Plasma renin measurements are unrelated to mineralocorticoid dose in patients with primary adrenal insufficiency

Pofi, Riccardo; Prete, Alessandro; Arlt, Wiebke

DOI:
10.1210/clinem/dgz055

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], Faisal S. Ahmed[4], Birgit Koehler[5], Antonio Balsamo[6], Federico Baronio[6], Carlo Acerini[7], Amalia Cannuccia[8], Ayla Guven[9], Tulay Guran[10], Feyza Darendeliler[11], Claire Higham[12], Walter Bonfig[13], Liat de Vries[14,15], Tania A.S.S Bachega[16], Mirela C Miranda[16], Berenice B. Mendonca[16], Violeta Iotova[17], Màrta Korbonits[18], Nils P. Krone[19], Ruth Krone[20], Andrea Lenzi[2], Wiebke Arlt[3,21], Richard J. Ross[19], Andrea M. Isidori[2] and Jeremy W. Tomlinson[1].

[1]Department of Endocrinology, Oxford Centre for Diabetes, Endocrinology and Metabolism (OCDEM) and NIHR Oxford Biomedical Research Centre, Churchill Hospital, University of Oxford, Oxford, OX37LE, UK;
[2]Department of Experimental Medicine, Sapienza University of Rome, Viale Regina Elena 324, 00161, Rome, Italy;
[3]Institute of Metabolism and Systems Research, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK;
[4]Developmental Endocrinology Research Group, University of Glasgow, UK;
[5]Department of Ecology and Genetics, Uppsala, Sweden;
[6]Department of Medical and Surgical Sciences, Paediatric Unit, S.Orsola-Malpighi University Hospital, Via Massarenti 11, 40138, Bologna, Italy;
[7]Department of Paediatrics, University of Cambridge, UK;
[8]Division of Endocrinology, Department of System Medicine, Section of Reproductive Endocrinology, Tor Vergata University of Rome, Fatebenefratelli Hospital San Giovanni Calibita, Rome, Italy;
[9]Saglik Bilimleri University, Zeynep Kamil Women and Children Hospital, Paediatric Endocrinology Clinic, Istanbul, Turkey;
[10]Marmara University, Department of Paediatric Endocrinology and Diabetes, Pendik, Istanbul, Turkey;
[11]Istanbul Faculty of Medicine, Department of Paediatrics, Paediatric Endocrinology Unit, Istanbul University, Çapa 34093, Istanbul, Turkey;
[12]Department of Endocrinology, Christie Hospital NHS Foundation Trust, Manchester, University Of Manchester, Manchester Academic Health Science Centre, Manchester, UK;
[13]Department of Paediatrics, Klinikum Wels-Grieskirchen, Wels, Austria; Department of Paediatrics, Technical University München, Munich, Germany;

[14]The Jesse Z and Sara Lea Shafer Institute for Endocrinology And Diabetes, Schneider Children's Medical Center Of Israel, Petah Tikva 49202, Israel;
[15]Sackler School of Medicine, Tel Aviv University, Israel;
[16]Unidade 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;
[17]Clinic Of Paediatric Endocrinology - UMHAT 'Sv. Marina', Medical University of Varna, Varna, Bulgaria;
[18]Centre for Endocrinology, William Harvey Research Institute, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, Charterhouse Square, London, ECM1 6BQ, UK.
[19]The University of Sheffield, Sheffield, UK;
[20]Birmingham Women's & Children's Hospital, Department for Endocrinology & Diabetes, Birmingham, UK;
[21]NIHR Birmingham Biomedical Research Centre, University Hospitals Birmingham NHS Foundation Trust and University of Birmingham, Birmingham, B15 3GW, UK;

Short Title: Renin and mineralocorticoid replacement.

Keywords: Congenital Adrenal Hyperplasia, salt-wasting CAH, primary adrenal insufficiency, Mineralocorticoid replacement, plasma renin concentration, Fludrocortisone

Word count: 3033

Number of Figures: 5

Number of Tables: 4

Address all correspondence and request for reprints:

Professor Jeremy Tomlinson,

Oxford Centre for Diabetes, Endocrinology and Metabolism

University of Oxford,

Oxford OX3 7LJ, UK

Email: Jeremy.tomlinson@ocdem.ox.ac.uk

Phone: +44 (0)1865857359

Fax: +44 (0)1865857213

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 (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.

Disclosure:

RJR is a Director of Diurnal Ltd, The other authors have nothing to disclose and have no relevant conflicts of interest

Acknowledgement:

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.

Abstract

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

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

Design: Observational, retrospective, longitudinal analysis.

Patients: 280 patients (with 984 clinical visits and plasma renin measurements) with primary adrenal insufficiency 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 (SW-CAH) (149 adults and 55 children) and 39 adult patients with Addison's 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 mineralocorticoid dose and PRC. Univariate analysis demonstrated a direct positive relationship between mineralocorticoid dose and PRC in adults and children. There was no relationship between mineralocorticoid dose and BP in adults, while BP increased with increasing mineralocorticoid dose in children. Using multiple regression modelling, sodium was the only measurement that predicted PRC in adults. Longitudinally, the change in mineralocorticoid dose was able to predict potassium, but not BP or PRC.

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

Précis: Plasma renin concentration is not related to mineralocorticoid dose. Serum electrolytes are associated with MC dose and should be considered to optimize MC dose in primary adrenal insufficiency.

Introduction

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 and is a crucial mechanism for maintaining blood pressure (BP) and electrolyte balance.

Primary adrenal insufficiency (PAI) is a life-threatening disease resulting from diseases directly involving the adrenal cortex. The clinical spectrum is characterised 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's disease, AD)(4,5), and the commonest 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 to mutations in genes encoding enzymes in pathways critical for adrenal steroid biosynthesis (6). The commonest 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 AI (9-11): so far only a few, small studies have investigated MC replacement in patients with primary AI (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)

147 within the upper limit of the local reference range (5,6). The most recent CAH Endocrine Society
148 guidelines suggests a fludrocortisone replacement dose of 50-200 µg/day (13). MC requirements
149 in infants and children decrease with age, reflecting changes in the capacity of the renal tubules to
150 reabsorb sodium over time. In adults, current guidance advocates titrating MC doses (and/or salt
151 supplementation) according to BP, serum sodium, potassium, and PRC appropriate for age.
152 Taking into account the complex regulation of PRC, for example with posture, as well as the
153 variability of timing of blood sampling with respect to the last fludrocortisone dose, we aimed to
154 explore the relationship between MC dose regimens and clinical and biochemical variables in *real-*
155 *life* clinical practice to determine whether they can usefully guide appropriate MC dose titration.
156

Patients and Methods

Patient selection

We performed a retrospective observational analysis of data from the International CAH Registry (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 (Figure 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) (Figure 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 mean arterial pressure (MAP) was calculated using the formula: diastolic blood pressure (DBP) + 1/3 of differential 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 median unless otherwise stated and change (Δ) for any variable was calculated by the difference: follow-up minus baseline. For the analysis in children, SDS (standard deviations scores) for BMI (sBMI), centiles for systolic blood pressure (cSBP) and diastolic blood pressure (cDBP) were calculated and the MC dose corrected by 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 was recorded with regards to the timing of the last fludrocortisone dose or adherence, and no centre adopted a standardised 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 centres that enrolled patients into the study (μIU/mL, ng/mL/h, nmol/L/h, pg/mL, ng/L). Every centre provided local reference range, which we used to categorise 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 a *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 3rd grade equation generated with MatLab (version 2017, MathWorks® Inc.) using the reference range of the different assay as intersection points.

Statistical Analysis

A Spearman's 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 Log_{10} 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 standard deviations, no leverage values greater than 0.2, and values for Cook's distance above 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) and 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 Figure 2. There was large variability in PRC, ranging from 0.6 to 3166 µUI/mL in adults (median 86µUI/mL) and 0.1 to 5090 µUI/mL in children (median 66µUI/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 (Figure 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 (Figure 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) (Figure 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 to those with low PRC. Patients with low and high PRC were younger and took higher MC_{BSA} dose compared to those with normal PRC (Figure 4f-j).

Plasma renin concentration, 17-OH progesterone and androstenedione

In order to determine if elevated PRC might be a reflection of under-treatment or non-adherence across both mineralocorticoid and glucocorticoid 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, 17OHP levels were similar in those 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 (17OHP: low PRC: 0.7 nmol/L (0.3-423.65); normal PRC: 48.41 nmol/L (6.35-1716); high PRC: 131 nmol/L (1.21-1424.1), p<0.01. Androstenedione: 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's 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 the assessments 433 days, range 33-2082). At follow-up, MC dose remained unchanged in 80 (67%) patients (*group A*) whilst in 9 (6%) patients (*group B*) MC dose was decreased (ΔMC dose -100µg/day, range -50 to -200) and in 23 (19%) patients (*group C*) MC dose was increased (ΔMC dose 50µg/day, range 25 to 100). Within each group, there was no significant change in ΔPRC ($\Delta PRC_{group\ A}$ 5 µUI/mL, z=0.783, p=0.434; $\Delta PRC_{group\ B}$ 0.1 µUI/mL, p=1.000; $\Delta PRC_{group\ C}$ -61 µUI/mL, p=0.405) (Figure 5). In addition, ΔPRC compared across groups were not different (p=0.560).

Multiple regression modelling significantly predicted ΔPRC (p=0.015). Only Na^+ concentration at 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 were found between ΔMC dose ΔPRC, ΔNa^+ or ΔMAP (data not show).

305

Discussion

In patients with adrenal insufficiency, there is an absolute requirement for lifelong steroid hormone replacement therapy. Almost 70 years have now 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 presence of peripheral edema (17). However, these are not always reliable and markers that are more objective are often used in addition. Compelling data 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.* showed that PRA was unable to distinguish between adequate and over-replacement and therefore raised doubts about its utility in MC dose optimization (19). Current expert consensus suggests that MC replacement should aim at normotension, normokalemia, and try to achieve PRC in the upper normal reference range (13,18,20,21).

In patients with AI, 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 under-replaced 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 in 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), perhaps suggesting relative MC under-replacement 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, with no internationally accepted standard reference range and interpretation of results that is dependent upon local reference intervals (17). In addition, there are many other factors that have a profound influence on PRC including volume status, salt intake, pregnancy, posture, ambient temperature, antihypertensive and non-steroidal anti-inflammatory drugs (25). Salt replacement is also a major confounding factor when PRC is evaluated and, for this reason, patients under salt supplementation were excluded from our analysis. In most centres, samples taken for PRC measurement are not standardised 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 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 a different mineralocorticoid 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 in whom up to one third of adult patients are non-adherent (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

362 global non-adherence (of both GC and MC replacement) may not be occurring and our data might
363 potentially point towards specific mineralocorticoid under-replacement. This contrasts with the
364 analysis in children, where we observed concordance between 17-OHP / androstenedione and
365 PRC levels. Taken together, these factors will undoubtedly contribute to the wide variability in PRC
366 values that we observed and the lack of relationship with MC replacement dose and biological
367 relevant clinical variables and endorses observations made in much smaller studies (27).
368 Our study does have limitations. It is a retrospective analysis from multiple centres, so there is the
369 potential for selection bias as well as high heterogeneity in our study population, and detailed,
370 extensive medical records were not available in many patients. Similarly, plasma renin has not
371 been measured centrally but was analysed by different assays in the participating centres. In
372 addition, we were unable to estimate the impact of glucocorticoid replacement therapy due to a
373 lack of information about preparation and dose. Also, we excluded the small number of patients
374 (n=7) who were taking salt supplementation as precise data on salt intake was not reported in the
375 records. The data we have analysed are from *'real-life'* clinic consultations and are not from a
376 standardized controlled clinical trial. This is particularly true for the longitudinal analysis where
377 titration of MC dose was made by physician preference rather than an established specific
378 algorithm. Prospective trials designed to reduce the effects of confounding factors through a
379 dedicated and rigorous approach are needed to clarify the contribution of different clinical and
380 biochemical variables on PRC and subsequently on MC dosage titration. While our study design is
381 clearly a limitation, this does offer a true reflection of the variables that are presented to clinicians
382 when trying to optimize the management of patients with PAI.
383 In conclusion, routine monitoring of serum electrolytes (alongside clinical assessment of symptoms
384 and BP) provides the most informative approach to add to PRC when MC replacement needs to be
385 adjusted. However, in the absence of the ability to standardise accurately the collection of samples
386 used to measure PRC, its routine measurement may conflict with other tools used to assess the
387 adequacy of MC replacement and decisions to modify MC dose should not be solely based on
388 PRC. There are many other questions that need to be addressed including under- or over-
389 replacement 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.

References

Bibliography

1. Ichikawi I, Harris RC. Angiotensin actions in the kidney: renewed insight into the old hormone. *Kidney Int*. 1991;40(4):583-596.
2. Cogan MG. Angiotensin II: a powerful controller of sodium transport in the early proximal tubule. *Hypertension*. 1990;15(5):451-458.
3. 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.
4. Charmandari E, Nicolaides NC, Chrousos GP. Adrenal insufficiency. *Lancet*. 2014;383(9935):2152-2167.
5. Bancos I, Hahner S, Tomlinson J, Arlt W. Diagnosis and management of adrenal insufficiency. *Lancet Diabetes Endocrinol*. 2015;3(3):216-226.
6. El-Maouche D, Arlt W, Merke DP. Congenital adrenal hyperplasia. *Lancet*. 2017;390(10108):2194-2210.
7. White PC, Speiser PW. Congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Endocr Rev*. 2000;21(3):245-291.
8. 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 E. Health status of adults with congenital adrenal hyperplasia: a cohort study of 203 patients. *J Clin Endocrinol Metab*. 2010;95(11):5110-5121.
9. 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.
10. Johannsson G, Nilsson AG, Bergthorsdottir R, Burman P, Dahlqvist P, Ekman B, Engstrom BE, Olsson T, Ragnarsson O, Ryberg M, Wahlberg J, Biller BM, Monson JP, Stewart PM, Lennernas 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.
11. 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.
12. Esposito D, Pasquali D, Johannsson G. Primary Adrenal Insufficiency: Managing Mineralocorticoid Replacement Therapy. *J Clin Endocrinol Metab*. 2018;103(2):376-387.
13. 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.
14. 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.
15. Bishop PM. The history of the discovery of Addison's disease. *Proc R Soc Med*. 1950;43(1):35-42.

16. 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.
17. 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.
18. Oelkers W, Diederich S, Bahr 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.
19. 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, Kampe O, Lovas 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 A, Wahlberg J, Ekwall O, Soderberg S, Ronnelid J, Olcen P, Winqvist O, Catrina SB, Kristrom B, Laudius M, Isaksson M, Halldin Stenlid M, Gustafsson J, Gebre-Medhin G, Bjornsdottir S, Janson A, Akerman AK, Aman J, Duchen K, Bergthorsdottir R, Johannsson G, Lindskog E, Landin-Olsson M, Elfving M, Waldenstrom E, Hulting AL, Kampe 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):R165-175.
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. Finkielstain 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.

Table 1: Baseline characteristics of 243 patients with adrenal insufficiency. Data are expressed as median (range). Abbreviations: SW-CAH=salt-wasting congenital adrenal hyperplasia, AD=Addison's disease, MC=mineralocorticoid, MC_{BSA}=MC daily dose corrected for body surface area (SBP=systolic blood pressure, DBP=diastolic blood pressure, MAP=mean arterial blood pressure, PRC=plasma renin concentration, BMI=body mass index, cSBP and cDBP=centile-corrected systolic and diastolic blood pressure, sBMI=SDS-corrected BMI.

	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/m^2)	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 (mmHg)	123(90-170)	123(90-169)	124(102-170)	101(62-150)
DBP (mmHg)	79(53-104)	79(57-104)	79(53-102)	62(32-88)
MAP (mmHg)	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 (mUI/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 (mmHg)	/	/	/	70(10-100)
cDBP (mmHg)	/	/	/	74(10-100)
sBMI (kg/m^2)	/	/	/	0.8(-1.8;3.5)

Table 2. Multiple regression modelling in adult patients with adrenal insufficiency (147 complete clinical assessments from 117 patients). The dependent variables assessed were plasma renin concentration (PRC), sodium (Na^+), potassium (K^+), mineralocorticoid (MC) dose, and mean arterial pressure (MAP). *p* value should be interpreted with Bonferroni correction, when significant, they are highlighted in bold and with asterisk.

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***

Table 3. Multiple regression modelling in adults with salt-wasting congenital adrenal hyperplasia (114 complete assessments from 82 patients). The dependent variables were plasma renin concentration (PRC), sodium (Na^+), potassium (K^+), mineralocorticoid (MC) dose, and mean arterial pressure (MAP). *p* value should be interpreted with Bonferroni correction, when significant, they are highlighted in bold and with asterisk.

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

Table 4. Multiple regression modelling in children with salt-wasting congenital adrenal hyperplasia (55 complete assessments from 11 patients). The dependent variables were plasma renin concentration (PRC), sodium (Na^+), potassium (K^+), SDS-corrected body mass index (sBMI), mineralocorticoid dose adjusted for body surface area, (MC_{BSA}), and centile-corrected systolic and diastolic blood pressure (cSBP and cDBP). *p* value should be interpreted with Bonferroni correction, when significant, they are highlighted in bold and with asterisk.

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

Figure Legends

Figure 1

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

Figure 2

Distribution of mineralocorticoid replacement dose in 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's disease (n=39) (a). Distribution of mineralocorticoid replacement dose in 55 children with salt-wasting congenital adrenal hyperplasia (b).

Figure 3

Baseline correlations of mineralocorticoid daily dose with clinical and biochemical variables 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) (a-d).

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 and cDBP=centile-corrected systolic and 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).

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).