Understanding clinical outcome in patients with pituitary disease: a biopsychosocial approach

Andela, C.D.; Andela C.D.

Author: Andela, C.D.
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CHAPTER 5

Increased hair cortisol concentrations and BMI in patients with pituitary- adrenal disease on hydrocortisone replacement

Sabine M. Staufenbiel*, Cornelie D. Andela*, Laura Manenschijn, Alberto M. Pereira, Elisabeth F.C. van Rossum, and Nienke R. Biermasz

**Equally contributed*

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ABSTRACT

Background: Intrinsic imperfections and lack of reliable biomarkers preclude optimal individual dosing of hydrocortisone replacement in adrenal insufficiency (AI). However, the clinical relevance of optimal dosing is exemplified by frequently occurring side effects of over-replacement and the dangers of under-replacement. Cortisol in scalp hair has been identified as a retrospective biomarker for long-term cortisol exposure. We compared hair cortisol concentrations ($CORT_{hair}$) of patients with primary or secondary AI on replacement therapy with those of patient controls with a pituitary disease without AI (PC) and of healthy controls (HC).

Methods: In this cross-sectional study, hair samples and anthropometric data were collected in 132 AI patients (52 males), 42 PC (11 males), and 195 HC (90 males). The proximal 3 cm of hair were used. $CORT_{hair}$ were measured using ELISA.

Findings: $CORT_{hair}$ were higher in AI patients than in HC and PC ($P < 0.001$), and hydrocortisone dose correlated with $CORT_{hair}$ ($P = 0.04$). Male AI patients demonstrated higher $CORT_{hair}$ than female patients ($P < 0.001$). AI patients had higher body mass index (BMI) than HC ($P < 0.001$), and BMI correlated with $CORT_{hair}$ in the whole sample ($P < 0.001$).

Interpretation: Physiological hydrocortisone replacement is associated with increased COR T_{hair}. The association between $CORT_{hair}$ and BMI could suggest a mild overtreatment that may lead to adverse anthropomorphic side effects, especially in males. $CORT_{hair}$ measurements may be a promising additional tool to monitor cumulative hydrocortisone replacement in AI.

INTRODUCTION

Adrenal insufficiency (AI) in which the adrenal corticosteroid synthesis, i.e. cortisol production, is insufficient can be primary in case of pathology of the adrenal glands, or secondary in case of hypopituitarism. Patients with AI need replacement therapy with exogenous glucocorticoids, preferably hydrocortisone, which is synthetically produced cortisol (1). In persons with intact adrenal function around 5 to 10 mg of cortisol per m^2 of body surface area per day is produced (2), with increased requirements during stress. The corresponding chronic oral replacement dosage is 15–25 mg per day, usually divided in three dosages in an attempt to mimic the circadian rhythm of natural cortisol secretion, with a peak in the morning and a gradual decrease during the day and evening (1, 3). It is recommended that hydrocortisone replacement should be individualized, taking into account blood pressure, metabolic derangements and sense of well-being (4). Various maintenance dosing strategies have been published (5). However, it is likely that there will be large individual variation in substitution requirements in view of differences in cortisol sensitivity due to polymorphisms of the glucocorticoid receptor gene (6). Currently available cortisol measurements in plasma, urine or saliva do not reflect cortisol action at tissue level. In accordance, plasma and salivary cortisol concentrations vary considerably between patients receiving hydrocortisone replacement, limiting the possibility to titrate individual hydrocortisone doses upon single plasma, or salivary measurements (7). A method to retrospectively assess cortisol for longer periods of time is the analysis of cortisol in scalp hair (8). As hair grows approximately one cm per month (9), a hair sample of for example three cm represents the long-term cortisol concentration of three months. Hydrocortisone is identical to human cortisol and has been shown to be measureable in scalp hair (8, 10).

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Hair cortisol levels ($CORT_{hair}$) have repeatedly been associated with body mass index (BMI) and increased risk of metabolic syndrome and cardiovascular disease in populations with endogenous cortisol metabolism (11-15). Until now, there is very limited data on the clinical utility of $CORT_{hair}$ measurements in patients with hydrocortisone replacement as is the case in AI. A recent study by Gow et al. demonstrated that hydrocortisone dose was significantly positively associated with $CORT_{hair}$ in patients with primary AI (10). Furthermore, they demonstrated a significant difference in $CORT_{hair}$ in male subjects between patients and controls, but no statistically significant difference in females. In addition, they did not observe a difference between male and female patients' $CORT_{hair}$. Thus, this study provided data indicative of a potential gender dependent effect in $CORT_{hair}$ in patients on hydrocortisone replacement. However, it should be acknowledged that this study included only 13 male patients *vs.* 80 female patients, which limits the generalizability of the results.

Therefore, we aimed to compare $CORT_{hair}$ in a large cohort of patients with primary and secondary AI on hydrocortisone replacement therapy (AI patients) with $CORT_{hair}$ of control patients with a pituitary disease but no hydrocortisone replacement therapy (PC) and healthy controls (HC). Furthermore, we aimed to explore possible determinants of $CORT_{hair}$ in hydrocortisone treated AI patients, i.e. self-reported hydrocortisone intake, sex, age, and weight. We hypothesized that AI patients would have higher $CORT_{hair}$ than PC and HC, and that AI patients show side effects associated with high cortisol levels. Moreover, we hypothesized that $CORT_{hair}$ are associated with doses of hydrocortisone replacement and BMI.

SUBJECTS AND METHODS

Study design

This study was designed as a cross-sectional assessment of patients seen at the outpatient clinic of the department of Endocrinology of the Leiden University Medical Center. This study was conducted between July 2012 and January 2014. Hair samples were collected and patients were asked to fill out two short self-developed questionnaires: one questionnaire about their hair treatment, and one questionnaire about their hydrocortisone intake (i.e. self-reported daily dose, time of intake, frequency of increasing/decreasing hydrocortisone dose) and/or the potential usage of other exogenous glucocorticoids. Clinical data of patients were obtained from their medical records.

Participants

Patients

We included two groups of patients: group I) patients with primary or secondary adrenal insufficiency using hydrocortisone (AI patients), and group II) patient controls (PC) with a pituitary disease not using hydrocortisone. A total of 184 patients were willing to participate. Patients could not participate in case of insufficient hair growth at the posterior vertex of the scalp. Ten patients were excluded from the analysis because of interpretative difficulty of their chronic steroid replacement scheme; three had high levels probably due to a hydrocortisone stress scheme for Addison's crisis in the three months prior to hair collection, three patients were excluded because of $CORT_{hair}$ >3 SD with no clear explanation, and four patients were excluded due to debatable AI diagnosis and inconsistent hydrocortisone use. The final sample comprised a total of 174 patients (i.e. 132 AI patients and 42 PC). Primary AI had been diagnosed by very low early morning cortisol concentrations (<120 nmol/l) or insufficient stimulation following ACTH test (below 550 nmol/l) usually in the presence of positive adrenal auto-antibodies or an alternative explanation. Secondary adrenal insufficiency was preferably diagnosed using an insulin tolerance test (ITT), or if contra-indicated, a CRH test using the same cut-off as for ACTH stimulation. Pituitary hormone replacement was prescribed dependent on the results of the annual evaluation of pituitary functions. In case of AI, hydrocortisone was prescribed (usually 20 mg/d divided into 3 dosages, with adjustments if clinically judged necessary by the treating physician) together with advices to increase the hydrocortisone dose in case of exposure to severe somatic and/or psychological stressors. In case of other hormone deficiencies, patients were substituted accordingly.

Healthy controls
To compare $CORT_{hair}$ between patients and healthy individuals, we used a group of 195 healthy controls (HC) previously described elsewhere (8).

The study was approved by the local ethics committee. All patients and controls gave written informed consent.

Hair cortisol assessment

A lock of approximately 150 hairs was cut as close to the scalp as possible from the posterior vertex. For analysis, the most proximal three cm of hair were used, corresponding to the most recent three months. Hair sample preparation and analysis has been described previously (8). In short, a minimum of 10 mg of hair was weighed and cut into small pieces in a glass vial. Extraction of cortisol took place in 1 mL of methanol for 16h at 52^{o}C while gently shaking. After extraction, the methanol was transferred to another vial and evaporated under a constant stream of nitrogen. The samples were dissolved in 250 µL of phosphate buffered saline (PBS, pH 8.0) for analysis. A commercially available ELISA Kit for salivary cortisol (DRG GmbH, Marburg, Germany) was used to measure cortisol levels. A correction factor was applied to the results to account for the potential influence of different hair weights. Cross reactivity of other steroids with the kit's antibodies was reported as follows: Corticosterone (29.00%), Cortisone (3.00%), 11-Deoxycortisol (<1.00%), 17-OH Progesterone (<0.50%), other hormones (<0.10%). Intra-assay variation was below 5% and the inter-assay variation below 8% as reported by the supplier. The recovery of the assay was described previously (8).

Statistical analysis

SPSS 20.0 for Windows was used for statistical analysis. Differences in demographic information between groups were tested with One-Way-ANOVAs and Pearson Chi Square tests. After logarithmic transformation, $CORT_{hair}$ were normally distributed. Analyses on $CORT_{hair}$ and differences between groups were performed by means of univariate general linear models. If groups differed on age, sex, BMI, or hair treatment (see Table 1 and Table 2), analyses on group differences were adjusted accordingly. For analyses of the etiologies of hydrocortisone use, post-hoc tests were applied. Pearson and spearman correlations were used for correlation analyses, depending on normality of the distribution. $CORT_{hair}$ are provided in pg/mg and are reported as median (Mdn) and interquartile range (IQR).

RESULTS

Participant characteristics (Table 1)

132 AI patients, 42 PC, and 195 HC were included in the analysis. The frequency of using glucocorticoid containing medication (other than hydrocortisone or maintenance dose) was 14.9% and did not differ between AI patients and PC (P = 0.26). Of these, 12.6% used one, and 2.3% used two kinds of glucocorticoid containing medication. The most frequent used products were ointments (n = 8) and inhalation aerosols (n = 8). Five patients used nasal spray, and one patient had received an injection into a joint. Patients that used externally applied glucocorticoid containing medication did not show different $CORT_{hair}$ than non-applying patients and were therefore not excluded (P = 0.75). The mean disease duration was not significantly different between AI patients (18.33 ± 13.54 years) and PC (15.06 ± 10.30 years), P = 0.16). Presence of hypertension (defined as either blood pressure above 140/90 or use of antihypertensive medication) and presence of diabetes mellitus (defined as use of oral medication and/or insulin injection) was not different between AI patients and PC, and in AI patients, frequencies of hypertension and diabetes mellitus were comparable between genders. Both AI and PC showed higher frequencies of diabetes mellitus than HC (both P < 0.05). Hypertension data were not available for HC.

Table 1. Baseline characteristics of patients, patient controls and HC

	AI patients (n = 132)	PC (n = 42)	HC (n = 195)	P-value[1]	P-value[2]	P-value[3]
Age	54.84 (14.99)	49.07 (12.94)	36.17 (12.23)	**0.05**	**0.001**	**0.001**
Sex (male)	52 (39.4%)	11 (26.2%)	90 (46.2%)	0.12	0.23	**0.02**
BMI	27.80 (5.12)	28.70 (7.45)	24.34 (3.85)	0.95	**0.001**	**0.001**
Use of exogenous glucocorticoids#	15 (11.5%)	2 (4.9%)	NA	0.21	NA	NA
Hypertension	56 (43.8%)	24 (60.0%)	NA	0.07	NA	NA
Diabetes mellitus	21 (9.2%)	4 (9.8%)	5 (2.6 %)	0.92	**0.008**	**0.029**
Hair dyed	42 (36.2%)	16 (42.1%)	37 (19.0%)	0.52	**0.001**	**0.002**
Hair bleached	13 (9.9%)	9 (22.0%)	13 (6.7%)	**0.04**	0.29	**0.002**
Hair permed	2 (1.5%)	3 (7.3%)	2 (1.0%)	**0.05**	0.69	**0.01**
Use hairproduct	66 (50.0%)	22 (53.7%)	90 (46.4%)	0.68	0.52	0.40
Frequency hair wash > 3 times/week	46 (35.1%)	17 (41.5%)	143 (74.1%)	0.46	**0.001**	**0.001**

P-value[1]: comparison between AI patients and PC, P-value[2]: comparison between AI patients and HC, P-value[3]: comparison between PC and HC

Data are presented as mean (standard deviation), and as n (valid percentage). AI, adrenal insufficiency; PC, patient control group; HC, healthy control group; BMI, Body Mass Index; NA, not applicable; #, use of other external glucocorticoids (besides hydrocortisone).

Table 2. Baseline characteristics of male and female AI patients

	Males (n = 52)	Females (n = 80)	P-value
Age	55.94 (16.01)	54.13 (14.35)	0.50
BMI	27.57 (3.85)	27.95 (5.80)	0.68
Duration of follow-up (years)	17.87 (12.69)	18.62 (14.11)	0.76
Daily hydrocortisone dose (mg)	21.58 (4.98)	20.39 (4.21)	0.15
Daily hydrocortisone dose mg/kg	0.25 (0.07)	0.27 (0.07)	0.12
Daily hydrocortisone dose mg/BSA	10.35(2.54)	10.84 (2.27)	0.26
Use of external glucocorticoids #	5 (10.0%)	10 (12.5%)	0.66
Hypertension	23 (46.9%)	33 (41.8%)	0.57
Diabetes Mellitus	4 (8.0%)	8 (10.0%)	0.70
Hair dyed	0	42 (58.3%)	**0.001**
Hair bleached	0	13 (16.5%)	**0.002**
Hair permed	1 (1.9%)	1 (1.3%)	0.76
Use hairproduct	19 (36.5%)	47 (58.8%)	**0.01**
Frequency hair wash			**0.03**
< 2 times/week	28 (53.8%)	57 (72.2%)	
> 3 times/week	24 (46.2%)	22 (27.8%)	

Data are presented as mean (standard deviation), and as n (valid percentage). #: use of other external glucocorticoids (besides hydrocortisone); BMI, Body Mass Index; BSA, body surface area.

$CORT_{hair}$ in AI patients, PC, and HC (Figure 1a-b)

Analyses showed a significant difference in $CORT_{hair}$ between the three groups, $F(2, 343) = 35.39$, $P < 0.001$, adjusted for age, gender, and dyeing of the hair. Post-hoc tests indicated that AI patients had higher $CORT_{hair}$ (33.89, 14.82 – 89.29) than PC (13.66, 6.22 – 26.58), P= 0.001, and HC (10.07, 3.52 – 17.83), P < 0.001, and that PC had higher $CORT_{hair}$ than HC, P= 0.04. In AI

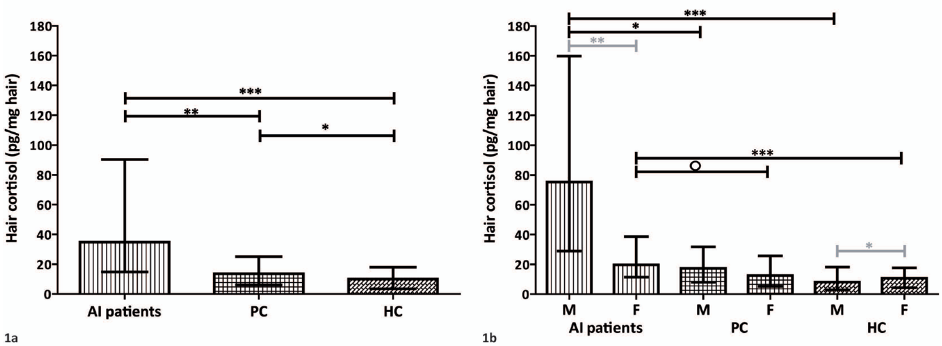


Figure 1. Median and IQR of $CORT_{hair}$. AI, adrenal insufficiency; PC, patient control group; HC, healthy control group. Untransformed data are shown. 1a) $CORT_{hair}$ of AI patients, PC, and HC. 1b) $CORT_{hair}$ for AI patients, PC, and HC, stratified for sex. *** = P < 0.001; ** = P < 0.01; * = P < 0.05, ○ = P < 0.1. Black lines represent differences between the participant groups, whereas grey lines represent sex differences within each participant group.

patients, 35.6% (61.5% males, 18.8% females) presented with $CORT_{hair}$ above our lab-internal cut-off for normal, as did 7.1% (9.1% males, 6.5% females) of PC and 3.1% (5.6% males, 1.0% females) of HC. Our lab-internal upper limit of normal is 52 pg/mg. For determination, we restricted our group of healthy controls to the ones with a BMI between 18.5 and 30.0, and used the 97.5 percentile as cut-off value.

In AI patients, men had significantly higher $CORT_{hair}$ (75.25, 28.91 – 159.81) than women (19.59, 11.49 – 38.49), $F(1, 112) = 8.17$, $P = 0.005$), adjusted for age and dyeing of the hair. No gender differences were observed in $CORT_{hair}$ in PC. In HC, females showed higher $CORT_{hair}$ than males, $F(1, 191) = 5.45$, $P = 0.02$. Stratified analysis for gender revealed that male AI patients had higher $CORT_{hair}$ than male PC and HC ($P = 0.02$ and $P < 0.001$, respectively), whereas for female AI patients, $CORT_{hair}$ was trend-significantly higher than in female PC (P= .07) and significantly higher than in female HC ($P < 0.001$). Males in the PC group did not show different $CORT_{hair}$ from males in the HC group, and females in the PC group had not different $CORT_{hair}$ compared to females in the HC group (all $P > 0.1$). Within AI patients, no difference in $CORT_{hair}$ was found for the various etiologies of AI.

Correlation between hydrocortisone dose and hair cortisol levels (Figure 2)

Self-reported daily hydrocortisone maintenance dose correlated with $CORT_{hair}$ ($\rho = 0.18$, P= 0.04). Stratification for gender showed that this correlation was primarily driven by the female AI patients ($\rho = 0.24$, $P = 0.04$), whereas the correlation was not significant in male AI patients. Neither incidental higher and/or lower hydrocortisone dosages nor the morning (peak) dose of hydrocortisone ($\rho = 0.15$, $P = 0.09$) were related to $CORT_{hair}$. The self-reported

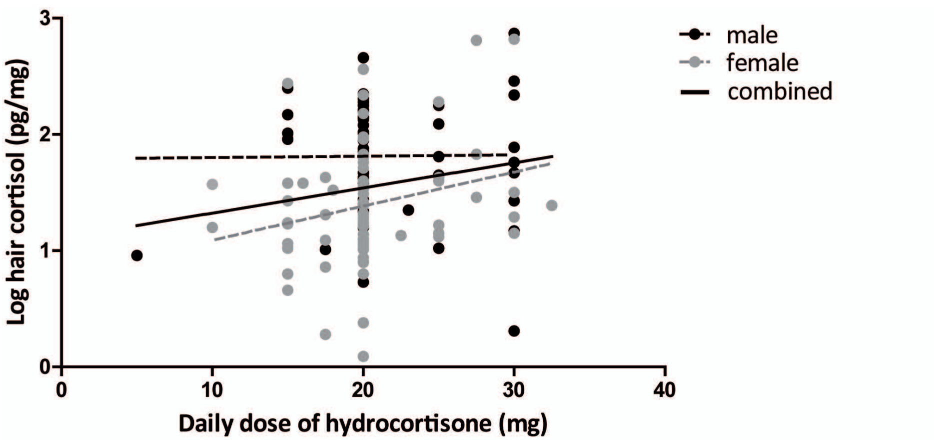
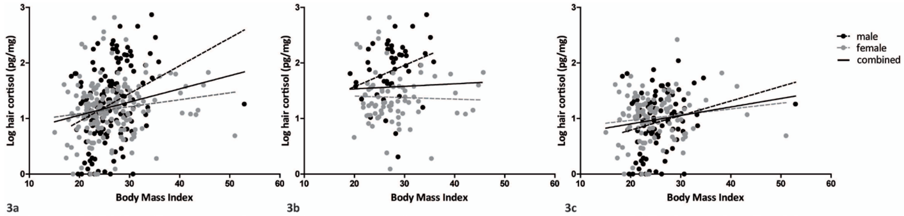


Figure 2. The relationship between daily hydrocortisone dose (mg/day) and $CORT_{hair}$ (pg/mg), $\rho = 0.18$, P = 0.04, as indicated with the black solid line, which is the regression line of the group analysis. Analyses stratified for sex show that this effect was driven by the female AI patients (grey; $\rho = 0.24$, $P = 0.04$), whereas no effect was observed for the male AI patients (black; $\rho = -0.04$, $P = 0.79$). $CORT_{hair}$ are shown on a log scale.



3a 3b 3c

Figure 3. The relationship between BMI and $CORT_{hair}$. 3a) The association between BMI and $CORT_{hair}$ for all participants was significant (ρ = 0.24, P < 0.001, black solid line); stratification for sex did not change the results (ρ = 0.35, p < 0.001 for male participants (black); ρ = 0.18, P = 0.02 for female participants (grey). 3b) In only the adrenal insufficiency (AI) patients, the association between BMI and $CORT_{hair}$ was not significant; ρ = 0.11, P = 0.23 (black solid line). Stratification for sex rendered a significant correlation for male AI patients (ρ = 0.34, P = 0.02, black) but no association for female AI patients (ρ = 0.04, P = 0.73, grey). 3c) In the control persons, the association did reach significance (ρ = 0.14, P = 0.04, black solid line). Stratified analyses showed that this effect was driven by the male controls (ρ = 0.24, P = 0.02, black) but was not significant for female controls (ρ = 0.10, P = 0.31, grey).

daily hydrocortisone dose in mg/kg or mg/m^2 was not related to $CORT_{hair}$ and stratification for sex did not render different results.

Correlations between anthropometrics and hair cortisol levels (Figure 3)

As indicated in Table 1, BMI differed significantly between AI patients, PC and HC (F2, 327) = 23.90, P < 0.001. Post-hoc tests revealed that the BMI of AI and PC patients was significantly higher compared to HC (P < 0.001), but there was no significant difference between AI and PC. For the whole group of participants, BMI showed a significant correlation with $CORT_{hair}$ (ρ = 0.24, P < 0.001). Stratification for sex and participant group revealed a significant correlation between BMI and $CORT_{hair}$ for male AI patients (ρ = 0.34, P = 0.02), but not for female AI patients nor for male or female PC or HC. Waist-to-hip ratio (WHR) information was only available in a subset of 50 AI patients, 12 PC, and 45 HC. WHR was not different between the groups. In the whole sample of participants, WHR and $CORT_{hair}$ correlated significantly (r = 0.20, P = 0.04). WHR and waist circumference were related to self-reported dose in mg/kg (r = -0.3, P = 0.04, and r = -0.58, P < 0.001, respectively) and to self-reported dose in mg/BSA (r = -0.36, P = 0.01, and r = -0.64, P < 0.001, respectively), but were not related to the total self-reported daily hydrocortisone maintenance dose (r = 0.11, P = 0.46, and r = 0.19, P = 0.17, respectively).

DISCUSSION

The present study showed that patients using hydrocortisone replacement for AI demonstrate higher $CORT_{hair}$ than pituitary patients and healthy controls with an intact HPA-axis. Furthermore, a gender-effect was identified, with male patients with AI demonstrating higher $CORT_{hair}$ than females, without differences in self-reported hydrocortisone intake. Intriguingly, this gender effect seems to be specific for hydrocortisone use, since it is not present in controls with an intact HPA-axis. In female patients, higher self-reported hydrocortisone intake was associated with higher $CORT_{hair}$, whereas this association was not found in male patients who demonstrated on average higher $CORT_{hair}$ even in the lower dose range.

In male, but not female AI patients, higher $CORT_{hair}$ were associated with higher BMI. This relation suggests that high $CORT_{hair}$ may reflect chronic overexposure to hydrocortisone, at least in male patients. However, further study is required to understand the role of gender in the determination of cortisol levels in hair and to confirm whether $CORT_{hair}$ are indeed representative for corticosteroid exposure in the rest of the organs. Furthermore, it is still unclear how exactly cortisol, and hence hydrocortisone, is incorporated into scalp hair (16). Therefore, the question remains whether it is the cumulative amount of cortisol or the cortisol peak that is most influential on $CORT_{hair}$. In our study, total dose appears to be associated with $CORT_{hair}$, and not a single/maximum dose. In contrast, the three patients who received a hydrocortisone bolus for an Addison crisis had extremely high values and were excluded from the study (data not shown), suggesting a role for a supraphysiological peak in determination of $CORT_{hair}$. In contrast to the positive correlation between the absolute hydrocortisone dose and $CORT_{hair,}$ we found no relation between body weight-adjusted dose and $CORT_{hair,.}$ This is an interesting finding, since previous research has reported that clearance of hydrocortisone in serum is faster in obese patients, and that adjusting the dose for body weight may be beneficial for the patient (5, 17). However, the current study may imply that tissue exposure following ingestion of hydrocortisone (at a physiological level) is independent of distribution volume, i.e. weight, at least for these patient groups as measured by $CORT_{hair}$, and thus questions the need to increase the hydrocortisone dose in obese patients. This is in accordance with recent guidelines pointing to no adjustment for weight (except for children) (3). Male patients reached higher $CORT_{hair}$ with considerably lower hydrocortisone dosages than female patients. This "higher sensitivity" to hydrocortisone is in accordance with the positive association between $CORT_{hair}$ and BMI in male patients. A possible explanation for this higher sensitivity in male patients might be that men seem to have lower corticosteroid binding globulin (CBG) levels while total cortisol levels are comparable to women's total cortisol (18). This may result in higher free cortisol levels in men upon hydrocortisone intake. As free cortisol is thought to be the cortisol fraction which is incorporated in hair (19), this might explain the sex difference found in our study and in the study of Gow and colleagues (10). However, the clearly increased $CORT_{hair}$ suggest that in general patients with AI are chronically over-replaced, despite prescribed hydrocortisone replacement dosages aiming at mimicking a “physiological” level. A higher daily hydrocortisone dose has been previously linked to a more adverse cardiometabolic risk profile, characterized by higher BMI (20). Steroid excess-related morbidity is well known from AI cohorts treated with higher doses, resulting in the awareness to replace hydrocortisone with the lowest dose possible, generally regarded to as a daily hydrocortisone dose of 20 mg (21-22). It is intriguing that AI patients treated with the currently advised low hydrocortisone dose have clearly increased $CORT_{hair}$ and additionally present with steroid-related side effects, such as increased BMI.

It appears unlikely that an increased perceived stress of being a patient influences $CORT_{hair}$ in this study. Some AI patients are known to occasionally increase their hydrocortisone doses in situations of increased psychological stress (23), which might result in higher $CORT_{hair}$. In population studies, $CORT_{hair}$ has been associated with perceived stress (24-26), but if this was the case in the present study, the increased stress of being a patient should then also be present in our PC group. However, $CORT_{hair}$ between PC and HC were comparable.

Several strengths and limitations of the present cross-sectional study need to be mentioned. In total, we included a considerable number of patients, which enabled us to examine $CORT_{hair}$ of patient groups with pituitary diseases due to different etiologies. Furthermore, we included a patient control group with a pituitary disease and normal adrenal function. All concurrent pituitary insufficiencies were treated, but we do acknowledge that, such as hydrocortisone replacement therapy, intrinsic imperfections of hormone replacement are also an important issue for gonadal steroids, thyroid hormone, and growth hormone replacement.

Besides the demonstrated association between $CORT_{hair}$ and anthropometrics, a considerable amount of studies demonstrated the association between high $CORT_{hair}$ and psychological symptoms (25). Furthermore, in a recent study it was demonstrated that a higher hydrocortisone intake in patients with primary adrenal insufficiency was associated with more impairments in quality of life, psychological morbidity, and maladaptive personality traits (27). For future research, it would be interesting to assess patients' perceived well-being in relation to $CORT_{hair}$.

In conclusion, patients on hydrocortisone replacement therapy have elevated $CORT_{hair}$, a finding which is predominantly present in male patients. Despite a low dose of on average 21 mg/day only 64.4% of patients had $CORT_{hair}$ in the normal range. This study provides important data on the fact that contemporary steroid replacement still results in clear supra-physiological (hair) cortisol levels, especially in males. However, it needs to be confirmed that $CORT_{hair}$ reflects cortisol (over)exposure in other organs of the body in exogenously treated patients, or that the incorporation of $CORT_{hair}$ is different from the reference population with normal HPA-axis. Next, it needs to be established which are safe, gender-specific $CORT_{hair}$ for patients to allow for the monitoring of hydrocortisone dose while avoiding the dangers of under- and over-replacement.

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