

Plasma Renin Measurements are Unrelated to Mineralocorticoid Replacement Dose in Patients With Primary Adrenal Insufficiency

Riccardo Pofi,^{1,2} Alessandro Prete,³ Vivien Thornton-Jones,¹ Jillian Bryce,⁴ Salma R. Ali,⁴ S. Faisal Ahmed,⁴ Antonio Balsamo,⁵ Federico Baronio,⁵ Amalia Cannuccia,⁶ Ayla Guven,⁷ Tulay Guran,⁸ Feyza Darendeliler,⁹ Claire Higham,¹⁰ Walter Bonfig,¹¹ Liat de Vries,^{12,13} Tania A. S. S. Bachega,¹⁴ Mirela C. Miranda,¹⁴ Berenice B. Mendonca,¹⁴ Violeta Iotova,¹⁵ Mária Korbonits,¹⁶ Nils P. Krone,¹⁷ Ruth Krone,¹⁸ Andrea Lenzi,² Wiebke Arlt,^{3,19} Richard J. Ross,¹⁷ Andrea M. Isidori,² and Jeremy W. Tomlinson¹

¹Department of Endocrinology, Oxford Centre for Diabetes, Endocrinology and Metabolism (OCDEM) and NIHR Oxford Biomedical Research Centre, Churchill Hospital, University of Oxford, Oxford, OX37LE, UK; ²Department of Experimental Medicine, Sapienza University of Rome, Viale Regina Elena 324, 00161, Rome, Italy; ³Institute of Metabolism and Systems Research, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK; ⁴Developmental Endocrinology Research Group, University of Glasgow, UK; ⁵Department of Medical and Surgical Sciences, Paediatric Unit, S.Orsola-Malpighi University Hospital, Via Massarenti 11, 40138, Bologna, Italy; ⁶Division of Endocrinology, Department of System Medicine, Section of Reproductive Endocrinology, Tor Vergata University of Rome, Fatebenefratelli Hospital San Giovanni Calibita, Rome, Italy; ⁷Saglik Bilimleri University, Zeynep Kamil Women and Children Hospital, Paediatric Endocrinology Clinic, Istanbul, Turkey; ⁸Marmara University, Department of Paediatric Endocrinology and Diabetes, Pendik, Istanbul, Turkey; ⁹Istanbul Faculty of Medicine, Department of Paediatrics, Paediatric Endocrinology Unit, Istanbul University, Çapa 34093, Istanbul, Turkey; ¹⁰Department of Endocrinology, Christie Hospital NHS Foundation Trust, Manchester, University Of Manchester, Manchester Academic Health Science Centre, Manchester, UK; ¹¹Department of Paediatrics, Klinikum Wels-Grieskirchen, Wels, Austria; Department of Paediatrics, Technical University München, Munich, Germany; ¹²The Jesse Z and Sara Lea Shafer Institute for Endocrinology and Diabetes, Schneider Children's Medical Center of Israel, Petah Tikva 49202, Israel; ¹³Sackler School of Medicine, Tel Aviv University, Israel; ¹⁴Unidade De Endocrinologia Do Desenvolvimento, Laboratório De Hormônios E Genética Molecular/Lim42, Hospital Das Clinicas Hcfmusp, Faculdade De Medicina, Universidade De Sao Paulo, Sao Paulo, Brazil; ¹⁵Clinic Of Paediatric Endocrinology - UMHAT 'Sv. Marina', Medical University of Varna, Varna, Bulgaria; ¹⁶Centre for Endocrinology, William Harvey Research Institute, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, Charterhouse Square, London, EC1M 6BQ, UK; ¹⁷The University of Sheffield, Sheffield, UK; ¹⁸Birmingham Women's & Children's Hospital, Department for Endocrinology & Diabetes, Birmingham, UK; and ¹⁹NIHR Birmingham Biomedical Research Centre, University Hospitals Birmingham NHS Foundation Trust and University of Birmingham, Birmingham, B15 3GW, UK.

ORCID numbers: 0000-0001-7808-5735 (Riccardo Pofi); 0000-0001-5106-9719 (Wiebke Arlt); 0000-0002-3170-8533 (Jeremy W. Tomlinson).

Context: No consensus exists for optimization of mineralocorticoid therapy in patients with primary adrenal insufficiency.

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in USA

© Endocrine Society 2019. All rights reserved. For permissions, please e-mail: journals.permissions@oup.com

Received 26 June 2019. Accepted 20 September 2019.

First Published Online 16 October 2019.

Abbreviations: 17-OHP, 17-OH progesterone; AD, Addison disease; BMI, body mass index; BP, blood pressure; CAH, congenital adrenal hyperplasia; cDBP, centile-corrected diastolic blood pressure; cSBP, centile-corrected systolic blood pressure; DBP, diastolic blood pressure; GC, glucocorticoid; MAP, mean arterial blood pressure; MC, mineralocorticoid; MC_{BSA}, mineralocorticoid daily dose corrected for body surface area; PAI, primary adrenal insufficiency; PRC, plasma renin concentration; sBMI, standard deviation score-corrected BMI; SBP, systolic blood pressure; SDS, standard deviation score; SW-CAH, salt-wasting congenital adrenal hyperplasia.

Objective: To explore the relationship between mineralocorticoid (MC) replacement dose, plasma renin concentration (PRC), and clinically important variables to determine which are most helpful in guiding MC dose titration in primary adrenal insufficiency.

Design: Observational, retrospective, longitudinal analysis.

Patients: A total of 280 patients (with 984 clinical visits and plasma renin measurements) with primary adrenal insufficiency were recruited from local databases and the international congenital adrenal hyperplasia (CAH) registry (www.i-cah.org). Thirty-seven patients were excluded from the final analysis due to incomplete assessment. Data from 204 patients with salt-wasting CAH (149 adults and 55 children) and 39 adult patients with Addison disease (AD) were analysed.

Main outcome measures: PRC, electrolytes, blood pressure (BP), and anthropometric parameters were used to predict their utility in optimizing MC replacement dose.

Results: PRC was low, normal, or high in 19%, 36%, and 44% of patients, respectively, with wide variability in MC dose and PRC. Univariate analysis demonstrated a direct positive relationship between MC dose and PRC in adults and children. There was no relationship between MC dose and BP in adults, while BP increased with increasing MC dose in children. Using multiple regression modeling, sodium was the only measurement that predicted PRC in adults. Longitudinally, the change in MC dose was able to predict potassium, but not BP or PRC.

Conclusions: The relationship between MC dose and PRC is complex and this may reflect variability in sampling with respect to posture, timing of last MC dose, adherence, and concomitant medications. Our data suggest that MC titration should not primarily be based only on PRC normalization, but also on clinical parameters such as BP and electrolyte concentration.

(*J Clin Endocrinol Metab* XX: 0–0, 2019)

The renin-angiotensin system plays a crucial role in the regulation of fluid volume status and electrolyte balance. Renin is released from the juxtaglomerular cells in the kidney in the presence of renal hypoperfusion and cleaves angiotensinogen to produce inactive angiotensin I. Angiotensin I is then converted to active angiotensin II by endothelial angiotensin-converting enzyme. Angiotensin II causes vasoconstriction of arteriolar vessels through inhibition of nitroxide synthetase and sodium retention, acting both in the proximal tubule (1, 2) and through stimulation of adrenal aldosterone release (3). Aldosterone, synthesized and released from the adrenal zona glomerulosa, acts through the nuclear mineralocorticoid (MC) receptor and enhances epithelial sodium channel activation, causing sodium and water retention with renal potassium loss; this is a crucial mechanism for maintaining blood pressure (BP) and electrolyte balance.

Primary adrenal insufficiency (PAI) is a life-threatening condition resulting from diseases directly involving the adrenal cortex. The clinical spectrum is characterized from deficient production or action of glucocorticoids (GCs), with or without concomitant deficiency of MC and adrenal androgens. In the majority of cases, PAI is caused by autoimmune adrenalitis (Addison disease, AD) (4, 5), and the most common symptoms include

weakness, fatigue, anorexia, abdominal pain, weight loss, orthostatic hypotension, and salt craving. Congenital adrenal hyperplasia (CAH) is a different form of PAI, caused by a group of rare autosomal recessive diseases resulting from mutations in genes encoding enzymes in pathways critical for adrenal steroid biosynthesis (6). The most common form is caused by mutations in the *CYP21A2* gene, accounting for approximately 95% of cases of CAH (7). Defective 21-hydroxylation can lead to decreased GC and MC synthesis. Specifically, salt-wasting CAH (SW-CAH) is characterized by both GC and MC deficiency. In SW-CAH and PAI, both GC and MC treatment are essential to avoid life-threatening adrenal crises (8).

However, much attention has focused mainly on optimization of GC replacement in adrenal insufficiency (9–11): so far only a few, small studies have investigated MC replacement in patients with PAI (12). Tailored and accurately titrated MC replacement therapy may be of crucial importance in patients with MC deficiency to improve long-term outcomes. MC replacement, usually in the form of fludrocortisone, is often administered with the aim of achieving plasma renin concentration (PRC) within the upper limit of the local reference range (5, 6). The most recent CAH guideline from the Endocrine Society suggests a fludrocortisone replacement dose of

50 to 200 µg/day (13). MC requirements in infants and children decrease with age, reflecting changes in the capacity of the renal tubules to reabsorb sodium over time. In adults, current guidance advocates titrating MC doses (and/or salt supplementation) according to BP, serum sodium, potassium, and PRC appropriate for age.

Taking into account the complex regulation of PRC, for example with posture, as well as the variability of timing of blood sampling with respect to the last fludrocortisone dose, we aimed to explore the relationship between MC dose regimens and clinical and biochemical variables in *real-life* clinical practice to determine whether they can usefully guide appropriate MC dose titration.

Patients and Methods

Patient selection

We performed a retrospective observational analysis of data from the International CAH Registry (I-CAH; www.i-cah.org) collected from 1982 to 2018, alongside that from local adrenal patient databases. The I-CAH Registry contains pseudoanonymized information on patients with CAH, and for this study we included patients from 14 centers in 7 countries (United Kingdom, Brazil, Italy, Turkey, Israel, Bulgaria, and Germany). Salt-wasting CAH was diagnosed on clinical grounds and/or on genetic testing. Patients were included if they had a diagnosis of CAH and were taking MC replacement. Records without MC replacement dose, or patients under salt replacement were excluded from the analysis.

A total of 984 visit records of 280 patients with PAI were recorded. 249 visits from 37 patients were excluded from the analysis due to incomplete medical records. The remaining 735 assessments of 243 patients were selected for the final analysis: 204 patients had SW-CAH (149 adults, 55 children) and 39 had AD (Fig. 1). The analyses were performed separately for adults (age ≥ 16 years) and children, a subsequent analysis was stratified by underlying disease etiology. A longitudinal analysis was performed in 112 patients (90 with SW-CAH and 22 with AD) (Fig. 1). Patient demographics are presented in Table 1.

Seven variables were considered in the final multivariate models: serum sodium (Na⁺), serum potassium (K⁺), mean arterial blood pressure (MAP), PRC, MC replacement dose, age and body mass index (BMI). Sitting MAP was calculated using the formula: diastolic blood pressure (DBP) + 1/3 of differential blood pressure (systolic blood pressure [SBP] – DBP). 17-OH Progesterone (17-OHP) and androstenedione of patients with SW-CAH were also considered in the baseline and univariate analysis, as indirect parameters of adherence to steroid replacement. For longitudinal analyses, data are expressed as medians unless otherwise stated, and change (Δ) for any variable was calculated by the difference: follow-up minus baseline. For the analysis in children, standard deviation score (SDS)-corrected BMI (sBMI) and centile-corrected systolic blood pressure (cSBP) and centile-corrected diastolic blood pressure (cDBP) were calculated, and the MC dose

was corrected for body surface area (MC_{BSA}). Data and samples were collected as part of “real-life” clinic consultations. Standard laboratory biochemical analyses were undertaken to measure electrolytes. No data were recorded regarding the timing of the last fludrocortisone dose or adherence, and no center adopted a standardized posture protocol prior to sampling for PRC.

Plasma renin concentration and renin assays

Different renin assays and units of measurement were used across the multiple centers that enrolled patients into the study (µIU/mL, ng/mL/h, nmol/L/h, pg/mL, ng/L). Every center provided local reference range, which we used to categorize results as “low,” “normal,” or “high.” Subsequently, all results were standardized according to the most frequently used reporting units (µIU/mL), using the following procedure: a) ng/L (n = 57), ng/mL/h (n = 70) and pg/mL (n = 3) values were converted using a factor of *1.67, *12, and *5.26 respectively as recommended within the Endocrine Society guidance (14); b) plasma renin activity values expressed as nmol/L/h (n = 33) were converted using a factor derived from a polyfit third-grade equation generated with MatLab (version 2017, MathWorks® Inc.) using the reference range of the different assay as intersection points.

Statistical analysis

A Spearman rank-order correlation was run to assess the relationship between individual variables. A *P* < 0.05 was considered indicative of a statistically significant difference. A Kruskal-Wallis test was conducted to determine if there were differences between groups that differed in their level of renin at baseline (the “low,” “normal,” and “high” PRC groups according to local reference range) or ΔMC dose for longitudinal analysis (“unchanged,” “decreased,” and “increased” dose). Distributions were similar for all groups, as assessed by visual inspection of a boxplot. When statistical significance was found, pairwise comparisons were performed with a Bonferroni correction for multiple comparisons. In the longitudinal analysis, a sign test with continuity correction was also conducted to determine the difference (within each group) between follow-up and baseline.

A first multiple regression model was run to assess the utility of clinical and biochemical variables to predict PRC. Six variables were initially inserted into the model: total MC daily replacement dose, Na⁺, K⁺, MAP, age, and BMI. All significant variables in the model were then tested as dependent variables in the subsequent multiple regression analyses. In order to have a linear relationship between all variables inserted into the models, a log₁₀ of PRC was computed and used for the multiple regression analysis.

In all the models generated, there was linearity as assessed by partial regression plots and a plot of studentized residuals against the predicted values. There was independence of residuals, as assessed by a Durbin-Watson value of approximately 2; there was homoscedasticity, as assessed by visual inspection of a plot of studentized residuals versus unstandardized predicted values; there was no evidence of multicollinearity, no studentized deleted residuals greater than ± 3 SD, no leverage values greater than 0.2, and values for Cook’s distance above

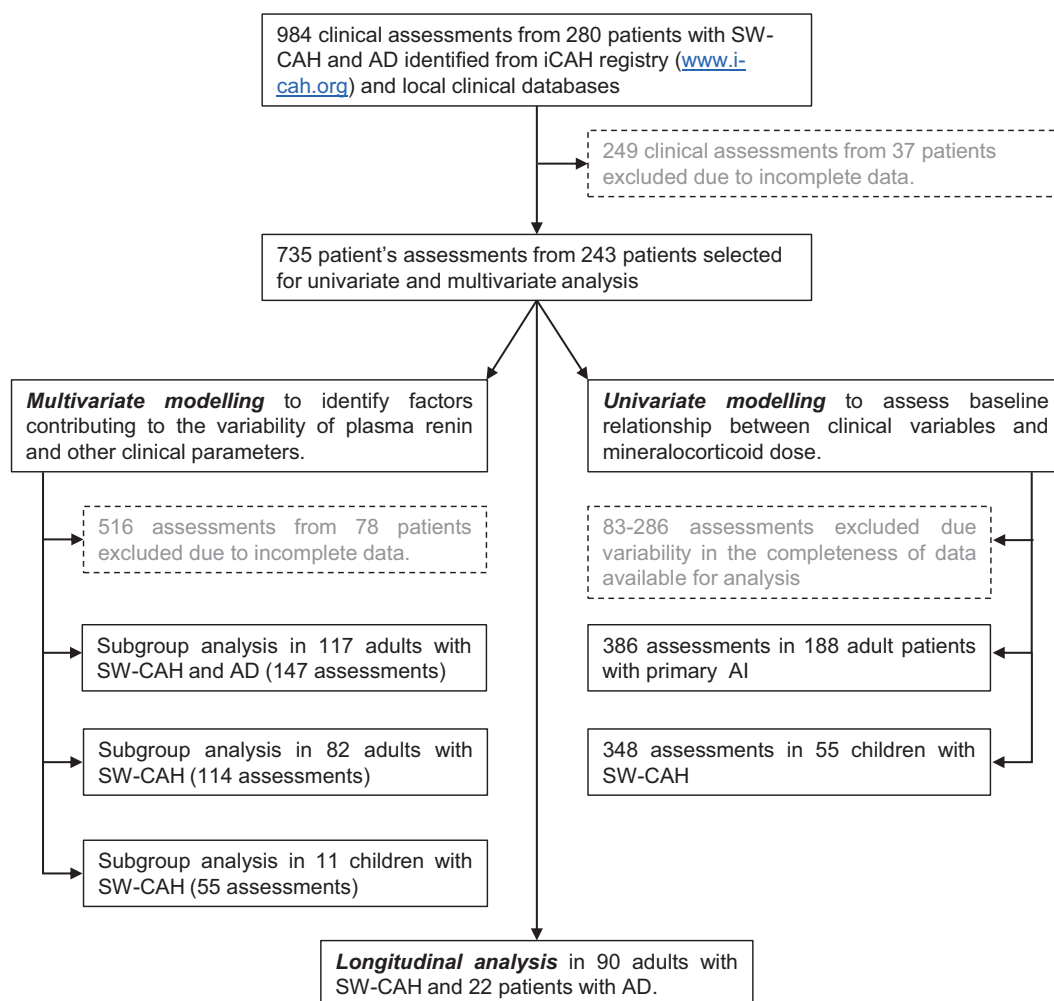


Figure 1. Flow chart for patient selection for the analysis of optimization of mineralocorticoid replacement. (SW-CAH, salt-wasting congenital adrenal hyperplasia; AD, Addison disease).

Table 1. Baseline Characteristics of 243 Patients With Adrenal Insufficiency

	Adults Only			Children Only
	Whole Adult Cohort	SW-CAH	AD	SW-CAH
n	188	149	39	55
Assessments(n)	386	347	39	348
Age (years)	27 (16–84)	25 (16–67)	49 (17–84)	2.3 (0–15)
Male	91 (48%)	72 (48%)	19 (49%)	26 (47%)
Female	97 (52%)	77 (52%)	20 (51%)	29 (53%)
BMI (kg/m²)	29 (15–50)	29 (15–50)	26 (17–33)	17 (12–37)
Na⁺ (mmol/L)	140 (126–146)	140 (130–146)	138 (126–143)	139 (104–148)
K⁺ (mmol/L)	4.2 (2.7–5.9)	4.2 (2.7–5.9)	3.9 (3.1–4.7)	4.3 (3.5–7.9)
SBP (mm Hg)	123 (90–170)	123 (90–169)	124 (102–170)	101 (62–150)
DBP (mm Hg)	79 (53–104)	79 (53–104)	79 (53–102)	62 (32–88)
MAP (mm Hg)	93 (70–125)	93 (70–125)	93 (75–120)	75 (42–103)
MC daily dose (μg/day)	150 (25–400)	150 (25–400)	100 (50–300)	100 (25–375)
PRC (μIU/mL)	86 (0.6–3166)	87 (0.6–3166)	82 (4.2–2879)	47 (0.1–5090)
MC_{BSA} dose (μg/day)	/	/	/	165 (15–965)
cSBP (mm Hg)	/	/	/	70 (10–100)
cDBP (mm Hg)	/	/	/	74 (10–100)
sBMI (kg/m²)	/	/	/	0.8 (–1.8–3.5)

Data are expressed as median (range). Abbreviations: AD, Addison disease; BMI, body mass index; cDBP, centile-corrected diastolic blood pressure; cSBP, centile-corrected systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial blood pressure; MC, mineralocorticoid; MC_{BSA}, mineralocorticoid daily dose corrected for body surface area; PRC, plasma renin concentration; sBMI, standard deviation score-corrected BMI; SBP, systolic blood pressure; SW-CAH, salt-wasting congenital adrenal hyperplasia.

1; the assumption of normality was met, as assessed by a Q-Q plot; finally, no outliers were found.

Statistical analyses were performed using SPSS (version 24, Chicago, IL, USA) and GraphPad Prism 7.0 software package (GraphPad Software, Inc. La Jolla, CA, USA).

Results

Patient characteristics, including clinical and biochemical variables, are presented in Table 1. A total of 243 patients with PAI currently taking MC replacement were included in the study. The analyses were performed in adult patients ($n = 188$) with SW-CAH and AD and in children with SW-CAH ($n = 55$). Separate subgroup analyses were performed in adults with SW-CAH ($n = 149$) and AD ($n = 39$). No children affected by AD were included in the analysis.

The distributions of MC doses in adults (stratified by underlying disease) and children are presented in Fig. 2. There was large variability in PRC, ranging from 0.6 to 3166 $\mu\text{IU/mL}$ in adults (median 86 $\mu\text{IU/mL}$) and 0.1 to

5090 $\mu\text{IU/mL}$ in children (median 66 $\mu\text{IU/mL}$). When stratified according to local reference ranges, 8%, 31%, and 61% of adults and 31%, 43%, and 26% of children had low, normal, and high PRC values, respectively.

Baseline correlations and univariate analysis—adults

Preliminary analysis showed the relationship to be monotonic, as assessed by visual inspection of a scatterplot. Univariate analysis demonstrated positive correlations between MC daily dose and BMI ($r = 0.233$, $P < 0.001$), age ($r = 0.116$, $P = 0.023$), and PRC ($r = 0.135$, $P = 0.051$), while there was no relationship with Na^+ , K^+ , or MAP (Fig. 3a–d). When adjusted to the local reference ranges, those patients with high PRC had lower serum Na^+ concentrations and higher concentrations of K^+ in comparison with those patients with low PRC. There was no relationship with the total MC replacement dose (Fig. 3e–h).

Baseline correlations and univariate analysis—children

Analysis of data from children showed a correlation of MC_{BSA} daily dose with sBMI ($r = -0.166$, $P = 0.023$), age ($r = -0.761$, $P < 0.01$), cSBP ($r = 0.364$, $P < 0.001$), cDBP ($r = 0.281$, $P = 0.005$), PRC ($r = 0.228$, $P = 0.002$), K^+ ($r = 0.308$, $P < 0.001$), and Na^+ ($r = -0.130$, $P = 0.035$) (Fig. 4a–e). When adjusted to the local reference ranges, as with the adults, those children with high PRC had lower serum Na^+ and higher K^+ concentrations in comparison with those with low PRC. Patients with low and high PRC were younger and took higher MC_{BSA} doses compared with patients with normal PRC (Fig. 4f–j).

Plasma renin concentration, 17-OH progesterone, and androstenedione

In order to determine if elevated PRC might be a reflection of undertreatment or nonadherence across both MC and GC replacement, we examined the 17-OHP and androstenedione levels in SW-CAH patients with low, normal, or high PRC. In adult patients with SW-CAH, 17-OHP levels were similar in patients with low, normal, or high PRC (low PRC: 29.2 nmol/L [1.6–117.9]; normal PRC: 38.2 nmol/L [1.2–1000]; high PRC: 46.3 nmol/L [0.8–862.4], median [min–max], $P = 0.39$). Data were similar for androstenedione levels (low PRC: 3.35 nmol/L [0.7–18.5]; normal PRC: 4.9 nmol/L [0.9–49.2]; high PRC: 7.5 nmol/L [0.4–86.2], $P = 0.06$). However, in children with SW-CAH, both 17-OHP and androstenedione were lowest in those individuals with low PRC: 17-OHP levels (low PRC: 0.7 nmol/L [0.3–423.65]; normal PRC: 48.41 nmol/L [6.35–1716]; high

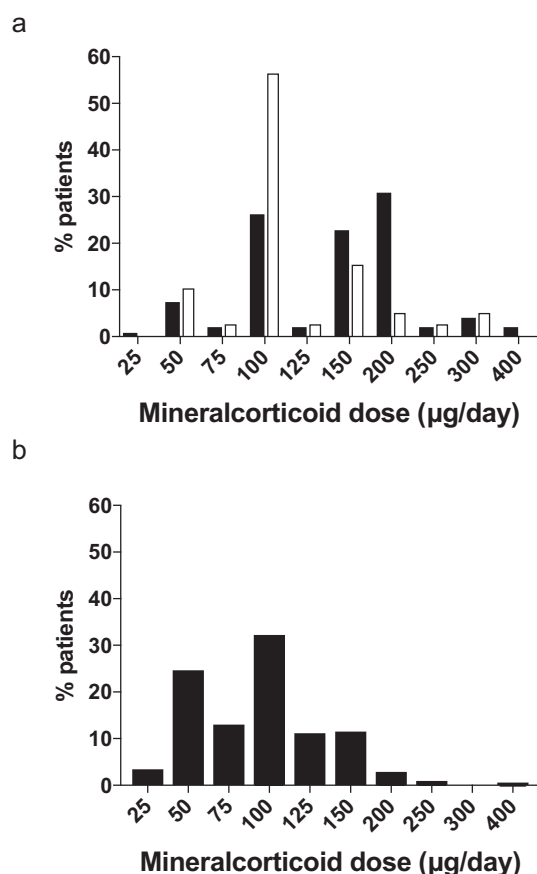


Figure 2. Distribution of mineralocorticoid replacement dose in (a) 188 adults with adrenal insufficiency. Black bars refer to patients with salt-wasting congenital adrenal hyperplasia ($n = 149$) and white bars to patients with Addison disease ($n = 39$). Distribution of mineralocorticoid replacement dose in (b) 55 children with salt-wasting congenital adrenal hyperplasia.

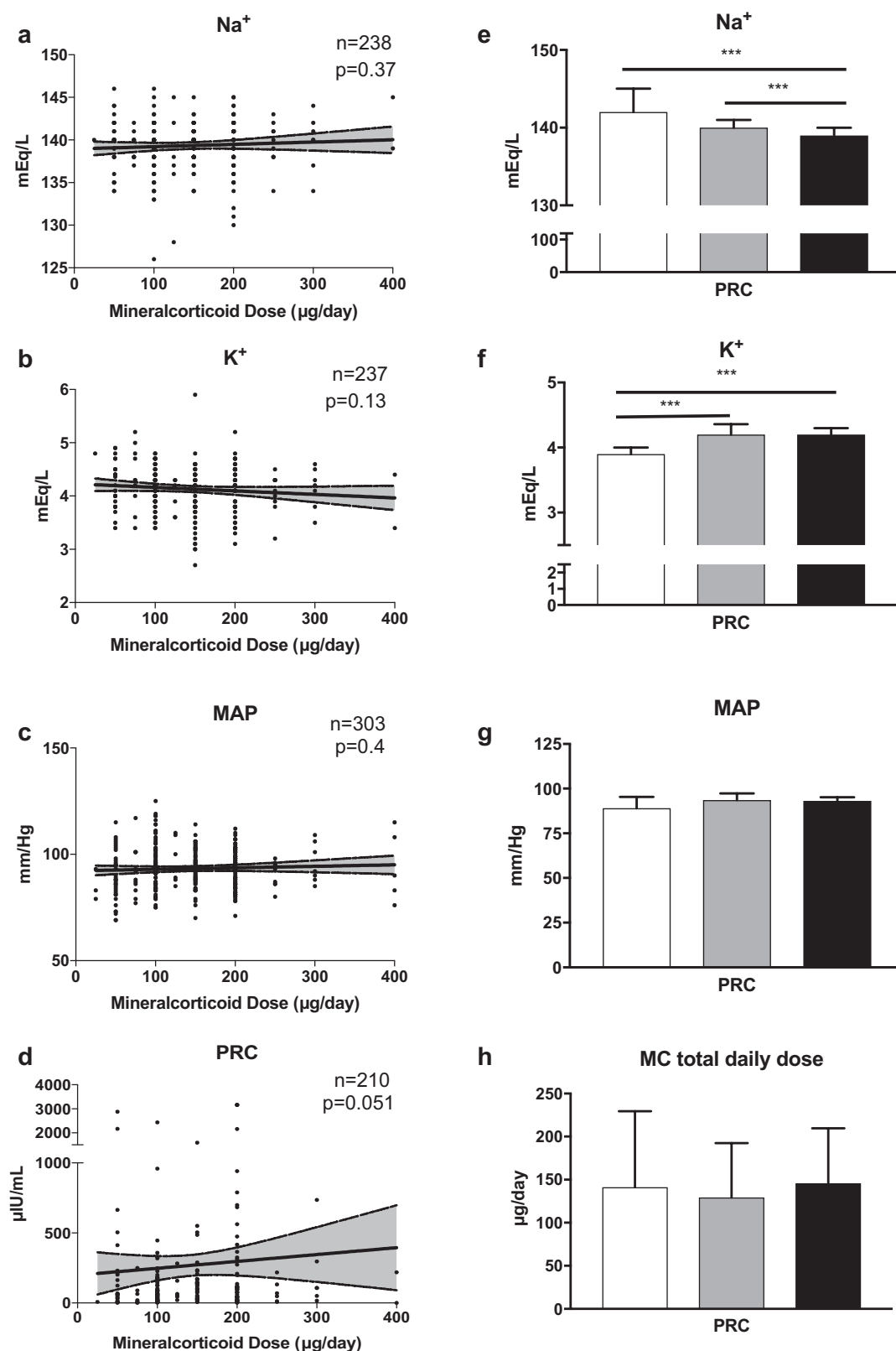


Figure 3. Baseline correlations of mineralocorticoid daily dose with clinical and biochemical variables (a-d) in adult patients with adrenal insufficiency (solid lines represent the regression analysis; shaded areas within dotted lines represent the 95% confidence intervals; n, number of individual clinical assessments included in the analysis; PRC, plasma renin concentration; Na^+ , serum sodium; K^+ , serum potassium; MAP, mean arterial pressure). When PRC is expressed as “low” (white bars), “normal” (grey bars), or “high” (black bars) according to local reference ranges, those patients with “high” PRC have lower Na^+ concentrations in comparison with individuals in whom PRC is “normal” or “low” (e). K^+ is lower in individuals with “low” PRC in comparison with individuals in whom PRC is “normal” or “high” (f). There is no difference in MAP or mineralocorticoid dose in groups when stratified by local PRC reference range (g and h) ($***P < 0.001$).

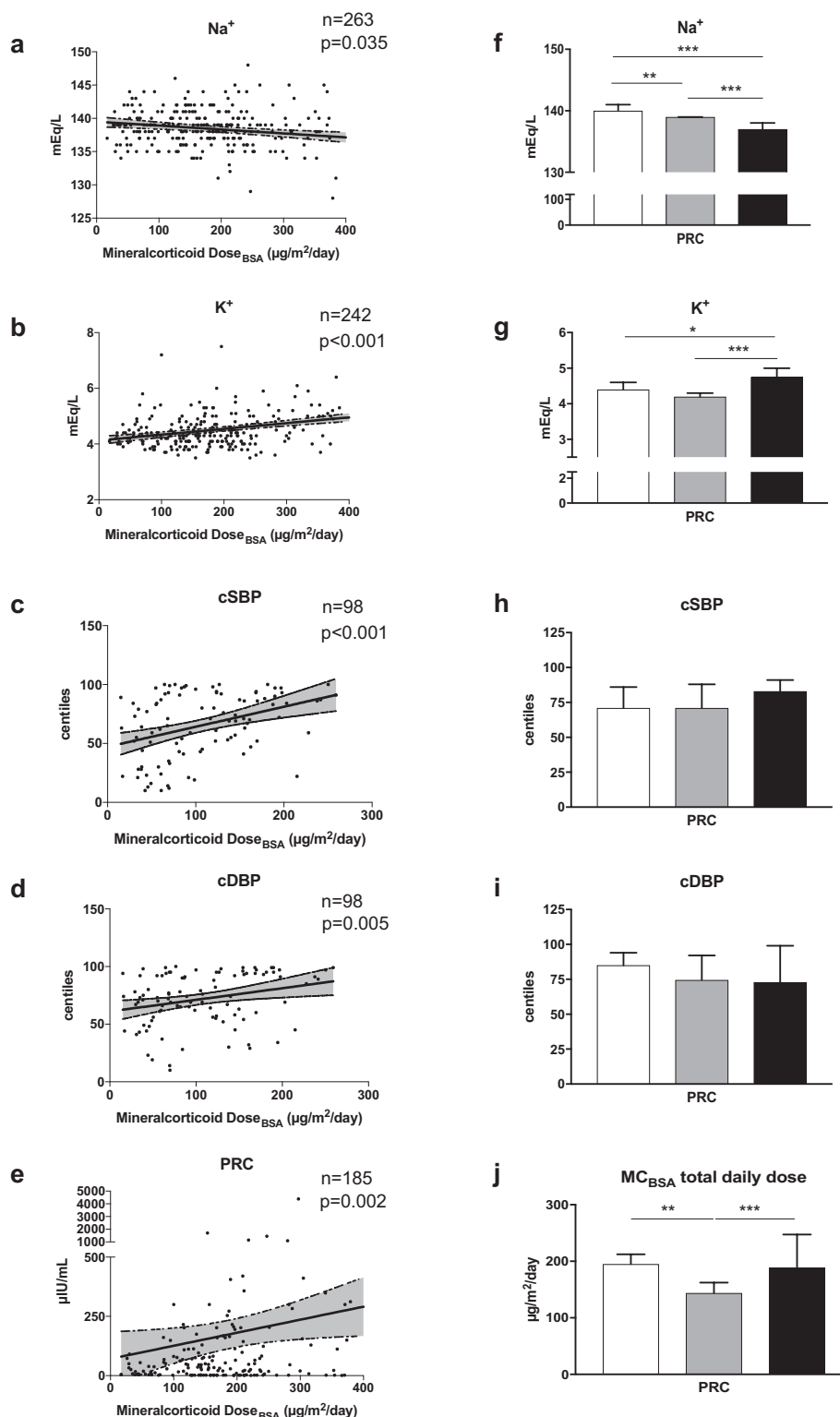


Figure 4. Baseline correlations of mineralocorticoid daily dose corrected for body surface area (MC_{BSA}) with clinical and biochemical variables in children with adrenal insufficiency due to salt-wasting congenital adrenal hyperplasia (solid lines represent the regression analysis; shaded areas within dotted lines represent the 95% confidence intervals; n , number of individual clinical assessments included in the analysis; PRC, plasma renin concentration; Na^+ , serum sodium; K^+ , serum potassium; cSBP, centile-corrected systolic blood pressure; cDBP, centile-corrected diastolic blood pressure) (a-e). When PRC is expressed as “low” (white bars), “normal” (grey bars), or “high” (black bars) according to local reference ranges, those children with “high” PRC have the lowest Na^+ concentrations (f). K^+ is highest in children with “high” PRC (g). There is no difference in cSBP or cDBP between groups when stratified by local PRC reference range (h and i). However, MC_{BSA} was lowest in those children with a “normal” PRC (j). (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

PRC: 131 nmol/L [1.21–1424.1], $P < 0.01$) and androstenedione levels (low PRC: 0.98 nmol/L [0.28–38.4]; normal PRC: 5.58 nmol/L [0.35–34.91]; high PRC: 4.75 nmol/L [1.01–45.39], $P < 0.01$).

Multiple regression models

All mineralocorticoid deficient patients—adults

When considered individually as dependent variables, our 6-variable multiple regression model was able to predict PRC ($P < 0.001$) and MC total daily dose ($P = 0.017$). Na^+ was the only variable weakly related to PRC ($B = -0.091$, $P < 0.001$). MC total daily dose was directly related to BMI ($B = 2.812$, $P = 0.001$), but not MAP ($B = 0.566$, $P = 0.34$) or PRC ($B = 5.846$, $P = 0.51$). All the computed and relative coefficients generated by the models are summarized in Table 2.

Salt-wasting congenital adrenal hyperplasia—adults

When data from adults with SW-CAH were analysed separately using the same strategy, results were comparable with the complete adult cohort analysis. The data are summarized in Table 3. The multiple regression model significantly predicted PRC ($P = 0.008$) MC total daily dose ($P < 0.001$). Na^+ was the only variable that was weakly associated (negatively) with PRC ($B = -0.097$, $P < 0.001$). Moreover, as physiologically expected, K^+ was strongly and inversely related to MC daily dose ($B = -41.180$, $P = 0.007$) (Table 3).

Salt-wasting congenital adrenal hyperplasia—children

The subgroup analysis on CAH children showed a similar pattern; Na^+ ($B = -0.142$, $P = 0.005$) and K^+ ($B = -0.697$, $P = 0.004$) were related to PRC; MC_{BSA} total daily dose, as expected, was inversely related to age ($B = -7.397$, $P < 0.001$), but not cSBP or cDBP ($B = 0.810$, $P = 0.2$ and $B = -0.405$, $P = 0.3$) or PRC ($B = 6.697$, $P = 0.5$) (Table 4).

Addison disease—adults

In the subgroup analysis on patients with AD, the multiple regression model significantly predicted PRC ($P = 0.050$) and Na^+ ($P = 0.004$). Once again, serum Na^+ was able to predict PRC ($B = -0.115$, $P = 0.002$). The model was not significant for prediction of MC total daily dose and serum K^+ (data not shown).

Longitudinal follow-up in adults with SW-CAH

Longitudinal analysis was performed in 112 adult patients (90 patients with SW-CAH and 22 patients with AD; median time between assessments 433 days [range, 33–2082]). At follow-up, MC dose remained unchanged in 80 (67%) patients (group A) while in 9 (6%) patients (group B), the MC dose was decreased (ΔMC dose $-100\mu\text{g/day}$ [range, -50 to -200]) and in 23 (19%) patients (group C), the MC dose was increased (ΔMC dose $50\mu\text{g/day}$ [range, 25 – 100]). Within each group, there was no significant change in ΔPRC ($\Delta\text{PRC}_{\text{group A}} 5 \mu\text{IU/mL}$, $z = 0.783$, $P = 0.434$; $\Delta\text{PRC}_{\text{group B}} 0.1 \mu\text{IU/mL}$,

Table 2. Multiple Regression Modeling in Adult Patients With Adrenal Insufficiency (147 complete clinical assessments from 117 patients)

Model 1 ($P < 0.001^*$)		Dependent: PRC		
Independent	B	95% CI lower bound	95% CI upper bound	P
MC total daily dose	0.001	-0.001	0.002	0.515
K^+	0.044	-0.201	0.289	0.723
Na^+	-0.091	-0.126	-0.055	<0.001*
MAP	0.005	-0.006	0.016	0.351
Age	-0.006	-0.013	0.001	0.072
BMI	0.004	-0.012	0.020	0.591
Model 2 ($P = 0.017^*$)		Dependent: MC total daily dose		
Independent	B	95% CI lower bound	95% CI upper bound	P
K^+	-17.523	-43.350	8.303	0.182
Na^+	1.026	-3.072	5.123	0.621
PRC	5.846	-11.873	23.565	0.515
MAP	0.566	-0.593	1.726	0.336
Age	0.030	-0.722	0.781	0.938
BMI	2.812	1.183	4.441	0.001*

The dependent variables assessed were plasma renin concentration, sodium, potassium, mineralocorticoid dose, and mean arterial pressure. P values should be interpreted with Bonferroni correction; when significant, they are highlighted in bold and marked with an asterisk. Abbreviations: BMI, body mass index; K^+ , serum potassium; MAP, mean arterial blood pressure; Na^+ , serum sodium; PRC, plasma renin concentration.

Table 3. Multiple Regression Modeling in Adults With Salt-Wasting Congenital Adrenal Hyperplasia (114 complete assessments from 82 patients)

Model 1 ($P = 0.008^*$)		Dependent: PRC		
Independent	B	95% CI lower bound	95% CI upper bound	P
MC total daily dose	0.000	−0.002	0.002	0.885
K ⁺	−0.064	−0.361	0.233	0.670
Na ⁺	−0.097	−0.143	−0.051	<0.001*
MAP	0.007	−0.007	0.021	0.343
Age	0.001	−0.010	0.012	0.850
BMI	0.002	−0.016	0.020	0.832

Model 2 ($P < 0.001^*$)		Dependent: MC total daily dose		
Independent	B	95% CI lower bound	95% CI upper bound	P
K ⁺	−41.180	−71.069	−11.290	0.007*
Na ⁺	−1.393	−6.561	3.776	0.594
PRC	1.461	−18.479	21.402	0.885
MAP	1.621	0.167	3.075	0.029
Age	1.143	0.036	2.251	0.043
BMI	2.362	0.577	4.147	0.010

The dependent variables assessed were plasma renin concentration, sodium, potassium, mineralocorticoid dose, and mean arterial pressure. *P* values should be interpreted with Bonferroni correction; when significant, they are highlighted in bold and marked with an asterisk. Abbreviations: BMI, body mass index; K⁺, serum potassium; MAP, mean arterial blood pressure; Na⁺, serum sodium; PRC, plasma renin concentration.

Table 4. Multiple Regression Modeling in Children With Salt-Wasting Congenital Adrenal Hyperplasia (55 complete assessments from 11 patients)

Model 1 ($P = 0.008^*$)		Dependent: PRC		
Independent	B	95% CI lower bound	95% CI upper bound	P
MC _{BSA} dose	0.002	−0.003	0.006	0.493
K ⁺	−0.694	−1.151	−0.237	0.004*
Na ⁺	−0.143	−0.242	−0.045	0.005*
cSBP	0.007	−0.003	0.017	0.178
cDBP	−0.008	−0.019	0.004	0.192
Age	−0.013	−0.073	0.047	0.662
sBMI	0.027	−0.203	0.257	0.814

Model 2 ($P < 0.001^*$)		Dependent: MC _{BSA} total daily dose		
Independent	B	95% CI lower bound	95% CI upper bound	P
K ⁺	−10.671	−43.913	22.570	0.521
Na ⁺	5.602	−1.347	12.550	0.111
cSBP	0.840	0.206	1.474	0.011
cDBP	−0.446	−1.214	0.322	0.248
Age	−7.275	−10.634	−3.917	<0.001*
sBMI	−11.440	−26.370	3.490	0.130
PRC	6.814	−13.039	26.667	0.493

The dependent variables assessed were plasma renin concentration, sodium, potassium, standard deviation score-corrected body mass index, mineralocorticoid dose adjusted for body surface area, and centile-corrected systolic and diastolic blood pressure. *P* values should be interpreted with Bonferroni correction; when significant, they are highlighted in bold and marked with an asterisk. Abbreviations: cDBP, centile-corrected diastolic blood pressure; cSBP, centile-corrected systolic blood pressure; K⁺, serum potassium; MAP, mean arterial blood pressure; Na⁺, serum sodium; PRC, plasma renin concentration; sBMI, standard deviation score-corrected body mass index; MC_{BSA}, mineralocorticoid daily dose corrected for body surface area.

$p = 1.000$; $\Delta\text{PRC}_{\text{group C}} -61 \mu\text{IU/mL}$, $P = 0.405$) (Fig. 5). In addition, ΔPRC values compared across groups were not different ($P = 0.560$).

Multiple regression modeling significantly predicted ΔPRC ($P = 0.015$). Only Na⁺ concentration at the final

follow-up visit was strongly associated with ΔPRC ($B = 59.465$, $P < 0.001$). There was no relationship between ΔPRC and final MAP, K⁺, or MC replacement dose. Finally, as expected, ΔMC dose was inversely related to ΔK^+ ($B = -3.104$, $P = 0.002$). No correlations

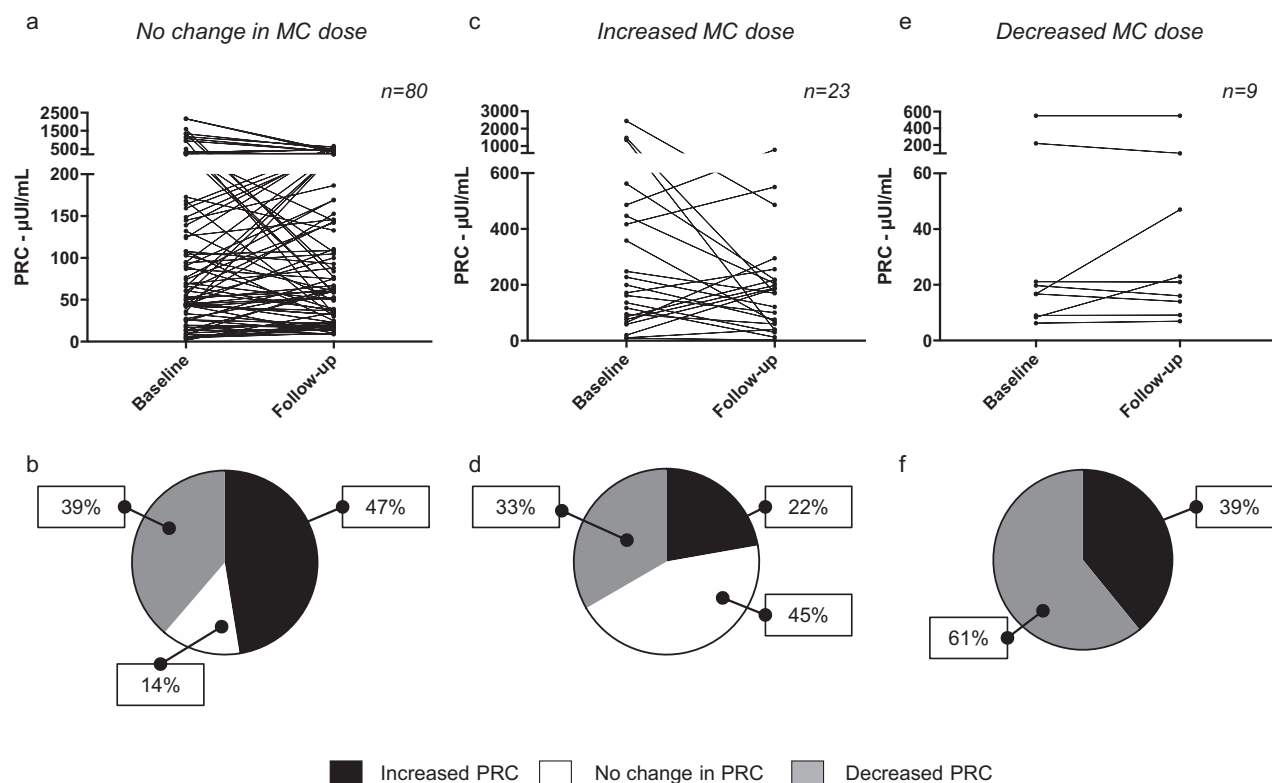


Figure 5. Longitudinal analysis of plasma renin concentration (PRC) in 112 patients with adrenal insufficiency at baseline and follow-up (median time between assessments = 433 days; range, 33–2082). Variation in PRC was defined as “increased” (>15% rise from baseline), “decreased” (>15% fall from baseline) or “no change” (<15% deviation from baseline). Longitudinal change in absolute PRC (a) and categorization of PRC change (b) in 80 patients with unchanged MC dose from baseline, 23 patients in whom MC dose was increased (c and d) and 9 patients with an decreased MC replacement dose (e and f).

were found between ΔMC dose ΔPRC , ΔNa^+ , or ΔMAP (data not shown).

Discussion

In patients with adrenal insufficiency, there is an absolute requirement for lifelong steroid hormone replacement therapy. Almost 70 years have passed since the introduction of MC replacement therapy for patients with PAI (15). MC treatment strategies have only been examined in a small number of studies and to date, there are limited data regarding dose optimization. Current standard replacement usually consists of fludrocortisone 50 to 200 μg (13) given once daily in the morning, reflecting the circadian rhythm of aldosterone, which is similar to that of cortisol (16). Guidance suggests that MC replacement dose should be tailored clinically by measuring BP, evaluating salt cravings, and assessment of the presence of peripheral edema (17). However, these are not always reliable and markers that are more objective are often used in addition. Compelling evidence to support the use of serum Na^+ , K^+ , and PRC levels is lacking (12).

Oelkers and colleagues found that, when targeted to the upper limit of the reference range, plasma renin activity (PRA) correlated more closely with MC dose than with Na^+ and K^+ levels alone (18). Conversely, Thompson et al demonstrated that PRA was unable to distinguish between adequate and overreplacement and therefore raised doubts about its utility in MC dose optimization (19). Current expert consensus suggests that MC replacement should aim to achieve normotension, normokalemia, and PRC in the upper normal reference range (13, 18, 20, 21).

In patients with adrenal insufficiency, much attention has focussed on optimization of GC replacement, but it can be hard to differentiate clinically between GC and MC under-replacement. It is important to avoid overtreatment with GCs, which is associated with significant adverse effects (22–24). Bearing in mind the MC activity of commonly used GCs (hydrocortisone, prednisolone), it is possible that increased doses of GCs are actually treating relative MC deficiency, therefore highlighting the possibility that many patients with PAI may actually be underreplaced with MC. This may well be an important contributing factor to the lack of

relationship between MC dose and PRC that we observed in our data.

In our cohort, PRC was weakly related to Na^+ , but had no relationship to other clinical variables (including BP) or, importantly, MC daily replacement dose. Furthermore, our longitudinal analysis suggests that MC dose changes are not associated with subsequently measured PRC. In contrast, serum electrolytes (notably K^+) are most closely and strongly related to MC dose both at baseline and in the longitudinal analysis. Our observations may well reflect underlying physiology, given that those patients with the highest PRC had lower Na^+ levels, (Na^+ was also the only variable associated with future change in PRC in the longitudinal analysis). This perhaps suggests relative MC underreplacement and consequent Na^+ loss, although it should be noted that we did not control for GC dose and therefore cannot assess the relative contribution of GCs to Na^+ balance. In parallel, the association between MC daily dose and K^+ suggested that higher MC doses were associated with lower serum K^+ concentrations, as expected.

The utility of PRC and aldosterone measurements in the diagnoses of MC deficiency is not in doubt. However, significant challenges arise when they are used for MC dose adjustment. There are challenges that relate to difficulties in sample collection and handling; additionally, there is no internationally accepted standard reference range, with interpretation of results dependent upon local reference intervals (17). Furthermore, there are many other factors that have a profound influence on PRC, including volume status, salt intake, pregnancy, posture, ambient temperature, and antihypertensive and nonsteroidal anti-inflammatory drugs (25). Salt replacement is a major confounding factor when PRC is evaluated; for this reason, patients under salt supplementation were excluded from our analysis. In most centers, samples taken for PRC measurement are not standardized with respect to posture or timing of last MC replacement dose and therefore meaningful interpretation and comparison of the results is difficult. Furthermore, there are cost implications that need to be considered if PRC assessments are routinely requested that cannot meaningfully help guide replacement strategies. Adherence to medication is a further factor that needs to be considered and in many cases, prescribed doses are not necessarily reflective of what is actually being taken. The effects of differing levels of salt consumption, together with different MC sensitivity and treatment adherence, all could potentially contribute to explain the findings of higher PRC in patients under higher MC doses, especially in children. This is particularly true in patients with CAH, among whom up to a third of adult

patients are nonadherent (26). However, in our cohort of adult patients with SW-CAH, there was no relationship between PRC and disease control (as measured by 17-OHP and androstenedione levels), suggesting that global nonadherence (to both GC and MC replacement) may not have been occurring, and our data might have pointed towards specific MC underreplacement. This contrasts with the analysis in children, where we observed concordance between 17-OHP/androstenedione and PRC levels. Taken together, these factors undoubtedly contributed to the wide variability in PRC values that we observed and the lack of relationship with MC replacement dose and biologically relevant clinical variables, endorsing observations made in much smaller studies (27).

Our study does have limitations. It is a retrospective analysis from multiple centers, so there is the potential for selection bias as well as high heterogeneity in our study population; in addition, detailed extensive medical records were not available in many patients. Similarly, plasma renin was not measured centrally but was analyzed by different assays in the participating centers. In addition, we were unable to estimate the impact of GC replacement therapy, due to a lack of information about preparation and dose. We excluded the small number of patients ($n = 7$) who were taking salt supplementation, as precise data on salt intake was not reported in the records. The data we have analyzed are from “real-life” clinic consultations and are not from a standardized controlled clinical trial. This is particularly true for the longitudinal analysis where titration of MC dose was made by physician preference rather than by an established specific algorithm. Prospective trials designed to reduce the effects of confounding factors through a dedicated and rigorous approach are needed to clarify the contribution of different clinical and biochemical variables on PRC and subsequently on MC dosage titration. While our study design is clearly a limitation, this does offer a true reflection of the variables that are presented to clinicians when trying to optimize the management of patients with PAI.

In conclusion, routine monitoring of serum electrolytes (alongside clinical assessment of symptoms and BP) provides the most informative approach to add to PRC when MC replacement needs to be adjusted. However, in the absence of the ability to accurately standardize the collection of samples used to measure PRC, its routine measurement may conflict with other tools used to assess the adequacy of MC replacement and decisions to modify MC dose should not be solely based on PRC. There are many other questions that need to be addressed including underreplacement or overreplacement

with MC and its clinical impact in patients with PAI. Dedicated large-scale prospective studies will be required to conclusively determine the role of PRC in monitoring MC replacement in PAI patients.

Financial support

This work was supported by the Medical Research Council (program grant to JWT ref. MR/P011462/1); the NIHR Oxford Biomedical Research Centre (JWT) and the NIHR Birmingham Biomedical Research Centre (BRC-1215-20009, WA); Exchange in Endocrinology Expertise Programme of the European Union of Medical Specialists 3E Fellowship (to R.P.), European Society of Endocrinology (ESE) Short-Term fellowship (to R.P.). The I-CAH Registry was developed using support from an unrestricted education grant from Diurnal Ltd, Medical Research Council (partnership award to SFA ref G1100236, the Seventh European Union Framework Program (201444) and the Research Unit of the European Society for Paediatric Endocrinology. SRA is supported by an unrestricted education grant from Diurnal and the Gardiner Lectureship at the University of Glasgow.

Acknowledgments

We would like to thank prof. Birgit Koehler and prof. Carlo Acerini for their contribution to the preparation of this manuscript.

This work was supported by the National Institute for Health Research (NIHR) Oxford Biomedical Research Centre (BRC, to JWT) and the NIHR Birmingham BRC (BRC-1215-20009, to WA), the Medical Research Council UK (program grant MR/P011462/1, to JWT); by the Exchange in Endocrinology Expertise programme of the European Union of Medical Specialists and the European Society of Endocrinology (3E fellowship and Short-Term Fellowship, to RP). The views expressed are those of the authors and not necessarily those of the NIHR or the Department of Health and Social Care UK.

Additional Information

Correspondence: Jeremy Tomlinson, Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, Oxford OX3 7LJ, UK. E-mail: Jeremy.tomlinson@ocdem.ox.ac.uk

Disclosure Summary: RJR is a Director of Diurnal Ltd. The other authors have nothing to disclose and have no relevant conflicts of interest.

References

- Ichikawa I, Harris RC. Angiotensin actions in the kidney: renewed insight into the old hormone. *Kidney Int.* 1991;40(4):583–596.
- Cogan MG. Angiotensin II: a powerful controller of sodium transport in the early proximal tubule. *Hypertension.* 1990;15(5):451–458.
- Kakiki M, Morohashi K, Nomura M, Omura T, Horie T. Expression of aldosterone synthase cytochrome P450 (P450aldo) mRNA in rat adrenal glomerulosa cells by angiotensin II type 1 receptor. *Endocr Res.* 1997;23(4):277–295.
- Charmandari E, Nicolaides NC, Chrousos GP. Adrenal insufficiency. *Lancet.* 2014;383(9935):2152–2167.
- Bancos I, Hahner S, Tomlinson J, Arlt W. Diagnosis and management of adrenal insufficiency. *Lancet Diabetes Endocrinol.* 2015;3(3):216–226.
- El-Maouche D, Arlt W, Merke DP. Congenital adrenal hyperplasia. *Lancet.* 2017;390(10108):2194–2210.
- White PC, Speiser PW. Congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Endocr Rev.* 2000;21(3):245–291.
- Arlt W, Willis DS, Wild SH, Krone N, Doherty EJ, Hahner S, Han TS, Carroll PV, Conway GS, Rees DA, Stimson RH, Walker BR, Connell JM, Ross RJ; United Kingdom Congenital Adrenal Hyperplasia Adult Study Executive (CaHASE). Health status of adults with congenital adrenal hyperplasia: a cohort study of 203 patients. *J Clin Endocrinol Metab.* 2010;95(11):5110–5121.
- Verma S, Vanryzin C, Sinaii N, Kim MS, Nieman LK, Ravindran S, Calis KA, Arlt W, Ross RJ, Merke DP. A pharmacokinetic and pharmacodynamic study of delayed- and extended-release hydrocortisone (Chronocort) vs. conventional hydrocortisone (Cortef) in the treatment of congenital adrenal hyperplasia. *Clin Endocrinol (Oxf).* 2010;72(4):441–447.
- Johannsson G, Nilsson AG, Berghorsdottir R, Burman P, Dahlqvist P, Ekman B, Engström BE, Olsson T, Ragnarsson O, Ryberg M, Wahlberg J, Biller BM, Monson JP, Stewart PM, Lennernäs H, Skrtic S. Improved cortisol exposure-time profile and outcome in patients with adrenal insufficiency: a prospective randomized trial of a novel hydrocortisone dual-release formulation. *J Clin Endocrinol Metab.* 2012;97(2):473–481.
- Pofi R, Feliciano C, Sbardella E, Argese N, Woods CP, Grossman AB, Jafar-Mohammadi B, Gleeson H, Lenzi A, Isidori AM, Tomlinson JW. The Short Synacthen (Corticotropin) test can be used to predict recovery of hypothalamo-pituitary-adrenal axis function. *J Clin Endocrinol Metab.* 2018;103(8):3050–3059.
- Esposito D, Pasquali D, Johannsson G. Primary adrenal insufficiency: managing mineralocorticoid replacement therapy. *J Clin Endocrinol Metab.* 2018;103(2):376–387.
- Speiser PW, Arlt W, Auchus RJ, Baskin LS, Conway GS, Merke DP, Meyer-Bahlburg HFL, Miller WL, Murad MH, Oberfield SE, White PC. Congenital adrenal hyperplasia due to steroid 21-hydroxylase deficiency: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab.* 2018;103(11):4043–4088.
- Funder JW, Carey RM, Mantero F, Murad MH, Reincke M, Shibata H, Stowasser M, Young WF Jr. The management of primary aldosteronism: case detection, diagnosis, and treatment: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab.* 2016;101(5):1889–1916.
- Bishop PM. The history of the discovery of Addison's disease. *Proc R Soc Med.* 1950;43(1):35–42.
- Williams GH, Cain JP, Dluhy RG, Underwood RH. Studies of the control of plasma aldosterone concentration in normal man. I. Response to posture, acute and chronic volume depletion, and sodium loading. *J Clin Invest.* 1972;51(7):1731–1742.
- Bornstein SR, Allolio B, Arlt W, Barthel A, Don-Wauchope A, Hammer GD, Husebye ES, Merke DP, Murad MH, Stratakis CA, Torpy DJ. Diagnosis and treatment of primary adrenal insufficiency: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab.* 2016;101(2):364–389.
- Oelkers W, Diederich S, Bähr V. Diagnosis and therapy surveillance in Addison's disease: rapid adrenocorticotropin (ACTH) test and measurement of plasma ACTH, renin activity, and aldosterone. *J Clin Endocrinol Metab.* 1992;75(1):259–264.
- Thompson DG, Mason AS, Goodwin FJ. Mineralocorticoid replacement in Addison's disease. *Clin Endocrinol (Oxf).* 1979;10(5):499–506.

20. Husebye ES, Allolio B, Arlt W, Badenhoop K, Bensing S, Betterle C, Falorni A, Gan EH, Hulting AL, Kasperlik-Zaluska A, Kämpe O, Løvås K, Meyer G, Pearce SH. Consensus statement on the diagnosis, treatment and follow-up of patients with primary adrenal insufficiency. *J Intern Med*. 2014;275(2):104–115.
21. Flad TM, Conway JD, Cunningham SK, McKenna TJ. The role of plasma renin activity in evaluating the adequacy of mineralocorticoid replacement in primary adrenal insufficiency. *Clin Endocrinol (Oxf)*. 1996;45(5):529–534.
22. Dalin F, Nordling Eriksson G, Dahlqvist P, Hallgren Å, Wahlberg J, Ekwall O, Söderberg S, Rönnelid J, Olcén P, Winqvist O, Catrina SB, Kriström B, Laudius M, Isaksson M, Halldin Stenlid M, Gustafsson J, Gebre-Medhin G, Björnsdóttir S, Janson A, Åkerman AK, Åman J, Duchon K, Bergthorsdóttir R, Johannsson G, Lindskog E, Landin-Olsson M, Elfving M, Waldenström E, Hulting AL, Kämpe O, Bensing S. Clinical and immunological characteristics of autoimmune Addison disease: a nationwide Swedish multicenter study. *J Clin Endocrinol Metab*. 2017;102(2):379–389.
23. Grossman A, Johannsson G, Quinkler M, Zelissen P. Therapy of endocrine disease: perspectives on the management of adrenal insufficiency: clinical insights from across Europe. *Eur J Endocrinol*. 2013;169(6):R165–R175.
24. Smith SJ, MacGregor GA, Markandu ND, Bayliss J, Banks RA, Prentice MG, Dorrington-Ward P, Wise P. Evidence that patients with Addison's disease are undertreated with fludrocortisone. *Lancet*. 1984;1(8367):11–14.
25. Stowasser M, Ahmed AH, Pimenta E, Taylor PJ, Gordon RD. Factors affecting the aldosterone/renin ratio. *Horm Metab Res*. 2012;44(3):170–176.
26. Jenkins-Jones S, Parviainen L, Porter J, Withe M, Whitaker MJ, Holden SE, Morgan CL, Currie CJ, Ross RJM. Poor compliance and increased mortality, depression and healthcare costs in patients with congenital adrenal hyperplasia. *Eur J Endocrinol*. 2018;178(4):309–320.
27. Finkelstein GP, Kim MS, Sinaii N, Nishitani M, Van Ryzin C, Hill SC, Reynolds JC, Hanna RM, Merke DP. Clinical characteristics of a cohort of 244 patients with congenital adrenal hyperplasia. *J Clin Endocrinol Metab*. 2012;97(12):4429–4438.