See COMMENTARY page 600

Age-dependent Decrease in 11β-Hydroxysteroid Dehydrogenase Type 2 (11β-HSD2) Activity in Hypertensive Patients

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BACKGROUND
The prevalence of arterial hypertension lacking a defined underlying cause increases with age. Age-related arterial hypertension is insufficiently understood, yet known characteristics suggest an aldosterone-independent activation of the mineralocorticoid receptor. Therefore, we hypothesized that 11β-HSD2 activity is age-dependently impaired, resulting in a compromised intracellular inactivation of cortisol (F) with F-mediated mineralocorticoid hypertension.

METHODS
Steroid hormone metabolites in 24-h urine samples of 165 consecutive hypertensive patients were analyzed for F and cortisone (E), and their TH-metabolites tetrahydro-F (THF), 5αTHF, TH-deoxycortisol (THS), and THE by gas chromatography-mass spectroscopy. Apparent 11β-HSD2 and 11β-hydroxylase activity and excretion of F metabolites were assessed.

RESULTS
In 72 female and 93 male patients aged 18–84 years, age correlated positively with the ratios of (THF + 5αTHF)/THE ($P = 0.065$) and F/E ($P < 0.002$) suggesting an age-dependent reduction in the apparent 11β-HSD2 activity, which persisted (F/E; $P = 0.020$) after excluding impaired renal function. Excretion of F metabolites remained age-independent most likely as a consequence of an age-dependent diminished apparent 11β-hydroxylase activity ($P = 0.038$).

CONCLUSION
Reduced 11β-HSD2 activity emerges as a previously unrecognized risk factor contributing to the rising prevalence of arterial hypertension in elderly. This opens new perspectives for targeted treatment of age-related hypertension.

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The prevalence of hypertension increases with age. Normotensive individuals at the age of 55 years have a 90% chance of becoming hypertensive over their remaining life span. At present, 80% of individuals aged ≥70 years in North America, Europe, Japan, and Australia have a blood pressure ≥140/90 mm Hg or are taking antihypertensive medication. The resulting cardiovascular disease is by far the leading cause of death and disability in persons aged >70 years in the United States and other westernized countries.

Antihypertensive therapy is effective even in very old patients by reducing cardiovascular complications, yet reaching blood pressure goals is more difficult in geriatric than in young patients. In most elderly patients, a nonspecific combination of multiple antihypertensive drugs is used to control blood pressure. Reduced adherence and increased frequency of side effects are the consequences of such a combination therapy. Both factors introduce a risk for undertreatment in up to 55% of elderly individuals with hypertension. Consequently, a more specific treatment approach targeting the causative mechanism of hypertension is warranted.

The underlying causes of age-related hypertension have not been completely elucidated so far. Increased salt sensitivity is a characteristic of hypertension in elderly.[1] Salt sensitivity in younger persons has been attributed at least in part to a reduced activity of the 11β-hydroxysteroid dehydrogenase type 2 enzyme (11β-HSD2).[2–5] The NAD-dependent 11β-HSD2 determines intracellular, but not systemic, cortisol availability for the mineralocorticoid receptor by inactivating cortisol into cortisone.[6,7] The 11β-HSD2 enzyme is a gatekeeper for the mineralocorticoid receptor preventing its inappropriate stimulation by the glucocorticoid cortisol. Stimulation of the mineralocorticoid receptor by cortisol as observed in subjects with a loss-of-function mutation of the 11β-HSD2 or with a pharmacologically

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inhibited 11β-HSD2 activity results in volume expansion and arterial hypertension.[6,8,9]

Ageing contributes to changes in enzyme activity and availability by various mechanisms including methylation of genes, a mechanism known to affect 11β-HSD2 expression.[10–12] Thus, it is conceivable that hypertension in older age is associated with an age-related reduction in overall 11β-HSD2 activity. To test the novel hypothesis of an age dependency of 11β-HSD2 activity, we conducted a study in a sample of adult subjects with arterial hypertension throughout a broad age range.

METHODS

Study population and study design. Patients were recruited from the Department of Nephrology and Hypertension, University Hospital Bern. Between 2002 and 2006, we screened 465 consecutive outpatients. The patients were referred for arterial hypertension, kidney diseases, endocrinological disorders, obesity, or osteopenia/osteoporosis and examined for urine steroid metabolite excretion. Inclusion criteria were a diagnosis of arterial hypertension as defined by a systolic and diastolic blood pressure ≥140 and ≥90 mm Hg, respectively, without or by antihypertensive therapy to control blood pressure, and a complete data set comprising basic patient's demographics, presence of associated diseases, laboratory results, blood pressure levels, and type of drugs taken at the time of steroid hormone metabolite analysis. The study was approved by the institutional review committee.

Patients were excluded if one or more of the following conditions were present: pregnancy, thyroidal, parathyroidal or other overt endocrinological disorders apart from diabetes mellitus, secondary forms of arterial hypertension other than chronic kidney disease, cytolytic or cholestatic hepatopathy, urinary protein excretion >1 g/24 h, and/or documented intake of one or more of the following xenobiotics: liquorice, diuretics, ACE inhibitors, angiotensin II receptor antagonists, antibiotics, β-blockers, drosperinone, or exogenous glucocorticoids. With these strict criteria, a study population of 165 patients was selected.

Serum and urine sampling. Creatinine, sodium, potassium, albumin, bilirubin, alkaline phosphatase, aspartate, and alanine aminotransferase were measured in serum samples with standard laboratory methods. Creatinine clearance (CrCl) was calculated using the Cockcroft-Gault's formula standardized for body surface area. A cutoff of <60 ml/min/1.73 m^2 for CrCl was used to define patients with moderately or severely impaired renal function. Urine sampling was performed to measure urinary protein and steroid hormone metabolites corrected for the urinary creatinine concentration. Antihypertensive medication was totally withdrawn 1 week (spironolactone 2 weeks) before and during urinary steroid hormone analysis, if reasonable according to clinical judgment by the physician in charge of the patient, or substituted by a calcium-channel blocker or an α-blocker or a combination hereof.

Urinary steroid hormone analysis by gas chromatography-mass spectroscopy. In 24-h urine samples, excreted cortisol (F), cortisone (E), tetrahydro-F (THF), 5αTHF, THE, and TH-deoxycortisol (THS) were measured with gas chromatography-mass spectroscopy as described previously by Shackleton and reported by our group.[13,14]

The apparent 11β-HSD2 enzyme activity was obtained by calculating the urinary ratio of F/E and the corresponding ratio of their TH-metabolites (THF + 5αTHF)/THE (**Table 1**). As an index for the 11β-hydroxylase enzyme activity, the THS/(THF + 5αTHF) ratio was analyzed. High ratios of the urine metabolites indicate high substrate and low product concentrations and reflect low enzyme activity. Excretion of F metabolites was determined as Σ(F + E) and Σ(THE + THF + 5αTHF) to obtain a measure for systemic F production and to exclude alterations of the availability of those substrates used to calculate changes in enzyme activity as a confounding variable. To correct for variations in urine collection, urinary excretion of steroid hormone metabolites was expressed as μg steroid hormone metabolite per mmol of urinary creatinine excretion.

Statistical analysis. Data were expressed as means ± s.d. unless otherwise stated. Age, CrCl, and systolic as well as diastolic blood pressure were successfully tested for standard normal distribution. First-order linear regression modeling and correlations were chosen to analyze the relationship between age and 11β-HSD2 enzyme activity expressed as F/E and (THF + 5αTHF)/THE ratios. The same approach was chosen to test for correlations between renal function and 11β-HSD2 enzyme activity as well as between age and apparent 11β-hydroxylase enzyme activity expressed as THS/(THF + 5αTHF) ratio. A multivariate linear regression model for the effect of age, renal function, and gender on F/E, (THF + 5αTHF)/THE, THS/(THF + 5αTHF) ratios and for F metabolite excretion expressed as the sums of (THF + 5αTHF + THE) and (F + E),

Table 1 | Mean (±s.d.) urinary steroid hormone metabolite levels and derived ratios of metabolites

Steroid hormone	Steroid hormone metabolite measured	$x \pm$ s.d.
11-Deoxycortisol	THS (μg/day)	75.5 ± 44.4
Cortisol	F (μg/day)	105 ± 61
	THF (μg/day)	2232 ± 1083
	5αTHF (μg/day)	2025 ± 1460
Cortisone	E (μg/day)	134 ± 63
	THE (μg/day)	5193 ± 2766
Derived indicators of steroid hormone metabolism		
F/E		0.82 ± 0.41
(THF + 5αTHF)/THE		0.85 ± 0.28
F + E (μg/mmol creatinine)		21.6 ± 9.64
THE + THF + 5αTHF (μg/mmol creatinine)		793 ± 373
THS/(THF + 5αTHF)		0.02 ± 0.01

respectively, was applied. χ^2 testing was used to identify differences on categorical and dichotomized data.

To deal with nonlinearity in the relationship between outcome and continuous predictors, a systematic multivariable model building approach based on fractional polynomials was used.[15] Power transformations are chosen from a restricted set S = {−2, −1, −0.5, 0, 0.5, 1, 2, 3}, where X^0 denotes log(X). The set includes no transformation and the reciprocal, logarithmic, square root, and square transformations. To fit the model, each of the eight values was tried, the best-fitting model being the one with the highest likelihood. Regression lines in figures are based on best-fitting fractional polynomials and are the partial linear predictor for the variable in question (age or CrCl). All statistical analyses were performed using STATA version 9.2. Significance was assigned at $P < 0.05$.

RESULTS

Demographics

A total of 165 patients (93 male, 72 female) with arterial hypertension were included in the final analysis and presented with a predominant white background (n = 156 patients); nine patients were of Asian origin. Major comorbidities consisted of chronic kidney disease (n = 44), coronary heart disease/heart failure (n = 25), and diabetes mellitus (n = 10).

Age distribution ranged from 18 to 84 years (mean age 49.6 years) with 36.7% of patients >60 years. The mean body mass index (BMI) was 27.1 ± 5 kg/m^2 (range 17.6–49.9 kg/m^2) and systolic, diastolic, and mean blood pressure were 155 ± 20 mm Hg (range 112–210 mm Hg), 96 ± 12 mm Hg (range 65–130 mm Hg) and 116 ± 12 mm Hg (range 87–157 mm Hg), respectively. The mean calculated CrCl was 91 ± 25.7 ml/min/1.73 m^2 (range 23–136 ml/min/1.73 m^2). Moderately or severely impaired renal function was present in 25 of 165 patients including 18 who were >60 years. In total, 102 patients received an antihypertensive therapy at the time of analysis consisting of either a calcium channel and/or an α-blocker.

Analysis of apparent 11β-HSD enzyme activity

The mean steroid hormone metabolite levels are given in Table 1. The ratio of urinary (THF + 5αTHF)/THE tended to increase (P = 0.06) and the ratio of urinary F/E clearly rose with increasing age (P = 0.002) (Figure 1a,b), suggesting a reduced apparent 11β-HSD2 activity in elderly persons. The activity of 11β-HSD2 was inversely correlated with renal function ((THF + 5αTHF)/THE, $P < 0.001$; F/E: $P < 0.001$) (Figure 2a,b). Best fit regression modeling applied as described in the Method's section was identical with a linear regression model except in the comparison of F/E and CrCl, where the consideration of a polynomial model indicated a pronounced decrease in apparent 11β-HSD2 activity with reduced renal function only (Figure 2b). Even after exclusion of patients with a CrCl <60 ml/min/1.73 m^2, no relation between (THF + 5αTHF)/THE ratios and age was found (P = 0.33) (Figure 3a); in contrast, the correlation of the F/E ratio with age remained after exclusion of patients with a CrCl <60 ml/min/1.73 m^2 (P = 0.002, Figure 3b).

A multivariate linear regression model was applied to test the association between age, gender, or renal function and the (THF + 5αTHF)/THE and the F/E ratios, respectively (Table 2). Elevated (THF + 5αTHF)/THE (P = 0.002) and F/E (P = 0.005) ratios indicating a diminished apparent 11β-HSD2 activity were associated with reduced renal function. In addition, the (THF + 5αTHF)/THE ratio was higher in men than in women (P = 0.001). Taking into account the unselected total study population, the correlation between age and (THF + 5αTHF)/THE or F/E were not significant in the multivariate models. After exclusion of individuals with a moderate to severely impaired renal function with a CrCl <60 ml/min/1.73m^2, the F/E ratio was correlated with age (P = 0.02) after adjusting for CrCl and gender (Table 2).

When the patients with a normal renal function (>70 ml/min/1.73 m^2) were dichotomized with respect to the 11β-HSD2 activity, 96% of patients with an F/E ratio >0.9 (indicating a reduced apparent 11β-HSD2 enzyme activity), but only 74% of patients with a ratio <0.9, had a systolic hypertension defined as ≥140 mm Hg (χ^2 12.7; $P < 0.001$), though both groups of patients had antihypertensive therapy with calcium channel and α-blockers prescribed as clinically required. No such cutoff was identified for an elevated diastolic blood pressure.

Analyses of systemic cortisol production as assessed by urinary cortisol metabolite excretion

To elucidate whether differences in steroid hormone production were explanatory for altered F/E or (THF + 5αTHF)/THE ratios, the urinary excretion of cortisol metabolites used to calculate the apparent 11β-HSD2 activity was quantified

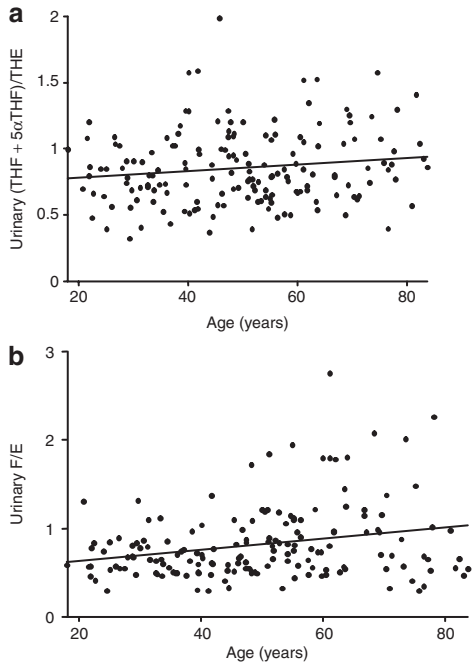

Figure 1 | Linear correlation between age and urinary ratios of (**a**) (THF + 5αTHF)/THE (r = 0.14, P = 0.06) or (**b**) F/E (r = 0.25, P = 0.002) (n = 165). 5αTHF, 5α-tetrahydrocortisol; E, cortisone; F, cortisol; THE, tetrahydrocortisone; THF, tetrahydrocortisol; THS, tetrahydrodeoxycortisol.

(Table 1). The cumulative amounts of urinary THF + 5αTHF + THE or F + E were independent of age, gender, or renal function (Table 3), which would best be explained by a reduced 11β-hydroxylase activity.

Analyses of the apparent 11β-hydroxylase enzyme activity

The apparent 11β-HSD2 activity decreased with age as shown in Figure 3b. Similarly, the apparent 11β-hydroxylase activity as assessed by the urinary ratio of THS/(THF + 5αTHF) diminished with age ($P = 0.005$; Figure 4), even in multivariate analyses ($P = 0.04$) correcting for gender and CrCl (Table 4).

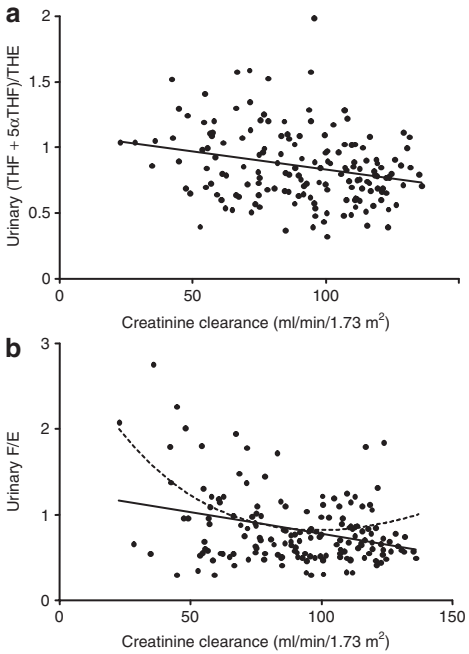

Figure 2 | Correlations between renal function (creatinine clearance = CrCl) and the urinary ratios of (**a**) (THF + 5αTHF)/THE ($r = -0.26$, $P < 0.001$) or (**b**) F/E (solid lines, linear: $r = -0.32$, $P < 0.001$; dotted lines, polynomial: $P < 0.001$) ($n = 165$). The linear and polynomial correlations are depicted by straight and dashed lines, respectively. 5αTHF, 5α-tetrahydrocortisol; E, cortisone; F, cortisol; THE, tetrahydrocortisone; THF, tetrahydrocortisol; THS, tetrahydrodeoxycortisol.

DISCUSSION

Uncontrolled arterial hypertension is extremely common in elderly persons and results in extensive cardiovascular morbidity and mortality. Here, we demonstrate for the first time that 11β-HSD2 activity as assessed by the urinary ratio of F/E declines with age in a hypertensive population and therefore might contribute to the development of hypertension in elderly patients.

Both the F/E and the (THF + 5αTHF)/THE ratios are established, though indirect markers, used to estimate the 11β-HSD activity. Several authors, but not all, suggested that the F/E ratio reflects the renal 11β-HSD2, and the (THF + 5αTHF)/THE ratio the overall 11β-HSD activity, which comprises both the renal 11β-HSD2 and the hepatic 11β-HSD1 activity.[9,16–22] This assumption is supported by the recent demonstration

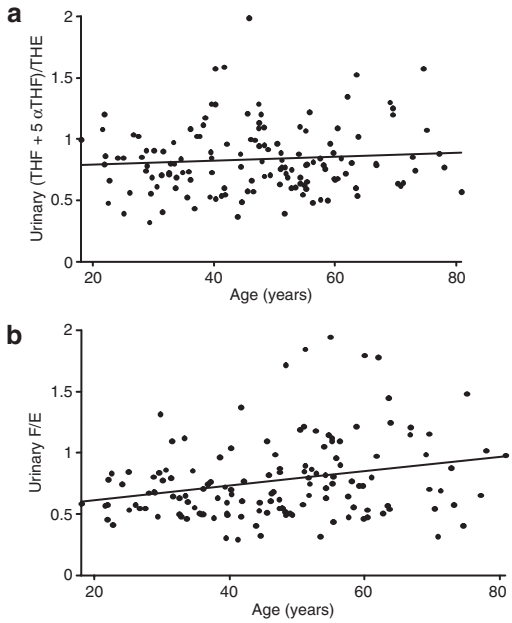

Figure 3 | Correlation between age and urinary ratios of (**a**) (THF + 5αTHF)/THE ($r = 0.08$, $P = 0.33$) or (**b**) F/E ($r = 0.26$, $P = 0.002$) in patients with preserved renal function (creatinine clearance >60 ml/min) ($n = 139$). 5αTHF, 5α-tetrahydrocortisol; E, cortisone; F, cortisol; THE, tetrahydrocortisone; THF, tetrahydrocortisol; THS, tetrahydrodeoxycortisol.

Table 2 | Multivariate linear regression analyses of the effect of age, gender, and creatinine clearance on apparent 11β-HSD activity

	F/E			(THF + 5αTHF)/THE		
	Regression coefficient ± s.e.	95 % CI	*P*	Regression coefficient ± s.e.	95 % CI	*P*
Total study population ($n = 165$)						
Age	0.002 ± 0.002	−0.002 to 0.007	0.31	<0.001 ± 0.002	−0.003 to 0.036	0.74
Female gender	−0.014 ± 0.063	−0.138 to 0.110	0.83	**−0.148 ± 0.04**	**−0.231 to −0.657**	**≤0.001**
CrCl	**−0.004 ± 0.001**	**−0.007 to −0.001**	**0.005**	**−0.003 ± 0.001**	**−0.005 to −0.001**	**0.002**
Subjects with CrCl >60 ml/min/1.73 m² ($n = 139$)						
Age	**0.005 ± 0.002**	**0.001 to 0.009**	**0.02**	<0.001 ± 0.002	−0.003 to 0.004	0.78
Female gender	−0.006 ± 0.056	−0.115 to 0.104	0.92	**−0.127 ± 0.047**	**−0.220 to −0.034**	**0.008**
CrCl	0.001 ± 0.002	−0.004 to 0.002	0.64	−0.003 ± 0.001	−0.005 to 0.001	0.06

Boldface indicates significant differences.
5αTHF, 5α-tetrahydrocortisol; CI, confidence interval; CrCl, creatinine clearance; E, cortisone; F, cortisol; THE, tetrahydrocortisone; THF, tetrahydrocortisol.

Table 3 | Multivariate linear regression analyses of the effect of age, gender, and creatinine clearance on cortisol production (*N* = 165)

	Σ(F + E)			Σ(THF + 5αTHF + THE)		
	Regression coefficient ± s.e.	95% CI	*P*	Regression coefficient ± s.e.	95% CI	*P*
Age	0.103 ± 0.083	−0.062 to 0.269	0.22	3.28 ± 3.38	−3.42 to 9.98	0.33
Female gender	1.28 ± 2.38	−3.44 to 5.99	0.59	63.5 ± 96.3	−128 to 255	0.51
CrCl	0.024 ± 0.048	−0.071 to 0.120	0.61	2.41 ± 1.95	−1.45 to 6.28	0.22

5αTHF, 5α-tetrahydrocortisol; CI, confidence interval; CrCl, creatinine clearance; E, cortisone; F, cortisol; THE, tetrahydrocortisone; THF, tetrahydrocortisol.

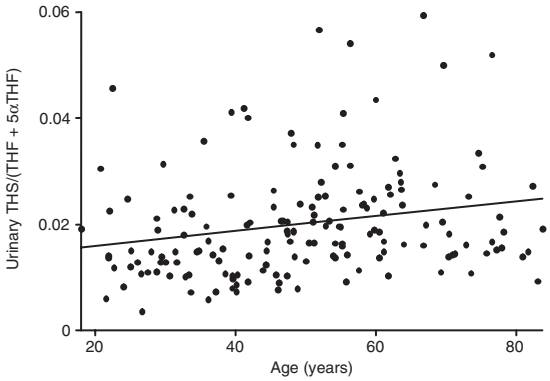

Figure 4 | Correlation between age and urinary ratios of THS/(THF + 5αTHF) ($r = 0.22$, $P = 0.005$) ($n = 162$). 5αTHF, 5α-tetrahydrocortisol; E, cortisone; F, cortisol; THE, tetrahydrocortisone; THF, tetrahydrocortisol; THS, tetrahydrodeoxycortisol.

Table 4 | Multivariate linear regression analyses of the effect of age, gender, and creatinine clearance on apparent 11β-hydroxylase activity (*N* = 165)

	THS/(THF + 5αTHF)		
	Regression coefficient ± s.e.	95% CI	*P*
Age	**0.0001 ± <0.001**	**0.000007 to 0.0002**	**0.04**
Female gender	**0.006 ± 0.002**	**0.003 to 0.009**	**<0.001**
CrCl	0.00001 ± <0.001	−0.00006 to <0.00009	0.75

Boldface indicates significant differences.
5αTHF, 5α-tetrahydrocortisol; CI, confidence interval; CrCl, creatinine clearance; E, cortisone; F, cortisol; THE, tetrahydrocortisone; THF, tetrahydrocortisol.

of a stronger correlation between renal 11β-HSD2 mRNA expression and urinary F/E than with urinary (THF + 5αTHF)/THE ratios.[23] Thus, our observation of an association between age and F/E rather than between age and (THF + 5αTHF)/THE ratio supports the conclusion that a reduced 11β-HSD2 contributes to the development of hypertension with increasing age. Obviously, the best proof of a link between 11β-HSD2 activity and blood pressure would be a direct association between F/E ratios and blood pressure. Such a relationship was obscured by the necessity to treat the majority of the patients and by the fact that the decision to discontinue antihypertensive drugs was also based on factors other than solely blood pressure values, such as the cardiovascular risk of the individual. In the multivariate analysis, antihypertensive medication was therefore not analyzed for its correlation with the apparent 11β-HSD2 activity. Nevertheless, in patients with an unaffected renal function, systolic blood pressure appears to be less well controlled in the presence of a reduced apparent 11β-HSD activity.

Age-related changes of apparent total body enzyme activity such as that observed in this study are potentially confounded by an age-dependent decline of specific organ functions. Previous studies demonstrated a deterioration in 11β-HSD2-activity with deteriorating renal function.[23,24] Here, this effect of renal function on 11β-HSD2 activity was experienced again. In addition, however, when only the group of subjects with a CrCl ≥60 ml/min/m^2 was considered, a decline of the 11β-HSD2 with age was observed, suggesting a strong influence of age on apparent 11β-HSD2 activity independent of renal function. The polynomial regression analysis used in our model identified a pronounced deterioration of apparent 11β-HSD2 activity with diminished renal function, an observation with the potential to camouflage the age effect in patients with severely reduced renal function. Thus, on the basis of present findings and the known effect of renal function on 11β-HSD2 activity, it is reasonable to conclude that both factors, age and the increasing prevalence of impaired renal function with age account for the observed reduction in 11β-HSD2 enzyme activity in elderly subjects with hypertension.[23,24]

Of interest, the multivariate linear regression model additionally identified a reduced apparent 11β-HSD2 activity as assessed by the (THF + 5αTHF)/THE ratio in male patients. A similar gender effect on the (THF + 5αTHF)/THE ratio has been observed in a black population, where an increased 11β-HSD2 activity was found in women when compared with men, suggesting an augmented risk for male individuals to develop volume-dependent hypertension, a finding in line with the enhanced risk of male subjects to progress to hypertension.[25]

In this study exogenous and endogenous factors associated with renal- or age-related disease states or treatment known to affect 11β-HSD2 activity such as furosemide, nephrotic range proteinuria, liquorice consumption, or cholestasis were excluded.[9,26–28] Renal function usually declines with age. Thus, a similar factor may account for the reduced 11β-HSD2 activity as a function of the declining glomerular filtration rate or the increasing age. A plausible mechanism is a concomitant quantitative reduction in enzyme availability as a consequence of decreasing renal tubular cells with age and/or with declining glomerular filtration rate. Our findings, however, suggest in addition and independently to the reduced number

of functioning nephrons the activity of 11β-HSD2 to decline age-related. The reason of this specific age-related impaired activity of the enzyme is unknown. A potential candidate mechanism for a decreased gene expression is an increased DNA methylation in the elderly.[12] This regulation might be relevant for the 11β-HSD2 enzyme, because we have recently shown that the degree of CpG-methylation in the promoter of the *11β-HSD2* gene determines activity and tissue specific expression of this enzyme.[11]

In summary, our results demonstrate a reduced 11β-HSD2-activity in hypertensive patients with advancing age, an effect causing cortisol-mediated mineralocorticoid receptor activation. As a corollary, antihypertensive therapy should include inhibitors of the mineralocorticoid pathway in elderly subjects with hypertension, a reasoning supported by a recent clinical study by Chapman and co-workers.[29] In that study comprising 1,411 elderly subjects resistant to three antihypertensive drugs, the aldosterone receptor antagonist spironolactone induced an average drop in blood pressure of ≈22/10 mm Hg.[29] In order to demonstrate unambiguously the role of the reduced 11β-HSD2 for this tremendous therapeutic efficacy of spironolactone, prospective future studies will have to compare the effect of spironolactone in aged hypertensives with a reduced 11β-HSD2 activity with that in subjects with a normal or high enzyme activity.

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