Chapter 6

Effects of hydrocortisone on the regulation of blood pressure: results from a randomized controlled trial

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ABSTRACT

Context
Cardiovascular risk is increased in patients with secondary adrenal insufficiency, which may be ascribed to an unfavorable metabolic profile consequent to a relatively high hydrocortisone replacement dose.

Objective
We determined the effects of a higher versus a lower glucocorticoid replacement dose on blood pressure (BP), the renin-angiotensin-aldosterone system, 11β-hydroxysteroid dehydrogenase enzyme activity and circulating (nor)metanephrines.

Design, setting and patients
Forty-seven patients with secondary adrenal insufficiency from the University Medical Center Groningen participated in this randomized double-blind crossover study.

Interventions
Patients randomly received 0.2–0.3 mg hydrocortisone/kg body weight followed by 0.4–0.6 mg hydrocortisone/kg body weight, or vice versa, each during 10 weeks.

Main outcome measure(s)
BP and regulating hormones were measured.

Results
The higher hydrocortisone dose resulted in an increase in systolic BP of 5 (12) mm Hg \((P = .011)\), diastolic BP of 2 (9) mm Hg \((P = .050)\), and a median [interquartile range] drop in plasma potassium of -0.1 [-0.3; 0.1] nmol/liter \((P = .048)\). The higher hydrocortisone dose led to decreases in serum aldosterone of -28 [-101; 9] pmol/liter \((P = .020)\) and plasma renin of -1.3 [-4.5; 1.2] pg/ml \((P = .051)\), and increased the ratio of plasma and urinary cortisol to cortisone (including their metabolites) \((P < .001\) for all). Furthermore, on the higher dose, plasma and urinary normetanephrine decreased by -0.101 [-0.242; 0.029] nmol/liter \((P < .001)\) and -1.48 [-4.06; 0.29] µmol/mol creatinine \((P < .001)\) respectively.

Conclusions
A higher dose of hydrocortisone increased systolic and diastolic BP and was accompanied by changes in the renin-angiotensin-aldosterone system, 11β-hydroxysteroid dehydrogenase enzyme activity, and circulating normetanephrine. This demonstrates that hydrocortisone dose even within the physiological range affects several pathways involved in BP regulation.
INTRODUCTION

Patients with adrenal insufficiency (AI) require life-long glucocorticoid (GC) replacement therapy, most commonly administered as oral hydrocortisone (HC). Despite replacement therapy, these patients have an increased overall mortality rate compared to the general population; this is predominantly attributable to an increased risk of cardiovascular and cerebrovascular diseases.\textsuperscript{1-4}

Inappropriate GC substitution therapy may be an explanation for the higher risk of mortality and cardiovascular disease found in patients with AI. GC substitution therapy aims to mimic the endogenous circadian rhythm of cortisol secretion, with peak values in the morning, slowly declining levels throughout the day, and a nadir at midnight. However, conventional oral GC substitution therapy is unable to accurately reproduce this physiological variation,\textsuperscript{3} inevitably resulting in over- and under-replacement during certain periods of the day.

The association between the level of GCs and a number of cardiovascular risk factors, of which hypertension is the most prominent, is well-recognized.\textsuperscript{6-8} Globally, elevated blood pressure (BP) is the cause of approximately 50% of deaths from stroke or cardiovascular disease.\textsuperscript{9} Both in animal models and in human studies, exogenous GCs have been shown to raise BP,\textsuperscript{10} and patients with AI treated with GCs show increased cardiovascular risk.\textsuperscript{11} However, to the best of our knowledge, the relationship between BP and different GC replacement doses has only been examined in two small studies, which did not reveal a significant change in BP after a change in GC dose.\textsuperscript{12,13}

Nevertheless, the GC dose-response relationship with BP is still poorly documented in humans, as are the pathogenic mechanisms that conceivably underlie a possible BP response. Patients with secondary AI (SAI) are characterized by a loss of endogenous cortisol production, making them a unique patient cohort because this group lacks the negative feedback mechanisms of the hypothalamic-pituitary-adrenal axis. As a result, cortisol levels can be controlled externally, allowing study of the direct effects of cortisol concentrations on BP and regulating systems.

The present randomized double-blind crossover study was initiated to compare the effect of two different doses of HC on cognition (primary endpoint),\textsuperscript{14} quality of life (QOL),\textsuperscript{15} metabolic parameters, somatosensory functioning, and pharmacokinetic parameters (secondary endpoints). Here we report the results of the ancillary analysis of the effect of HC dose on BP and pathways related to BP regulation: the renin-angiotensin-aldosterone-system (RAAS), the 11\beta-hydroxysteroid dehydrogenase (11\beta-HSD) enzymes catalyzing the cortisol and cortisone interconversion, the sympathetic nervous system, and vasopressin.
MATERIALS AND METHODS

Patients
Reporting of the study conforms to the Consolidated Standards of Reporting Trials 2010 statement.\(^{16}\) This study is part of a randomized double-blind crossover study, of which the effects on cognition,\(^{14}\) pain, depressive symptoms, and QOL have been reported previously.\(^{15}\) Patients with SAI for which they received GC substitution therapy were eligible for this study. They were recruited from the endocrine outpatient clinic of the University Medical Center Groningen, a tertiary referral and expertise center for pituitary disease in the Netherlands. A total of 63 patients were included in the study, 60 of whom completed the run-in phase and the baseline measurement. The diagnosis of SAI was based on internationally accepted biochemical criteria. Early morning cutoff cortisol levels for AI in our center were validated for patients with hypothalamic-pituitary disorders, as published previously.\(^{17}\) Other inclusion and exclusion criteria and reasons for withdrawal have been described earlier.\(^{14}\)

The study protocol was approved by the local medical ethics review committee at the University Medical Center Groningen. Patients provided informed written consent before entering the study.

Intervention
Patients were randomly assigned to either group 1 or group 2. Group 1 first received a physiological lower dose of HC for 10 weeks, followed by a physiological higher dose for another 10 weeks. Group 2 received the two doses in reverse order. Patients were treated in a double blind fashion with oral tablets containing either 5 mg HC (lower dose) or 10 mg HC (higher dose). In the lower dose condition, patients received a cumulative daily dose of 0.2–0.3 mg HC/kg body weight, divided in three doses administered before breakfast, before lunch and before dinner, resulting in total daily doses between 15 and 20 mg HC depending on body weight. In the higher dose condition this was 0.4–0.6 mg HC/kg body weight, resulting in total daily doses between 30 and 40 mg HC dependent on body weight. For the exact dosing scheme, see Werumeus Buning et al.\(^{14}\) Compliance with study medication was assessed using daily diaries in which patients had to report every day whether they had forgotten or doubled their medication, and if so, how often. In addition, HC tablets returned by the patient at the end of each treatment period were counted. In cases of intercurrent illness or fever, patients were allowed to double or triple their HC dose according to a fixed protocol for a maximum of seven days (ie, 10% of the study time and of the cumulative HC dose). Adjustments of the dose of HC were not allowed in the week preceding testing. Randomization to one of the two treatment groups was performed by Tiofarma Inc., with a block size of four.
Protocol

After each 10-week treatment period, patients returned to the hospital for evaluation. On testing days, they were instructed to take their morning dose of HC at 07:00 am. At 08:00 am, the first blood samples were drawn in a fasting state in sitting position after a short period of rest for the measurement of sodium, potassium, creatinine, cortisol, cortisone, renin concentration, aldosterone, copeptin, and metanephrines (metanephrine, normetanephrine and 3-methoxytyramine). In addition, 24-hour urinary samples were collected for determination of sodium, potassium, osmolarity, cortisol, cortisone, GC metabolites, and metanephrines. Breakfast was provided for the patients, followed by a physical examination including the measurement of body weight, height and BP. Body mass index was calculated as the ratio between the weight (in kilograms) and height (in meters) squared (kg/m²). Blood pressure was measured three times with an automated device (Dinamap XL Model 9300; Johnson & Johnson Medical), with the patient in a sitting position, and reported as the mean of the three measurements. Approximately 5 hours after intake of the morning dose of HC, a second blood sample was drawn for the measurement of cortisol and cortisone.

Biochemical measurements

Total and free cortisol and cortisone in plasma, as well as free cortisol and free cortisone in urine, were all measured by isotope dilution liquid chromatography tandem mass spectrometry (LC-MS/MS). Total and free cortisol and cortisone in plasma were performed essentially as described by Hawley et al, using cortisol-13C₃ and cortisone-D₇ as internal standards.¹⁸ For free cortisol and cortisone, intra- and interassay coefficients of variation (CVs) were less than 6% and less than 5%, respectively. For total cortisol and cortisone, intra- and interassay CVs were less than 2.6% and less than 5.3%, respectively. Plasma equilibrium dialysis for free cortisol and cortisone was performed as described by Fiers et al,¹⁹ the only difference being that we used 10-kD cellulose membranes (Harvard Apparatus). Free cortisol and cortisone in urine were performed essentially as described by Jones et al.²⁰ Plasma renin concentration was measured with an immunoradiometric renin assay (Renin III Generation; Cisbio). The intra-assay CVs were 5.5, 4.7, and 1.6% at 6.0 ng/liter, 19.9 ng/liter, and 52.2 ng/liter, respectively. The interassay CVs were 14, 9.2, and 4.0% at 5.1 ng/liter, 19.8 ng/liter, and 54.7 ng/liter, respectively.²¹ Aldosterone in serum was measured by LC-MS/MS, essentially as described by Van der Gugten et al,²² but using additional online solid-phase extraction in combination with LC-MS/MS analysis. Intra- and interassay CVs were less than 6%. Osmolarity in serum and urine was measured by freezing-point depression on an osmometer (Menarini Diagnostics). Plasma sodium, potassium, creatinine, and urinary sodium and potassium were all measured routinely using a Roche Modular ISE/P system (Roche Diagnostics). Serum corticosteroid-binding
globulin concentrations were determined in duplicate using a RIA (IBL International GmbH). GC metabolites (tetrahydrocortisol [THF], allo-tetrahydrocortisol [alloTHF], tetrahydrocortison [THE]) in urine were measured using gas chromatography in combination with mass spectrometry, as previously described. Free metanephrines in plasma were analyzed by LC-MS/MS. Urinary free metanephrine concentrations were also determined by LC-MS/MS, and concentrations were normalized to the urinary excretion of creatinine, measured using an enzymatic method (Roche Diagnostics), and expressed in units of µmol/mol creatinine. Plasma copeptin was measured using a sandwich immunoassay on a Kryptor immunoassay analyzer (Thermo Fischer) based on the assay described by Morgenthaler et al. Copeptin can be considered a surrogate marker of vasopressin and has the same rapid in vivo response to osmotic changes as vasopressin but has higher ex vivo preanalytical stability than vasopressin and measurement is faster and easier.

11β-HSD enzyme activity

The urinary cortisol to cortisone metabolite ratio (THF+alloTHF)/THE and plasma cortisol to cortisone ratio are regarded as an overall measure of 11β-HSD activity and determined by the combined enzymatic activities of both 11β-HSD types 1 and 2. The ratio of urinary free cortisol (UFF) to urinary free cortisone (UFE) is considered specifically to reflect renal 11β-HSD type 2 activity. In the liver 11β-HSD type 1 predominantly converts the inactive cortisone to the biologically active cortisol, whereas 11β-HSD type 2, which is highly expressed in mineralocorticoid target tissues, converts active cortisol to inactive cortisone to protect mineralocorticoid receptors (MR) from stimulation by GC.

Statistics

As described previously, a prestudy power analysis revealed that a study design with two arms of 25 patients each was required to detect a change in primary or secondary endpoints with an effect size of 0.4 (two-sided α = 0.05 and β = 0.80), even when between-test correlations are poor (0.50). An effect size of 0.4 was chosen because it is considered a relevant change with a small to moderate effect size. To allow for a dropout rate of about 20%, a total of 60 patients needed to be included.

Normally distributed data were presented as mean (SD), non-normally distributed data were presented as median [interquartile range], and categorical data were presented as number or percentage. Normality of data was analyzed by visual inspection of Q-Q plots and histograms.

To test for period and carryover effects, the procedure developed by Altman was used. In this procedure, to test for a carry-over effect, the average response to both treatments (ie, of the low dose and high dose combined) was compared between the
two treatment groups. If these average responses were not different between the treatment groups, the effect of the treatment was considered the same irrespective of the order in which the treatments were administered. All variables were compared using the Wilcoxon signed rank test for paired observations. Statistical significance was set at a \( P < .05 \). All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS, Inc.), version 22.

RESULTS

Study population
A total of 63 patients were included in this trial. Forty-seven of them completed both study periods (29 men, age 51 (14) years [range, 19–73]). The patient flow through the study is shown in Supplemental Figure 1. Thirteen patients withdrew from the study, with various reasons for withdrawal that have been described previously. The number of patients that did not complete the study was comparable between the two doses and the two treatment groups (data not shown). Furthermore, subjects completing the study did not differ from those who did not with regard to age, sex, educational level, age at diagnosis, childhood onset or adult onset, body weight, or number of patients taking antihypertensive medication (data not shown). One patient was excluded from the analysis because of starting BP-lowering medication during the study; therefore a total of 46 patients were included in the present analysis. The patients’ characteristics are given in Supplemental Table 1. Fourteen of the patients used BP-lowering medication before the study. Of those, four received angiotensin-converting enzyme inhibitors, one received thiazides, nine received beta blockers, six received calcium antagonists, two received angiotensin II receptor antagonists, and one received alpha blockers (some patients used multiple BP-lowering drugs). All other medication apart from the study medication remained stable during the study periods.

Effects of HC dose on cortisol and cortisone concentrations in blood and urine
During the low dose condition, patient received on average 17.9 (2.2) mg HC/day, during the high dose condition they received on average 35.8 (4.5) mg HC/day. Administration of the higher HC dose resulted in significantly higher plasma total cortisol levels compared to the administration of the lower HC dose, both 1 hour after ingestion (median difference, 233 [136; 367] nmol/liter) and approximately 5 hours after ingestion (median difference, 100 [10; 156] nmol/liter, both \( P < .001 \) (Table 1). Plasma free cortisol levels were significantly increased after the higher dose of HC, both 1 hour after administration (median difference, 31 [14; 43] nmol/liter) and approximately 5 hours after administration (median difference, 4 [1; 7] nmol/liter, both
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Table 1. Cortisol concentrations in plasma and urine (n = 46)

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<th>Lower dose</th>
<th>Higher dose</th>
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<td><strong>Plasma total cortisol levels</strong></td>
<td></td>
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</tr>
<tr>
<td>1 h after ingestion (nmol/liter)</td>
<td>500 [389; 602]</td>
<td>745 [676; 880]</td>
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<tr>
<td>5 h after ingestion (nmol/liter)</td>
<td>117 [76; 211]</td>
<td>232 [171; 311]</td>
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<tr>
<td>1 h after ingestion (nmol/liter)</td>
<td>65 [53; 70]</td>
<td>63 [55; 71]</td>
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</tr>
<tr>
<td>5 h after ingestion (nmol/liter)</td>
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<td>5 h after ingestion (nmol/liter)</td>
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<td>7 [5; 8]</td>
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<td><strong>Urinary free cortisol (nmol/24 h)</strong></td>
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<td>281 [200; 411]</td>
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<tr>
<td><strong>Urinary free cortisone (nmol/24 h)</strong></td>
<td>229 [147; 374]</td>
<td>445 [326; 663]</td>
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Data are median [interquartile range].

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Data are median [interquartile range].

* P < .01 (Table 1). UFF median excretion levels were increased after receiving the higher dose of HC by 209 [134; 320] nmol/24 hours compared to the lower dose (P < .001) (Table 1). Plasma total cortisone levels did not differ between the two HC doses 1 hour after ingestion of the morning dose, whereas 5 hours after the morning dose of HC a significant increase was observed after the higher dose (median difference, 17 [-1; 27] nmol/liter, P < .001) (Table 1). For plasma free cortisone levels, a significant increase was found both 1 hour and 5 hours after intake of the higher HC dose (median difference, 1 [-1; 2] nmol/liter and 3 [-1; 4] nmol/liter, respectively, both P < .05) (Table 1). Furthermore, UFE median excretion levels were increased with 230 [133; 370] nmol/24 hours (P < .001) after receiving the higher dose of HC (Table 1).

**Effects of HC dose on BP and body weight**

An increase in systolic BP of 5 (12) mm Hg (P = .011) and a borderline significant increase in diastolic BP of 2 (9) mm Hg (P = .050) were observed in patients using the higher compared with the lower HC dose (Table 2). The effect of HC dose on BP was not different between patients treated with BP-lowering medication and those who were not (Supplemental Table 2). Body weight and body mass index tended to increase by 0.5 (1.7) kg (P = .060) and 0.2 (0.5) kg/m² points (P = .045), respectively, in response to the higher HC dose (Table 2).
Effects of Hydrocortisone on Blood Pressure

The RAAS

The higher dose of HC led to a borderline significant median decrease in plasma renin of -1.3 [-4.5; 1.2] pg/ml (P = .051, Figure 1A), a median decrease in aldosterone of -28 [-101; 9] pmol/liter (P = .020, Figure 1B) and a median decrease in the aldosterone-to-renin ratio of -2.7 [-5.7; 1.5] pmol/ng (P = .044), compared to the lower dose of HC (Table 2). Treatment with the higher HC dose was followed by a small, albeit significant, reduction of plasma potassium concentrations compared to the lower HC dose (median difference, -0.1 mmol/liter [-0.3; 0.1] mmol/liter, P = .048). Plasma sodium and creatinine concentrations, as well as the transtubular potassium gradient, did not differ between the two doses. The higher dose of HC led to higher 24-hour urine potassium excretion (median difference, 10 [-9; 28] mmol/24 hours, P = .032), but sodium excretion remained unchanged. Of note, a significant treatment by period interaction effect was found for plasma renin concentration (P = .039). Thus, the effect of dose was different for the two treatment groups, depending on the order in which the doses were given (Supplemental Figure 2). We performed a sensitivity analysis

Table 2. Anthropometric measures and biochemical and hormonal analysis (n = 46)

<table>
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<tr>
<th>Measure</th>
<th>Lower dose</th>
<th>Higher dose</th>
<th>P value</th>
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<tr>
<td>Systolic BP (mm Hg)</td>
<td>133 (14)</td>
<td>138 (16)</td>
<td>.011</td>
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<tr>
<td>Diastolic BP (mm Hg)</td>
<td>76 (10)</td>
<td>78 (9)</td>
<td>.050</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>82.8 (14.0)</td>
<td>83.3 (14.3)</td>
<td>.060</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.9 (4.0)</td>
<td>27.1 (4.0)</td>
<td>.045</td>
</tr>
<tr>
<td>Plasma sodium (mmol/liter)</td>
<td>142 [141; 143]</td>
<td>142 [141; 143]</td>
<td>.099</td>
</tr>
<tr>
<td>Plasma potassium (mmol/liter)</td>
<td>3.9 [3.7; 4.0]</td>
<td>3.8 [3.6; 4.0]</td>
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</tr>
<tr>
<td>Plasma creatinine (µmol/liter)</td>
<td>82 [66; 88]</td>
<td>80 [68; 89]</td>
<td>.109</td>
</tr>
<tr>
<td>Serum CBG (µg/ml)</td>
<td>53.2 [49.1; 63.0]</td>
<td>56.5 [49.0; 62.5]</td>
<td>.102</td>
</tr>
<tr>
<td>Plasma renin concentration (pg/ml)</td>
<td>11.6 [6.7; 17.3]</td>
<td>8.6 [5.9; 14.9]</td>
<td>.051</td>
</tr>
<tr>
<td>Serum aldosterone (pmol/liter)</td>
<td>150 [77; 256]</td>
<td>107 [43; 235]</td>
<td>.020</td>
</tr>
<tr>
<td>ARR (pmol/ng)</td>
<td>13.8 [7.3; 21.3]</td>
<td>11.0 [6.1; 19.8]</td>
<td>.044</td>
</tr>
<tr>
<td>Plasma copeptin (pmol/liter)</td>
<td>3.7 [2.5; 5.0]</td>
<td>3.4 [2.5; 4.9]</td>
<td>.819</td>
</tr>
<tr>
<td>Urine sodium (mmol/24 h)</td>
<td>142 [119; 206]</td>
<td>161 [112; 200]</td>
<td>.534</td>
</tr>
<tr>
<td>Urine potassium (mmol/24 h)</td>
<td>77 [64; 96]</td>
<td>83 [69; 103]</td>
<td>.032</td>
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<tr>
<td>Creatinine clearance calculated (ml/min)</td>
<td>117 (37)</td>
<td>117 (29)</td>
<td>.700</td>
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Abbreviations: ARR, aldosterone to renin ratio; CBG, corticosteroid-binding globulin; TTKG, transtubular potassium gradient.

Data are mean (SD) or median [interquartile range]. Fourteen patients received blood pressure lowering medication. Diabetes mellitus treated with medication known to be able to induce hypoglycaemia was an exclusion criterion.

a n = 45, b n = 43, c n = 44.
to further study the effects of HC dose on the RAAS. To this end, we excluded all patients with RAAS-influencing medication (n = 13). Results are shown in Supplemental Table 3. The effects on BP, plasma renin, serum aldosterone, plasma potassium and the aldosterone-to-renin ratio remained significantly different and were even more pronounced than in the entire group.

**11β-HSD enzyme activity**

A shift toward higher cortisol exposure on the higher HC dose was found as calculated by the ratios of plasma cortisol to cortisone (overall 11β-HSD enzyme activity) (median increase of 4 [2; 6] for total cortisol to cortisone; median increase of 3 [1; 4] for free cortisol to cortisone, Figure 2, A and B), urinary (THF+alloTHF)/THE ratio (overall 11β-HSD enzyme activity) (median increase, 0.41 [0.22; 0.69], Supplemental Figure 3A) and the ratio UFF/UFE (11β-HSD type 2 enzyme activity) (median increase, 0.30 [0.20; 0.47], Supplemental Figure 3B).

**Figure 1.** Plasma renin concentration (A) and serum aldosterone (B) on the two doses of hydrocortisone. Box plots represent median and interquartile ranges, whiskers represent fifth and 95th percentiles. HD, high-dose hydrocortisone; LD, low-dose hydrocortisone. ***P < .05.
Sympathetic activity

Plasma free normetanephrine concentrations showed a median decrease of -0.101 [-0.242; 0.029] nmol/liter \((P < .01)\) on the higher dose of HC (Figure 3A). Plasma free metanephrine and 3-methoxytyramine levels remained unchanged (Figure 3, B and C). Free normetanephrine in 24-hour urinary samples decreased on the higher dose of HC (median difference, -1.48 [-4.06; 0.29] µmol/mol creatinine, \(P < .001\), Figure 4A), as well as free 3-methoxytyramine (median difference, -2.20 [-3.99; -0.82] µmol/mol creatinine, \(P < .001\), Figure 4C), whereas urinary free metanephrine concentrations remained unchanged (Figure 4B).

Copeptin

Copeptin concentrations were not different on the two doses of HC (Table 2).
DISCUSSION

The present double-blind randomized controlled trial showed that higher levels of HC substitution doses lead to increased BP together with changes in several related

**Figure 3.** Plasma free normetanephrine (NMN) (A), metanephrine (MN) (B), and 3-methoxytyramine (3-MT) (C) concentrations on the two doses of hydrocortisone. Box plots represent median and interquartile ranges, whiskers represent fifth and 95th percentiles. HD, high-dose hydrocortisone; LD, low-dose hydrocortisone.

**P < .01.**
systems such as the RAAS, 11β-HSD enzyme activity and sympathetic nerve activity.

A major strength of this study is its crossover design. Patients were their own controls, thereby minimizing interindividual effects. Furthermore, patients with SAI have an intact RAAS, but lack the negative feedback mechanism of the hypothalamic-pituitary-adrenal axis that is normally present in healthy individuals. This enabled us to control the cortisol levels externally and study the effects of cortisol levels on

![Figure 4](image)

**Figure 4.** Urinary free normetanephrine (NMN) (A), free metanephrine (MN) (B), and free 3-methoxytyramine (3-MT) (C) concentrations on the two doses of hydrocortisone. Box plots represent median and interquartile ranges, whiskers represent fifth and 95th percentiles. HD, high-dose hydrocortisone; LD, low-dose hydrocortisone. ***P < .001.
BP and regulating mechanisms. Furthermore, elaborate biochemical measurements enabled us to explore the effect on mechanisms involved in BP regulation. A limitation of the study is that the BP value is based on three office BP measurements, whereas 24 hour ambulatory BP measurement is reported to be more accurate and is more related to cardiovascular events.\textsuperscript{33,34}

The effects of HC on BP are likely to be caused by increased MR activation. The higher HC dose resulted in an increase in BP together with a decrease in plasma potassium, an increase in urinary potassium excretion, and a decrease in plasma renin and serum aldosterone concentrations that, all taken together, are compatible with activation of the MR.\textsuperscript{35} These effects were even more pronounced when studied in patients without RAAS-influencing medication. Our results are in contrast to those found by Dunne and colleagues.\textsuperscript{12} They found no change in BP in 13 patients after reduction of a cumulative dose of HC from 30 to 15 mg/d. It is possible that because of the small number of patients, this study may have lacked power to detect an effect on BP. Our results are also in contrast to the findings of Pettersons and colleagues.\textsuperscript{13} In this study, among 17 subjects with SAI, BP was unaltered after increasing the HC daily replacement dose equivalent to less than 20 mg/d to 30 mg/d for 7 days. That study, however, was much smaller, had a nonrandomized design, used a different GC dosing protocol, and had a considerable shorter intervention period compared to our study. Further, in another open-label randomized trial, Johannsson and colleagues were able to show a beneficial effect of reduced cortisol exposure on BP.\textsuperscript{36} Using an oral dual-release HC formulation and compared to thrice daily dosing, a similar daily dose of the dual-release HC resulted in a more physiological cortisol profile and a 20\% reduction in 24-hour cortisol exposure. The investigators describe a reduction in BP of 6 mm Hg systolic and 2 mm Hg diastolic; however, mechanisms leading to this reduction in BP were not studied.

Sodium is an important determinant of the RAAS and sodium retention results in decreased renin concentrations. In addition to MR, glucocorticoid receptors also mediate sodium retention\textsuperscript{37}; thus the observed suppression of renin concentration could also be the result of activation of the glucocorticoid receptors. No information on sodium intake is available for the present patient group. However, plasma sodium concentrations as well as sodium excretion remained unchanged under the two doses of HC. Therefore, it is unlikely that sodium intake has influenced our results.

Exogenous estrogen may interfere with cortisol level measurements because of estrogen induced elevation of serum corticosteroid-binding globulin levels.\textsuperscript{38} However, to correct for this effect, several measurements of cortisol were included that are not influenced by corticosteroid-binding globulin, most importantly plasma free cortisol. An additional analysis was performed in women with and without estrogen substitution and showed that both plasma total cortisol levels and plasma free cortisol levels did not differ, neither on the two different HC doses, nor at the different time points after HC intake (data not
shown). Furthermore, estrogen substitution therapy remained unchanged throughout the study. Even though we cannot fully exclude the effects of possible confounding covariates, because of the crossover design of the study, probable effects are reduced.

It is known that cortisol and aldosterone have similar affinity for the MR in vitro, but 11β-HSD type 2 protects the MR in vivo from stimulation by cortisol through catalyzing the conversion of biologically active cortisol to inactive cortisone. The increased MR effects we observed may be caused by a change in set point of 11β-HSD enzyme activity, thereby favoring enhanced cortisol exposure. This seems attributable to a change in 11β-HSD type 2 enzyme activity as indicated by the increased UFF/UFE ratio, whereas changes in 11β-HSD type 1 activity cannot be excluded. Intriguingly, 11β-HSD activity may thus contribute to a feed-forward system enhancing systemic cortisol exposure. In accordance with these findings, Sherlock and colleagues showed that patients receiving exogenous HC therapy demonstrate a significantly altered corticosteroid metabolism with respect to urinary (THF+alloTHF)/THE ratios, with the highest ratios found in patients receiving the highest dose HC of 30 mg HC/day. Although the use of metabolite ratios is widely accepted as a tool to assess enzyme activity, newer study methodology, in particular using isotope tracer infusions (ie deuterated cortisol and cortisone), may allow improved delineation of the activity of 11β-HSD type 1 and 2 in vivo.

Plasma and urinary normetanephrine levels were decreased during treatment on the higher HC replacement dose. Normetanephrine is a metabolite of norepinephrine, which is a neurotransmitter released by sympathetic nerves. Normetanephrine concentrations are widely accepted to reflect changes in sympathetic nerve activity in normal physiology. The most likely explanation for the decrease in sympathetic activity is the baroreceptor reflex which is influenced by (a change in) BP. Elevated BP results in an increase in the sensory impulses transmitted from the baroreceptors to the vasomotor center in the brainstem, enhancing parasympathetic and decreasing sympathetic activity in order to reestablish normal BP values. In this context, it is also relevant that increased effector organ responsiveness towards norepinephrine under high GC doses is observed. Of note, supraphysiological doses of GCs were used in the described studies.

We found no changes in copeptin as surrogate marker for vasopressin. Vasopressin is involved in homeostasis by regulating water absorption in the renal tubules and by vasoconstriction mediated by two different vasopressin receptors. Hypocortisolism changes the free water clearance by corticotropin-releasing hormone-stimulated vasopressin release. Potentially, we were unable to demonstrate an effect of HC dose on copeptin concentrations because our study subjects were deficient in ACTH and possibly also in part in corticotropin-releasing hormone. The observed response may thus be different in healthy individuals.

A complex balancing of the harms and benefits needs to be made in the individualization of substitution therapy in patients with SAI. We suggest to use the lowest dose
possible that relieves symptoms of GC deficiency to prevent the observed negative side effects of higher doses of GCs. However, in light of our previous paper, which showed improved QOL after higher substitution doses, an increase in dose should be considered when necessary or desirable.

In conclusion, a higher dose of HC increased BP and resulted in changes in BP-regulating mechanisms, with most pronounced effects found in the RAAS, 11β-HSD enzyme activity, and sympathetic nerve activity.

ACKNOWLEDGEMENT

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REFERENCES


Chapter 6

SUPPLEMENTAL DATA

Supplemental Figure 1. Patient flow diagram

624 pituitary patients underwent medical-record review

488 were ineligible for screening
- 337 had no adrenal insufficiency
- 38 were > 75 years old
- 22 had diabetes mellitus
- 15 had body weight > 100 kg at screening
- 13 used anti-epileptics
- 74 had other reasons

135 were eligible for screening
- 72 declined to participate

68 were randomized and assigned to run-in phase
- 60 completed
- 3 did not complete
- 2 withdrew consent
- 1 withdrawn by investigator

30 received low dose hydrocortisone
- 25 completed
- 5 did not complete
- 3 violated protocol
- 1 withdrew consent
- 1 withdrawn by investigator

First treatment period (10 weeks)

30 received high dose hydrocortisone
- 28 completed
- 2 did not complete
- 1 violated protocol
- 1 withdrew consent

25 received high dose hydrocortisone
- 22 completed
- 3 did not complete
- 3 violated protocol

Second treatment period (10 weeks)

28 received low dose hydrocortisone
- 25 completed
- 3 did not complete
- 1 violated protocol
- 2 withdrew consent

Analysis

47 were included in the analysis
Supplemental Figure 2. Period by treatment interaction effect
Group 1 received first the lower dose of hydrocortisone followed by the higher dose; Group 2 first received the higher dose followed by the lower dose. The mean values for renin concentration of the two groups differed significantly ($P = 0.039$), indicating a period by treatment interaction effect.
Supplemental Figure 3. Ratio of (THF+alloTHF)/THE (A) and the ratio of UFF/UFE (B) on the two doses of hydrocortisone. Box plots represent median and interquartile ranges, whiskers represent 5th and 95th percentile. HD: high dose hydrocortisone, LD low dose hydrocortisone, THE: tetrahydrocortisone, THF: tetrahydrocortisol, allo-THF: allo-tetrahydrocortisol, UFE: urinary free cortisone, UFF: urinary free cortisol. *** $P < 0.001$. 
**Supplemental Table 1.** Clinical characteristics at baseline of pituitary patients treated for adrenal insufficiency (N = 46)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (men/women), n</td>
<td>28/18</td>
</tr>
<tr>
<td>Age (y), median [IQR]</td>
<td>54 [42; 61]</td>
</tr>
<tr>
<td>Age at diagnosis (y), median [IQR]</td>
<td>31 [20; 46]</td>
</tr>
<tr>
<td>Body weight (kg), median [IQR]</td>
<td>82.0 [72.1; 93.1]</td>
</tr>
<tr>
<td>Hydrocortisone treatment prior to randomization</td>
<td></td>
</tr>
<tr>
<td>Total daily dose (mg/day), median [IQR]</td>
<td>25 [20; 30]</td>
</tr>
<tr>
<td>Dose/kg body weight (mg/kg), median [IQR]</td>
<td>0.32 [0.25; 0.35]</td>
</tr>
<tr>
<td>Number of daily dosages (1/2/3), n</td>
<td>3/33/10</td>
</tr>
<tr>
<td>Duration of glucocorticoid treatment (y), median [IQR]</td>
<td>12 [5; 23]</td>
</tr>
<tr>
<td>No. of hormonal replacements (1/2/3/4/5)</td>
<td>3/9/21/10/3</td>
</tr>
<tr>
<td>Thyroid hormone deficiency (% of patients)</td>
<td>91</td>
</tr>
<tr>
<td>Growth hormone deficiency (% of patients)</td>
<td>66</td>
</tr>
<tr>
<td>Growth hormone deficiency (% of patients receiving substitution)</td>
<td>67</td>
</tr>
<tr>
<td>Sex hormone deficiency (% of patients)</td>
<td>57</td>
</tr>
<tr>
<td>Men: testosterone (% of patients receiving substitution)</td>
<td>79</td>
</tr>
<tr>
<td>Premenopausal women, n = 8: estrogens (% of patients receiving substitution)</td>
<td>50</td>
</tr>
<tr>
<td>Postmenopausal women, n = 10: estrogens</td>
<td>NA</td>
</tr>
<tr>
<td>Desmopressin (% of patients)</td>
<td>20</td>
</tr>
</tbody>
</table>

Abbreviations: IQR: Interquartile range, NA: Not applicable.
**Supplemental Table 2.** Effect of dose of HC on blood pressure for patients with and without blood-pressure lowering medication

<table>
<thead>
<tr>
<th>Blood-pressure lowering medication (n = 13)</th>
<th>No blood-pressure lowering medication (n = 33)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ Systolic blood pressure (mm Hg)</td>
<td>5 (17)</td>
<td>5 (10)</td>
</tr>
<tr>
<td>Δ Diastolic blood pressure (mm Hg)</td>
<td>3 (10)</td>
<td>1 (8)</td>
</tr>
</tbody>
</table>

One patient was excluded from the analysis due to the introduction of a new blood-pressure lowering drug during the study.

Data are mean (SD).

* *P*-value blood-pressure lowering medication versus no blood-pressure lowering medication by Mann-Whitney U Test

**Supplemental Table 3.** Effect of HC dose on blood pressure and the RAAS for patients not using medication influencing the RAAS (N = 33)

<table>
<thead>
<tr>
<th></th>
<th>Lower dose</th>
<th>Higher dose</th>
<th>P-value *</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP (mmHg)</td>
<td>129 (12)</td>
<td>135 (14)a</td>
<td>0.007</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>76 (9)</td>
<td>77 (8)</td>
<td>0.108</td>
</tr>
<tr>
<td>Plasma potassium (mmol/L)</td>
<td>3.9 [3.7; 4.1]</td>
<td>3.7 [3.6; 4.0]</td>
<td>0.006</td>
</tr>
<tr>
<td>Plasma renin concentration (pg/mL)</td>
<td>11.9 [7.6; 19.0]</td>
<td>8.6 [6.0; 5.0]</td>
<td>0.021</td>
</tr>
<tr>
<td>Serum aldosterone (pmol/L)</td>
<td>157 [68; 278]</td>
<td>91 [39; 220]</td>
<td>0.002</td>
</tr>
<tr>
<td>ARR (pmol/ng)</td>
<td>12.3 [7.3; 21.3]</td>
<td>9.5 [6.4; 18.6]</td>
<td>0.004</td>
</tr>
<tr>
<td>TTKG</td>
<td>7.9 [6.5; 9.8]</td>
<td>8.0 [6.2; 10.1]</td>
<td>0.570</td>
</tr>
<tr>
<td>Urine potassium (mmol/24hr)</td>
<td>82 [61; 100]</td>
<td>83 [68; 106]a</td>
<td>0.116</td>
</tr>
</tbody>
</table>

Patients using angiotensin-converting-enzyme inhibitors, angiotensin II receptor blockers, beta-blockers and diuretics were excluded.

Abbreviations: ARR: aldosterone to renin ratio, DBP: diastolic blood pressure, SBP: systolic blood pressure, TTKG: transtubular potassium gradient.

Data are mean (SD) or median [interquartile range].

* *P*-value lower dose versus higher dose by Wilcoxon Signed Rank Test for paired data.