ORIGINAL ARTICLE

11-Beta Dehydrogenase Type 2 Activity Is Not Reduced in Treatment Resistant Hypertension

Lama Ghazi,[1] Tanja Dudenbostel,[1] Maria El Hachem,[1] Mohammed Siddiqui,[1] Chee Paul Lin,[2] Suzanne Oparil,[1] and David A. Calhoun[1]

BACKGROUND AND OBJECTIVE
Decreased renal 11-beta dehydrogenase type 2 (11β-HSD2) activity, as reflected by an increased urinary free cortisol to cortisone ratio (UFF/UFE), is associated with having hypertension (HTN). The current study was conducted to determine if reduced 11β-HSD2 activity is also associated with having resistant HTN.

METHOD
We evaluated 55 consecutive patients with RHTN, defined as blood pressure (BP) ≥140/90 mm Hg despite using ≥3 antihypertensive medications including a diuretic, and 38 patients whose BP was controlled on ≤3 medications to serve as a non-RHTN comparator group. All patients underwent biochemical evaluation, including measurement of 24-hour urinary UFF/UFE.

RESULTS
The 2 study groups had similar demographic characteristics. Systolic, diastolic BP, and number of antihypertensive medications were greater in patients with uncontrolled RHTN vs. the control group (167.5 ± 28.2/91.2 ± 18.8 vs. 126.6 ± 11.4/77.8 ± 8.65 mm Hg and 4.31 ± 1.23 vs. 2.74 ± 0.6, respectively). The 24-hour UFF was 13.6 ± 11.8 vs. 14.3 ± 10.7 μg/24 h and UFE was 64.9 ± 36.3 vs. 76.1 ± 44 μg/24 h such that the UFF/UFE was 0.22 ± 0.16 vs. 0.19 ± 0.09 in RHTN vs. the control group. This ratio was not associated to age, race, gender, and body mass index.

CONCLUSION
An elevated UFF/UFE was not present in this large cohort of patients with uncontrolled RHTN. This suggests that reduced conversion of cortisol to cortisone does not contribute to the development of RHTN.

Keywords: 11-beta dehydrogenase type 2; blood pressure; cortisol/cortisone ratio; hypertension; resistant hypertension.

doi:10.1093/ajh/hpx002

Cortisol and aldosterone are both secreted by the adrenal cortex and bind with similar affinity to the mineralocorticoid receptor (MR) *in vitro*.[1] Circulating concentrations of cortisol *in vivo* are 100–1000 fold higher than aldosterone. MR selectively binds to aldosterone due to the inactivation of cortisol to cortisone by 11-beta hydroxysteroid dehydrogenase type 2 (11β-HSD2).[2] Therefore, aldosterone is the major MR agonist. 11β-HSD2 is expressed in distinct MR rich tissues, specifically in sodium transporting epithelia such as distal nephron, colon, salivary glands, and in the placenta. 11β-HSD2 in the kidneys is primarily in the cortical collecting duct and has a protective role as it prevents cortisol activation of the MR.[2–5]

Apparent mineralocorticoid excess, is a rare form of salt-dependent hypertension (HTN)[6] that is caused by 11β-HSD2 mutation resulting in MR activation by cortisol leading to sodium retention, hypokalemia, and HTN. The diagnosis of apparent mineralocorticoid excess is based on urinary cortisol metabolites with high ratio of urinary cortisol (UFF) to cortisone (UFE).[2,5] Interestingly, normotensive healthy individuals may develop HTN with elevation of UFF/UFE through pharmacologic inhibition of the 11β-HSD2 activity with licorice or carbenoxolone.[7–11]

Inactivating mutations and competitive inhibition of 11β-HSD2 cause inherited or acquired HTN, leading to a state of excess MR activation in the kidneys that is characterized by an elevated UFF/UFE. These observations have lead to a growing interest in the role of 11β-HSD2 as a cause of HTN. In patients with primary HTN the half-life of cortisol is prolonged and UFF/UFE metabolites have been shown to be increased. These findings suggest that 11β-HSD2 may be mutated or inhibited in these patients.[2,5] Some studies have observed that UFF/UFE ratios were significantly greater in primary hypertensive patients when compared to normotensive individuals; however, UFF/UFE values vary greatly in primary hypertensive patients (0.8 ± 0.04[12] to 8.6 ± 4.75[13]). A UFF/UFE, if ≥3, corresponds to 50% reduction in 11β-HSD2 activity and is though to contribute to blood pressure (BP) elevation. Data regarding a higher than normal ratio (≥1) but <3 has been inconsistent.[14] Whether

Correspondence: Lama Ghazi (lamaghazi@gmail.com).

Initially submitted July 1, 2016; date of first revision November 9, 2016; accepted for publication January 5, 2017; online publication February 9, 2017.

[1]Vascular Biology and Hypertension Program, Cardiovascular Disease, Department of Medicine, University of Birmingham at Alabama, Birmingham, Alabama, USA; [2]Center for Clinical and Translational Science, University of Birmingham at Alabama, Birmingham, Alabama, USA.

alterations in 11β-HSD2 activity contributes to BP remains under discussion.[15–17]

Resistant HTN defined as a lack of BP control despite using 3 or more antihypertensive medications, including a diuretic, is present in 10–20% of the hypertensive population.[18,19] We hypothesized that reduced 11β-HSD2 activity as reflected by an increase in UFF/UFE might contribute to the development of RHTN. The aim of this study was to determine if reduced 11β-HSD2 activity is associated with having RHTN.

METHODS

The study is a retrospective analysis of consecutive patients referred to the University of Alabama at Birmingham Hypertension Clinic for evaluation and treatment of RHTN between 2012 and 2014.

Study population

The analysis included 55 patients with RHTN defined as having clinic BP ≥140/90 mm Hg despite use of a ≥3 drug regimen, including a diuretic[17] and 38 patients with controlled, non-RHTN (control group), defined as having BP <140/90 and on ≤3 medications. The majority of RHTN patients were on a 4-drug treatment regimen including chlorthalidone, an angiotensin converting enzyme inhibitor or angiotensin receptor blocker, a calcium channel blocker, and a beta blocker. All patients had been on stable BP antihypertensive regimen for at least 4 weeks prior to biochemical evaluation.

Baseline parameters

Assessed variables included demographics and anthropometrics (age, gender, body mass index—BMI), number of antihypertensive medications, duration of HTN, and biochemical analysis (serum potassium, creatinine, serum aldosterone concentration (SAC—ng/dl), plasma renin activity (PRA—ng/ml/h), aldosterone–renin ratio, and 24-hour urinary aldosterone (U-Aldo, μg/24 h), sodium ($U\text{-}Na^+$, mEq/24 h), potassium ($U\text{-}K^+$, mEq/24 h), cortisol (UFF, μg/24 h), cortisone (UFE, μg/24 h), UFF/UFE ratio, and urinary creatinine(mg/24 h). UFF and UFE levels were obtained by gas chromatography and mass spectrometry using a stable isotope-labeled internal standard.[20] Aldosterone–renin ratio was calculated by the formula aldosterone–renin ratio = SAC/PRA. SAC, PRA, and 24-hour urine levels were analyzed by mass spectrometry at Mayo Clinic (Mayo Medical Laboratories, Rochester, MN).[21–24] Adequacy of the 24-hour urine collection was assessed by measuring 24-hour U-Cr by comparing total creatinine in the sample to predicted creatinine.[25] As per routine clinical care, 24-hour urine collections were done while patients were consuming their usual diet and without change in their level of physical .[18]Patients were advised to return the collections early in the morning. Adherence was measured by patient self-report and by monitoring of medication refill rates.

BP assessment

Clinic BP was measured according to guidelines by a trained physician using the auscultatory method with a manual sphygmomanometer (Welch Allyn, Skaneateles Falls, NY). Patients were seated for 5 minutes in a rested position with both feet on the ground and with their back supported while the arm was supported at the level of the heart. An appropriate sized cuff was used with a cuff bladder encircling at least 80% of the arm. Three BP readings were taken at intervals of 2 minutes by the physician and the second and third reading was used to average BP. The BP was measured in both arms and the arm with the higher BP was used for further BP measurements.[18]

Exclusion criteria

Exclusion criteria for this analysis included: missing baseline demographics, suspicion of being nonadherent with prescribed medications, documented chronic kidney disease, treated with antidepressants, thyroid replacement hormones, or mineralocorticoid antagonist (spironolactone or eplerenone), diagnosed with primary aldosteronism (defined as PRA <1 ng/ml/hr and 24-hour U-Aldo >12 μg).

The study was approved by the University of Alabama at Birmingham Institutional Review Board and conducted according to institutional guidelines.

Statistical analysis

Demographics and clinical characteristics were summarized using descriptive statistics with mean ± SD[10] for continuous variables, frequency, and percentage for categorical variables. A chi-squared test and Student's *t*-test were performed to make the statistical comparisons of demographics, clinical, and laboratory measures between uncontrolled RHTN and non-RHTN groups. The reference ranges from University of Alabama at Birmingham and Mayo Clinic were also used for comparisons of laboratory measures and 24-hour urinary analysis. As an exploratory analysis, the associations of UFF/UFE ratio to select patients' characteristics were evaluated using the Student's *t*-test and linear regression. A P value of <0.05 was considered statistically significant in 2-tailed statistical tests. All analyses were conducted using SAS 9.4 software (SAS Institute, Cary, NC). The box and whisker plot was created using R 3.3.0.

RESULTS

There were no significant differences in age, gender, race, BMI, duration of HTN, in patients with uncontrolled RHTN vs. with non-RHTN (Table 1). The entire cohort, both study groups combined, were on average 56 years old, with slightly more woman and more Caucasians, and mostly obese with BMI ≥30 kg/m^2. By definition, systolic BP, diastolic BP, and number of antihypertensive medications were significantly higher in patients with uncontrolled RHTN versus non-RHTN (167.5 ± 28.2/91.2 ± 18.8 mm Hg vs. 126.6 ± 11.4/77.8 ± 8.65 mm Hg and 4.31 ± 1.23 vs. 2.74 ± 0.6, respectively).

Biochemical analysis revealed no significant abnormalities in serum potassium, serum creatinine or SAC, and a nonsuppressed PRA in wither study group. The aldosterone–renin ratio was significantly higher in the uncontrolled RHTN group. (Table 2) All 24-hour urine collections were adequate. Mean U-Na^+ was 176.1 ± 91.4 mEq/24 h for RHTN patients compared to 175.5 ± 96.8 mEq/24 h (P = 0.97) in the non-RHTN group. Overall, 36.4% (20/55) of the patients with RHTN and 33.3% of the non-RHTN patients (6/18) had U-Na^+ >200 mEq/24 h indicative of high sodium intake. Mean UFF and UFE were within normal limits in both groups, with nonsignificant higher trends observed in the control subjects.[26] The activity of 11β-HSD2 estimated by UFF/UFE showed that both uncontrolled RHTN and non-RHTN patients had a low ratio, i.e., <1.5 (0.22 ± 0.16, and 0.19 ± 0.1 [P = 0.36], respectively), indicating normal enzyme activity. The distribution of UFF/UFE was similar in both groups, with the ratio ranging between 0.06–0.59 for RHTN and 0.067–0.44 in control (Figure 1). There was no significant association between UFF/UFE ratio and age, gender, race, BMI, U-Aldo, presence of primary aldosteronism, or U-Na^+ levels.

DISSCUSSION

11β-HSD2 activity, as indexed by UFF/UFE ratio is not different in patients with uncontrolled RHTN vs. patients with controlled, non-RHTN. We observed a mean UFF/UFE of 0.22 in patients with RHTN and 0.19 in patients without RHTN, indicating normal conversion of cortisol to cortisone in both study groups. To our knowledge this is the first study to assess 11β-HSD2 activity in RHTN. The findings are consistent with normal enzyme activity in this population suggesting that RHTN is not attributable to variation in conversion of cortisol to cortisone.

The role of 11β-HSD2 activity in the development of HTN has been evaluated in several studies comparing normotensive patients to patients with primary HTN (Table 3). In

Table 1. Baseline characteristics of uncontrolled resistant hypertension (RHTN) patients and the control group (non-RHTN)

Demographics, mean (SD)	Uncontrolled RHTN (n = 55)	Non-RHTN (n = 38)	P
Age (years)	56.4 ± 14.7	55.8 ± 12.8	0.84
Women, n (%)	22 (40.0)	22 (57.9)	0.09
African American, n (%)	30 (54.6)	16 (42.1)	0.24
Body mass index (kg/m^2)	33.6 ± 8.71	34.3 ± 8.67	0.72
Number of antihypertensive medications	4.31 ± 1.23	2.74 ± 0.6	<0.0001*
HTN duration (years)	20.7 ± 10.8	16.3 ± 8.86	0.052
Systolic blood pressure (mm Hg)	167.5 ± 28.2	126.6 ± 11.4	<0.0001*
Diastolic blood pressure (mm Hg)	91.2 ± 18.8	77.8 ± 8.65	<0.0001*

*Denotes statistical significance at P <0.05.

Table 2. Baseline biochemical analysis of uncontrolled resistant hypertension (RHTN) patients and the control group (non-RHTN)

Laboratory measures[a]	Uncontrolled (n = 55)	Reference range	Controls (n = 38)	P
Serum creatinine (mg/dl)	1.05 ± 0.33	0.7–1.3	1.06 ± 0.24	0.95
Serum potassium (Eq/dl)	3.91 ± 0.54	3.1–5.1	11.8 ± 48.7	0.32
Serum aldosterone concentration—SAC (ng/dl)	9.98 ± 9.35	≤21.0	9.62 ± 5.81	0.83
Plasma renin activity—PRA (ng/ml/hr)	9.41 ± 20.9	>1	11.1 ± 21.3	0.72
Aldosterone–renin ratio (SAC/PRA)	6.52 ± 6.67	NA	3.83 ± 41.2	0.027*
24-Hour urinary analysis[b]				
Aldosterone (μg/24 h)	12.6 ± 12.7	2.0–20.0	13.8 ± 11.7	0.66
Sodium (mEq/24 h)	176.1 ± 91.4	75.0–200.0	175.5 ± 96.8	0.97
Potassium (mEq/24 h)	63.3 ± 42.3	40.0–80.0	60.4 ± 34.8	0.72
Creatinine (mg/24 h)	1609 ± 575.5	1000.0–2000.0	1706.2 ± 703.5	0.48
Cortisol—UFF (μg/24 h)	13.6 ± 11.8	3.5–45.0	14.3 ± 10.7	0.75
Cortisone—UFE(μg/24 h)	64.9 ± 36.3	17–129	76.1 ± 44	0.18
Ratio (UFF/UFE)	0.22 ± 0.16	0.7–1.3[38–39]	0.19 ± 0.09	0.3

Abbreviations: UFF: 24-hour urinary free cortisol; UFE: 24-hour urinary free cortisone. *Denotes statistical significance at P <0.05.
[a]Reference range as per our lab at University of Alabama at Birmingham.
[b]Reference range as per Mayo Clinic[35].

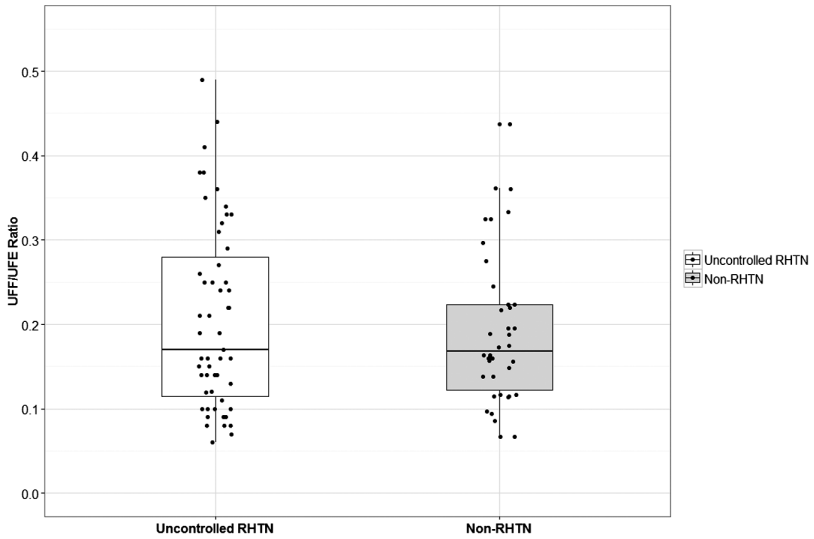

Figure 1. Boxplot of distribution of urinary cortisol to cortisone ratio (UFF/UFE) in uncontrolled resistant hypertension (RHTN), $n = 55$ and control non-RHTN group, $n = 38$.

Table 3. 11-beta dehydrogenase type 2 assessment indexed by cortisol to cortisone ratios in essential hypertensive vs. normotensive patients in different studies

	Ratio used	Primary hypertensives	Normotensives	*P* value
Mariniello *et al.* (2005)[12]	UFF/UFE	0.80 ± 0.04	0.57 ± 0.03	0.01
Campino *et al.* (2010)[36]	UFF/UFE	0.9 [0.8–0.9][a,b]	0.4 [0.2–0.5]	<0.05
Mongia *et al.* (2012)[13]	UFF/UFE	8.60 ± 4.75	3.66 ± 1.60	<0.005
Kosicka *et al.* (2013)[39]	UFF/UFE	0.363 [0.216–0.459][§]	0.330 [0.279–0.493]	NS

Abbreviations: UFF: 24-hour urinary free cortisol; UFE: 24-hour urinary free cortisone.
[a]Values refer to essential hypertensive patients who had a high UFF/UFE.
[b]Median (range).

around 20% of patients with primary HTN with no overt symptoms of apparent mineralocorticoid excess, a mild defect in 11β-HSD2 activity is found.[27,28] Previous studies assessed 11β-HSD2 activity by measuring conjugated urinary steroids metabolites tetrahydrocortisol/tetrahydrocortisone (THF/THE). However, these measurements have low sensitivity, especially if 11β-HSD activity elsewhere in the body is abnormal. UFF/UFE is therefore thought to provide the most sensitive index of 11β-HSD2 activity.[2,5,29–32] We assessed 11β-HSD2 activity by UFF/UFE, finding a mean of 0.22 ± 0.16 in RHTN group and 0.19 ± 0.09 in non-RHTN patients, indicating enzyme activity capable of converting cortisol to cortisone at a normal rate. We did not find any association between any biochemical laboratory parameters and UFF/UFE levels.

A recent study by Mariniello *et al.* of White Italian patients compared UFF/UFE in 292 patients with primary HTN vs. 163 normotensive controls to assess whether variants of 11β-HSD2 might be associated with the development of HTN.[12] There were significant differences in both groups-hypertensive patients were younger (47 vs. 57 years), had a higher mean BMI (27 vs. 25 kg/m^2). However, UFF/UFE was significantly higher in the hypertensive population than normotensives, 0.80 ± 0.04 vs. 0.57 ± 0.03, respectively ($P < 0.01$). The investigators concluded that the increase in UFF/UFE, a sensitive marker for renal 11β-HSD2 activity, in patients with HTN might be due to genetic alterations consistent with the growing evidence that mutations or polymorphisms of this enzyme may play a role in causing HTN.[14,30,33,34] The data also showed that the UFF/UFE ratio was significantly higher in salt-sensitive patients (0.9 ± 0.05) compared to salt-resistant patients (0.65 ± 0.05), reflecting reduced 11β-HSD2 activity. UFF and UFE mean values in this study were not reported. Our cohort included participants older than the primary HTN group in Mariniello's cohort and more obese individuals. The mean UFF/UFE in both RHTN patients and in the control group were lower than the ratios found by Mariniello *et al.* Note that, African Americans tend to be more salt-sensitive than Caucasians, such that and one would expect a difference in the ratio when analyzing RHTN cohort.[35] However, in the current study, the UFF/UFE was similar in African Americans and Caucasians indicating normal 11β-HSD2 activity.

Campino *et al.* estimated activity of 11β-HSD2 by UFF/UFE and compared the enzyme activity in 102 patients with primary HTN to 18 normotensive individuals in a Chilean population.[36] Of the primary hypertensive population, 15.7% had a high UFF/UFE due to an increase in cortisol

excretion. However, a clear cutoff for UFF/UFE ratio was not provided. UFF levels were significantly greater in the hypertensive group compared to normotensives (30.1 [20.8–43.7] vs. 10.5 1 [6.1–18.7] μg/24 h, $P < 0.05$); values we obtained for patients with RHTN were similar to normotensives. UFF/UFE was 0.9 [0.8–0.9] in the hypertensives vs. 0.4 [0.2–0.5] for the normotensives ($P < 0.05$); in our current analysis we observed similar ratios to that of normotensives. Campino *et al.* showed no association between PRA or SAC and UFF/UFE, which is in agreement with our findings. Furthermore, some studies have shown that partial deficiency in 11β-HSD2 in primary HTN can be amplified by high salt intake as reflected by a high U-Na^+ excretion.[37] In this study by Campino *et al.*, U-Na^+ values were similar across all studied groups (141.0 in the hypertensive population vs. 141.5 mEq/l in the normotensives) and were not associated with UFF/UFE. Similarly, we did not find any effect of U-Na^+ on UFF/UFE in our cohort, although mean U-Na^+ levels were higher than levels observed by Campino *et al.*

Based on a study on children with primary HTN, Mongia *et al.*[13] hypothesized that a defect in renal cortisol metabolism, as indicated by THF/THE or UFF/UFE elevation, secondary to a decrease in 11β-HSD2 activity, stimulates MR through cortisol and leads to development of HTN. Study limitations included using spot urine a less accurate assessment of urine metabolites UFF and UFE than 24-hour urine collection. The population consisted mostly of African American children. UFF/UFE ratio was significantly higher in the patients with HTN (8.60 ± 4.75) compared to controls (3.66 ± 1.60, $P < 0.005$). They, however, did not use UFF/UFE to index 11β-HSD2 activity and that may be due to the poor quality of measurement using spot urine.[38] Despite that, these study findings further supports the significance of 11β-HSD2 activity deficiency in the development of primary HTN. This was in contrast to our findings in which we did not observe any deficiency in enzyme activity in RHTN patients.

Not all studies have found higher UFF/UFE in primary HTN patients compared to normotensives. Kosicka *et al.* found no significant differences in UFF/UFE between hypertensives and controls.[39] However, the median of UFF/UFE was higher in hypertensives ($n = 79$) than the controls ($n = 70$), but not significant. UFF/UFE exceeded the reference range, ≤0.6 in 15.9% of hypertensive subjects and 7.1% of normotensives. The patients with HTN were more obese with a mean BMI of 30.5 kg/m^2 and had a mean age of 44 years. UFF and UFE in the hypertensive group were significantly lower than normotensives, 18.5 μg/24 h vs. 28.5, $P = 0.0002$ and 58.9 vs. 79.4, $P = 0.0015$, respectively. This cohort has very similar baseline characteristics to the current cohort. Our results are in agreement with Kosicka *et al.* as we found no association between UFF/UFE ratio and RHTN. The mean UFF/UFE value of 0.22 observed by us in patients with RHTN was less than the cutoff used by Kosicka, i.e., 0.6. The values we observed in our study groups overlapped with the UFF/UFE range in both normotensive and primary hypertensive patients seen by Kosicka. Our findings, however, do not confirm those earlier observations. We observed a low ratio both in patients with RHTN and without RHTN, providing no evidence of alterations in 11β-HSD2 activity in either group.

Strengths of the current analysis include evaluation of a diverse cohort of patients with confirmed RHTN, comparison to a control group of patients without RHTN, and use of 24-hour urine values to index UFF/UFE. Limitations include the cross-sectional study design, having evaluated all patients during stable but ongoing antihypertensive treatment, having measured only 11β-HSD2 and not 11β-HSD type 1 activity, and having not confirmed medication adherence by measurement of serum or urinary drug levels.

In summary, 11β-HSD2 activity is not reduced in patients with RHTN suggesting that variation in the conversion of cortisol to cortisone does not contribute to development of antihypertensive treatment resistance. The negative findings provide important insight into the mechanisms of RHTN.

ACKNOWLEDGMENTS

This work was supported by NIH grant NIH RO1 HL113004. Statistical analysis of research reported in this publication was partially supported by National Center for Advancing Translational Sciences of the National Institutes of Health under award number UL1TR001417.

DISCLOSURE

The authors declared no conflict of interest.

REFERENCES

1. Arriza JL, Weinberger C, Cerelli G, Glaser TM, Handelin BL, Housman DE, Evans RM. Cloning of human mineralocorticoid receptor complementary DNA: structural and functional kinship with the glucocorticoid receptor. *Science* 1987; 237:268–275.
2. Hammer F, Stewart PM. Cortisol metabolism in hypertension. *Best Pract Res Clin Endocrinol Metab* 2006; 20:337–353.
3. Edwards CR, Stewart PM, Burt D, Brett L, McIntyre MA, Sutanto WS, de Kloet ER, Monder C. Localisation of 11 beta-hydroxysteroid dehydrogenase–tissue specific protector of the mineralocorticoid receptor. *Lancet* 1988; 2:986–989.
4. Funder JW, Pearce PT, Smith R, Smith AI. Mineralocorticoid action: target tissue specificity is enzyme, not receptor, mediated. *Science* 1988; 242:583–585.
5. Ferrari P. The role of 11β-hydroxysteroid dehydrogenase type 2 in human hypertension. *Biochim Biophys Acta* 2010; 1802:1178–1187.
6. Bhatt DL, Kandzari DE, O'Neill WW, D'Agostino R, Flack JM, Katzen BT, Leon MB, Liu M, Mauri L, Negoita M, Cohen SA, Oparil S, Rocha-Singh K, Townsend RR, Bakris GL; SYMPLICITY HTN-3 Investigators. A controlled trial of renal denervation for resistant hypertension. *N Engl J Med* 2014; 370:1393–1401.
7. Epstein MT, Espiner EA, Donald RA, Hughes H, Cowles RJ, Lun S. Licorice raises urinary cortisol in man. *J Clin Endocrinol Metab* 1978; 47:397–400.
8. MacKenzie MA, Hoefnagels WH, Jansen RW, Benraad TJ, Kloppenborg PW. The influence of glycyrrhetinic acid on plasma cortisol and cortisone in healthy young volunteers. *J Clin Endocrinol Metab* 1990; 70:1637–1643.
9. Kageyama Y, Suzuki H, Saruta T. Glycyrrhizin induces mineralocorticoid activity through alterations in cortisol metabolism in the human kidney. *J Endocrinol* 1992; 135:147–152.

10. Sigurjonsdottir HA, Manhem K, Axelson M, Wallerstedt S. Subjects with essential hypertension are more sensitive to the inhibition of 11 beta-HSD by liquorice. *J Hum Hypertens* 2003; 17:125–131.
11. Whitworth JA, Williamson PM, Brown MA, Edwards CR. Haemodynamic and metabolic effects of carbenoxolone in normal subjects and patients with renal impairment. *Clin Exp Hypertens* 1994; 16:431–450.
12. Mariniello B, Ronconi V, Sardu C, Pagliericcio A, Galletti F, Strazzullo P, Palermo M, Boscaro M, Stewart PM, Mantero F, Giacchetti G. Analysis of the 11beta-hydroxysteroid dehydrogenase type 2 gene (HSD11B2) in human essential hypertension. *Am J Hypertens* 2005; 18:1091–1098.
13. Mongia A, Vecker R, George M, Pandey A, Tawadrous H, Schoeneman M, Muneyyirci-Delale O, Nacharaju V, Ten S, Bhangoo A. Role of 11βHSD type 2 enzyme activity in essential hypertension and children with chronic kidney disease (CKD). *J Clin Endocrinol Metab* 2012; 97:3622–3629.
14. Wilson RC, Dave-Sharma S, Wei JQ, Obeyesekere VR, Li K, Ferrari P, Krozowski ZS, Shackleton CH, Bradlow L, Wiens T, New MI. A genetic defect resulting in mild low-renin hypertension. *Proc Natl Acad Sci USA* 1998; 95:10200–10205.
15. White PC, Mune T, Agarwal AK. 11 beta-Hydroxysteroid dehydrogenase and the syndrome of apparent mineralocorticoid excess. *Endocr Rev* 1997; 18:135–156.
16. Ferrari P, Lovati E, Frey FJ. The role of the 11beta-hydroxysteroid dehydrogenase type 2 in human hypertension. *J Hypertens* 2000; 18:241–248.
17. Farman N, Bocchi B. Mineralocorticoid selectivity: molecular and cellular aspects. *Kidney Int* 2000; 57:1364–1369.
18. Calhoun DA, Jones D, Textor S, Goff DC, Murphy TP, Toto RD, White A, Cushman WC, White W, Sica D, Ferdinand K, Giles TD, Falkner B, Carey RM; American Heart Association Professional Education Committee. Resistant hypertension: diagnosis, evaluation, and treatment: a scientific statement from the American Heart Association Professional Education Committee of the Council for High Blood Pressure Research. *Circulation* 2008; 117:e510–e526.
19. McAdam-Marx C, Ye X, Sung JC, Brixner DI, Kahler KH. Results of a retrospective, observational pilot study using electronic medical records to assess the prevalence and characteristics of patients with resistant hypertension in an ambulatory care setting. *Clin Ther* 2009; 31:1116–1123.
20. Palermo M, Shackleton CH, Mantero F, Stewart PM. Urinary free cortisone and the assessment of 11 beta-hydroxysteroid dehydrogenase activity in man. *Clin Endocrinol (Oxf)* 1996; 45:605–611.
21. Fredline VF, Taylor PJ, Dodds HM, Johnson AG. A reference method for the analysis of aldosterone in blood by high-performance liquid chromatography-atmospheric pressure chemical ionization-tandem mass spectrometry. *Anal Biochem* 1997; 252:308–313.
22. Fredline VF, Kovacs EM, Taylor PJ, Johnson AG. Measurement of plasma renin activity with use of HPLC-electrospray-tandem mass spectrometry. *Clin Chem* 1999; 45:659–664.
23. Taylor RL, Machacek D, Singh RJ. Validation of a high-throughput liquid chromatography-tandem mass spectrometry method for urinary cortisol and cortisone. *Clin Chem* 2002; 48:1511–1519.
24. Taylor PJ, van Rosendal SP, Coombes JS, Gordon RD, Stowasser M. Simultaneous measurement of aldosterone and cortisol by high-performance liquid chromatography-tandem mass spectrometry: application to dehydration-rehydration studies. *J Chromatogr B Analyt Technol Biomed Life Sci* 2010; 878:1195–1198.
25. Ix JH, Wassel CL, Stevens LA, Beck GJ, Froissart M, Navis G, Rodby R, Torres VE, Zhang YL, Greene T, Levey AS. Equations to estimate creatinine excretion rate: the CKD epidemiology collaboration. *Clin J Am Soc Nephrol* 2011; 6:184–191.
26. Mayo Foundation for Medical Education and Research. http://www.mayomedicallaboratories.com/test-catalog/Overview/88903. 2015. Accessed 23 October 2015.
27. Walker BR, Stewart PM, Shackleton CH, Padfield PL, Edwards CR. Deficient inactivation of cortisol by 11 beta-hydroxysteroid dehydrogenase in essential hypertension. *Clin Endocrinol (Oxf)* 1993; 39:221–227.
28. Soro A, Ingram MC, Tonolo G, Glorioso N, Fraser R. Evidence of coexisting changes in 11 beta-hydroxysteroid dehydrogenase and 5 beta-reductase activity in subjects with untreated essential hypertension. *Hypertension* 1995; 25:67–70.
29. Best R, Walker BR. Additional value of measurement of urinary cortisone and unconjugated cortisol metabolites in assessing the activity of 11 beta-hydroxysteroid dehydrogenase in vivo. *Clin Endocrinol (Oxf)* 1997; 47:231–236.
30. Draper N, Stewart PM. 11beta-hydroxysteroid dehydrogenase and the pre-receptor regulation of corticosteroid hormone action. *J Endocrinol* 2005; 186:251–271.
31. Quinkler M, Stewart PM. Hypertension and the cortisol-cortisone shuttle. *J Clin Endocrinol Metab* 2003; 88:2384–2392.
32. Palermo M, Delitala G, Mantero F, Stewart PM, Shackleton CH. Congenital deficiency of 11beta-hydroxysteroid dehydrogenase (apparent mineralocorticoid excess syndrome): diagnostic value of urinary free cortisol and cortisone. *J Endocrinol Invest* 2001; 24:17–23.
33. Mune T, Rogerson FM, Nikkilä H, Agarwal AK, White PC. Human hypertension caused by mutations in the kidney isozyme of 11 beta-hydroxysteroid dehydrogenase. *Nat Genet* 1995; 10:394–399.
34. Wilson RC, Krozowski ZS, Li K, Obeyesekere VR, Razzaghy-Azar M, Harbison MD, Wei JQ, Shackleton CH, Funder JW, New MI. A mutation in the HSD11B2 gene in a family with apparent mineralocorticoid excess. *J Clin Endocrinol Metab* 1995; 80:2263–2266.
35. Wright JT Jr, Rahman M, Scarpa A, Fatholahi M, Griffin V, Jean-Baptiste R, Islam M, Eissa M, White S, Douglas JG. Determinants of salt sensitivity in Black and White normotensive and hypertensive women. *Hypertension* 2003; 42:1087–1092.
36. Campino C, Carvajal CA, Cornejo J, San Martín B, Olivieri O, Guidi G, Faccini G, Pasini F, Sateler J, Baudrand R, Mosso L, Owen GI, Kalergis AM, Padilla O, Fardella CE. 11β-Hydroxysteroid dehydrogenase type-2 and type-1 (11β-HSD2 and 11β-HSD1) and 5β-reductase activities in the pathogenia of essential hypertension. *Endocrine* 2010; 37:106–114.
37. Ingram MC, Wallace AM, Collier A, Fraser R, Connell JM. Sodium status, corticosteroid metabolism and blood pressure in normal human subjects and in a patient with abnormal salt appetite. *Clin Exp Pharmacol Physiol* 1996; 23:375–378.
38. Esler MD, Krum H, Sobotka PA, Schlaich MP, Schmieder RE, Böhm M; Symplicity HTN-2 Investigators. Renal sympathetic denervation in patients with treatment-resistant hypertension (The Symplicity HTN-2 Trial): a randomised controlled trial. *Lancet* 2010; 376:1903–1909.
39. Kosicka K, Cymerys M, Majchrzak-Celińska A, Chuchracki M, Główka FK. 11β-Hydroxysteroid dehydrogenase type 2 in hypertension: comparison of phenotype and genotype analysis. *J Hum Hypertens* 2013; 27:510–515.