–19.7]
THFs/THE ratio	2.5 [1.6–3.1]	2.0 [1.7–2.5]	2.1 [1.5–3.1]
E/THE ratio	3.1 [1.9–5.0]	3.2 [2.3–4.5][a]	2.5 [1.3–3.3]

Values correspond to median [Q_1–Q_3]. *n* number of subjects, *M* men, *W* women, *BMI* body mass index, *SA* serum aldosterone, *PRA* plasma renin activity. THFs represent 5αTHF + 5βTHF

[a] $P < 0.05$ respect to normotensives, [b] $P < 0.05$ respect to hypertensives with high F/E ratio, Kruskal−Wallis test and Dunn's multiple comparisons test

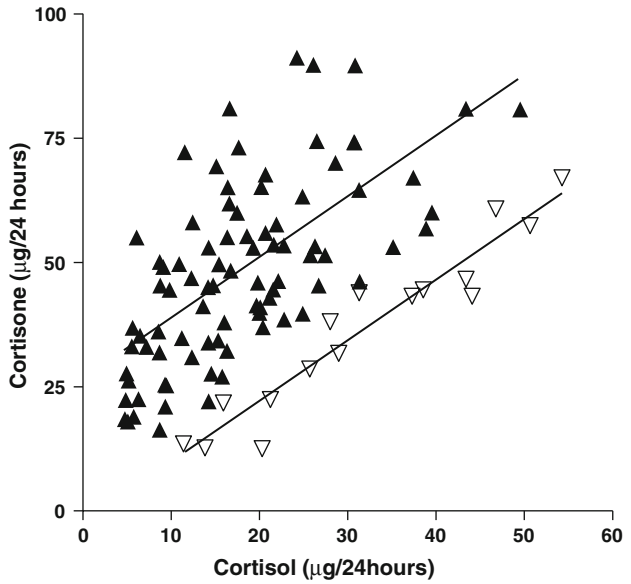

Fig. 4 The relationship of cortisol–cortisone in essential hypertensive patients with normal (*closed triangles*) and deficient (*open triangles*) activity of the enzyme 11β-HSD2. The equations of the lines are described in the text

the equation is THE = 0.511THFs−0.277 ($r^2 = 0.584$, $P < 0.0001$). These results indicate that for equal amount of THFs excreted in either of the two groups of hypertensive patients, there is a corresponding proportional amount of THE independent of renal 11β HSD2 activity.

Activity of enzymes 5β-reductase and 3α-hydroxy steroid dehydrogenase (3α-HSD)

The activity of 5β-reductase and 3α-HSD, inferred by calculating the ratio E/THE, showed that 16 of 102 essential hypertensive patients (15.7%) had a high E/THE ratio, suggesting an alteration of the enzymes 5β-reductase and/or the 3αHSD. These 16 patients were distributed as follows: four patients also had deficient activity of the enzyme 11β-HSD-2 (high F/E ratio), one suggested an enhanced activity of 11β-HSD1 (high THFs/THE ratio) and 11 patients belonging to the group of hypertensive patients with normal balance of the enzymes 11β-HSD type 1 and 2 activities (normal F/E and normal THFs/THE ratios).

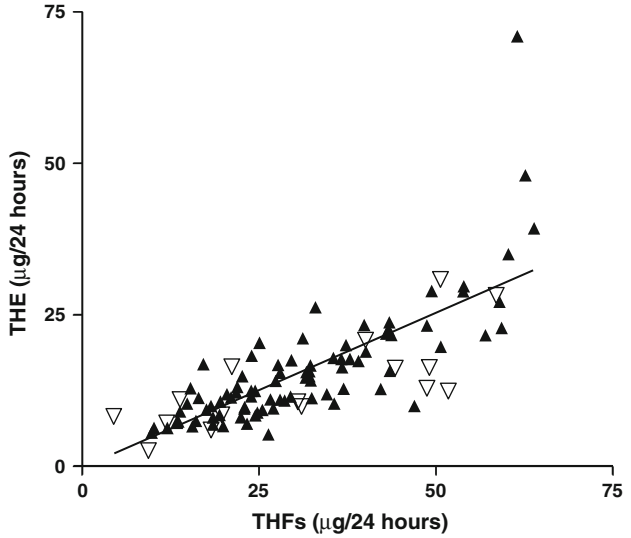

Fig. 5 The relationship of THFs–THE in essential hypertensive patients with normal (*closed triangles*) and deficient (*open triangles*) activity of the enzyme 11β-HSD2. The equation of the regression line is described in the text. THFs represent 5αTHF + 5βTHF

Discussion

The results of the present study show that 29 (28.4%) of this cohort of 102 essential hypertensive patients demonstrated a change, either in isolation or in combination, in the activities of the main enzymes involved in cortisol metabolism.

A partial deficit in the activity of the enzyme 11β-HSD2 was observed in 16 of the 102 essential hypertensive patients studied. These finding were similar to those previously described by our group evaluating the F/E ratio in serum, where we found a prevalence of 10.5% [24]. We found no correlation between the increase of the F/E ratio and the levels of PRA, despite lower values of PRA detected in hypertensives in comparison with normotensive controls. This could be due to the multiplicity of factors that regulate the biosynthesis of renin and factors that influence their levels, by enhanced activation of mineralocorticoid receptor or through changes in the expression of the sodium channel as was recently reported by our group [47].

There are several reasons that may explain a deficient activity of the enzyme 11β-HSD2. Mutations have been reported in the coding regions that trigger the onset of arterial hypertension, severe and early, with suppression of plasma renin activity and aldosterone levels [6, 20, 21]. Our group recently reported the case of a child carrying such a mutation who showed short stature, renal failure with nephrocalcinosis, and hypertension [22]. In some cases, however, the hypertensive profile begins later and is less severe, possibly manifesting as hyporeninemic hypertension of the adult [21]. Furthermore, polymorphic markers have been reported in the *HSD11B2* gene which associates to hypertension development. Among these polymorphisms is the presence of a CA-repeat microsatellite in intron 1 of the *HSD11B2* gene, which has been correlated to high F/E ratios and a higher salt sensitivity [24, 25]. In addition, other polymorphism markers had been described such as Thr156/Thr(C468A) in exon 2 (ex2) and Glu178/Glu(G534A) in exon 3 (ex3) present in a higher prevalence in hypertensive patients than in normotensives subjects [48, 49]. However, this association was not correlated with salt sensitivity and only C468A correlated significantly with hypertension [49]. Other studies have focused on the 5′-regulatory region, identifying that polymorphisms G-209A and G-126A in the promoter region produce a diminished HSD11B2 transcription, favoring salt sensitivity and hypertension with higher urinary ratios of cortisol to cortisone metabolites [50].

Herein we report an imbalance in 11β-HSDs activities in favor to type 1 compared to type 2 in two patients of the 86 hypertensive patients with normal F/E ratio. In a recent study carried out by our group in 49 morbidly obese individuals, we found that those patients who had hypertension had a higher hepatic 11β-HSD1 mRNA expression than non-hypertensive obese subjects [29]. Other authors have found that transgenic rats which over express the 11 β-HSD1 mRNA in adipose tissue develop obesity and hypertension and those which demonstrate overexpression in the liver develop hypertension but not obesity [51]. These new data highlights the importance of studying the hypothesis that central obesity and metabolic syndrome are associated with splanchnic and portal hypercortisolism through higher reactivation of cortisol from cortisone by 11-βHSD1 in visceral adipose tissue (VAT), which could trigger hypertension and other associated metabolic disorders.

Some of the causes that could explain the increased activity of 11β-HSD1 in relation to 11β-HSD2 might be a higher expression of mRNA and protein of 11β-HSD1, associated with a higher generation of its cofactor NADPH. The generation of NADPH is dependent on the enzyme glucose-6 phosphate dehydrogenase and its activity determines the concentration of NADPH which is critical for the activity of 11β-HSD1 as a reductase [52]. In addition, recently it has been reported, that in humans the rate of cortisol regeneration in peripheral tissues is of similar magnitude to adrenal diurnal secretion of cortisol and occurs principally in the splanchnic circulation by the 11β-HSD1 of VAT [53]. These data suggest that increasing VAT during the lifetime of the patient associates with higher expression of 11β-HSD1, and this initiates a vicious circle, as this enzyme is shown in turn to promote increased visceral adiposity, leading to hypertension and metabolic

disorders [54]. Another alternative is the decrease in the concentration of endogenous inhibitors which may also increase the activity of 11β-HSD1 [55].

The deficiency of the enzyme 5β-reductase was observed in 15.7% of our cohort of essential hypertensive patients. This percentage is higher than those published by other authors [56, 57] who found about 5% of essential hypertensive patients had altered 5β-reductase activity inferred by 5βTHF/5αTHF ratio in the urine. 5α/5β-reductase enzymes are limiting enzymes in the metabolism of cortisol and their activities can also be modified by endogenous inhibitors. The presence in the urine of variable concentrations of endogenous inhibitors of 11β-HSDs and reductases opens another field of research, as these inhibitors may modulate the activity of these enzymes in vivo [55–57]. These finding could also explain the co-existence of alterations in the activities of the 11β-HSD type 2 and 1 with alterations in the activity of 5β-reductase, as observed in this study. In relation to the controversy about sexual dimorphism in 11β-HSDs, we found in our recently published study, no gender differences in 11βHSD1 expression in hepatic and adipose tissue [29]. To complement these expression data with the activity of 11β-HSDs, analysis of our current data for gender specificity showed consistent results demonstrating, in accordance with other author, no correlation with sexual dimorphism [58].

In conclusion this study shows a high percentage of alterations in cortisol metabolism at pre-receptor level in hypertensive patients, previously misclassified as essential. Further studies in confirmation of our hypothesis, could reclassify essential hypertensive patients, as affected by a partial deficit of 11β-HSD2 activity (suggestive of a "subclinical Apparent Mineralocorticoid Excess"), an imbalance in 11β-HSD type 1 in comparison to type 2 or a deficiency of 5β-reductase. This new classification could permit treatment of hypertensive patients with specific monotherapy, such as blockage of the mineralocorticoid receptor or inhibitors of 11β-HSD1, thus avoiding unspecific multidrug treatment, side effects, and reducing costs [59].

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