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

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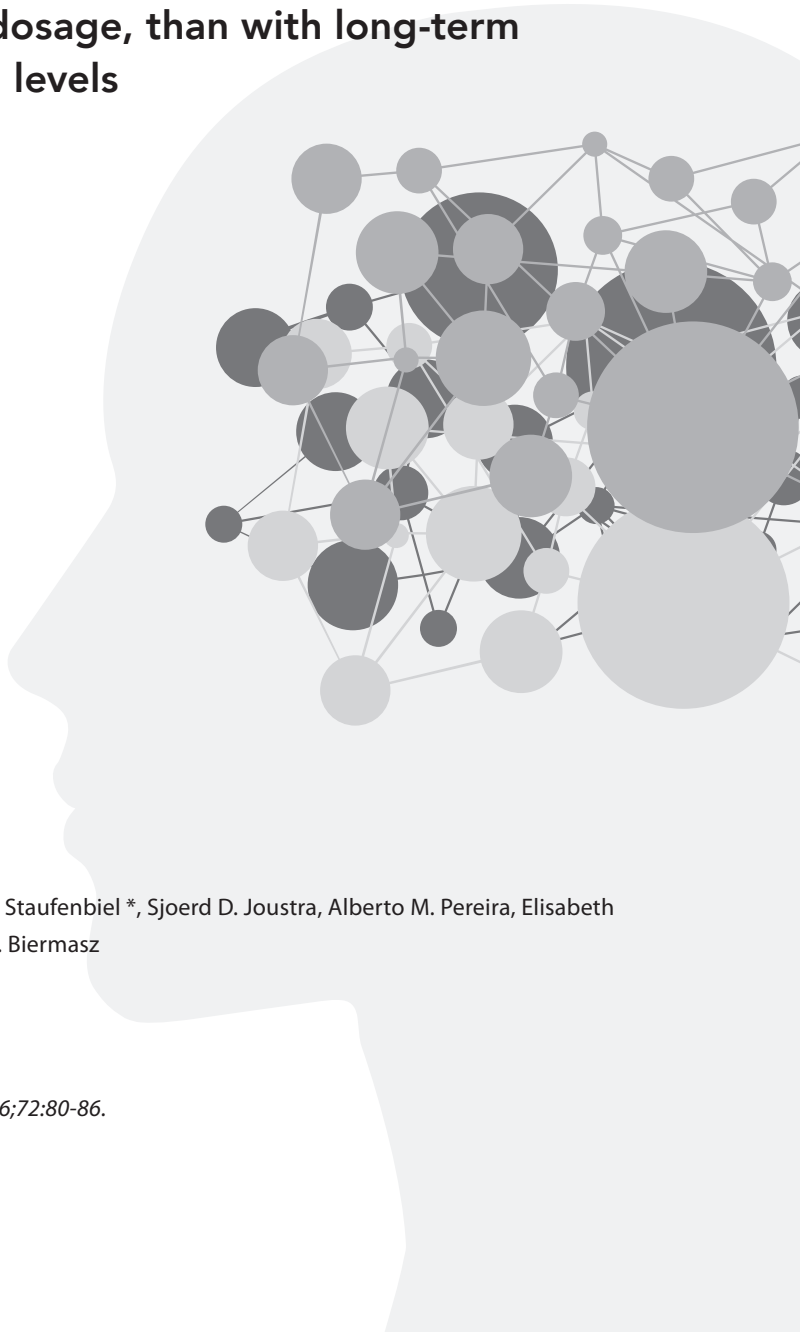
CHAPTER 6

Quality of life in patients with adrenal insufficiency correlates stronger with hydrocortisone dosage, than with long-term systemic cortisol levels



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ABSTRACT

In patients with adrenal insufficiency (AI) a higher hydrocortisone intake has been associated with more impairment in Quality of Life (QoL). Irrespective of age, sex and severity of AI the dosage of hydrocortisone is titrated around 20 mg/D in all patients with AI based on physical and mental signs and symptoms. However, until now it is unknown whether these QoL impairments are related to increased systemic cortisol exposure. Measurement of hair cortisol levels ($CORT_{hair}$) can be used to assess chronic systemic cortisol exposure. This study aimed to explore whether QoL in patients with AI is associated with $CORT_{hair}$ and daily hydrocortisone intake. We performed a cross-sectional study in 120 patients with AI on stable hydrocortisone replacement, in whom hair samples and QoL data were collected. $CORT_{hair}$ were measured with ELISA, and QoL was assessed with validated questionnaires (SF-36, EQ-5D, HADS, MFI-20). Patients reported impairments in 14 of 15 QoL subscales ($P < .001$). More impairments in physical aspects of QoL correlated with higher $CORT_{hair}$ and higher daily hydrocortisone intake ($P < .05$), an effect that was more pronounced in female patients. Regression analyses including both $CORT_{hair}$ and hydrocortisone intake revealed a significant negative contribution of higher hydrocortisone intake on physical aspects of QoL ($P \leq .046$), whereas no significant contribution was found for $CORT_{hair}$.

The present study showed that patients with AI report several impairments in QoL which are associated with hydrocortisone intake, and to a lesser extent reflected by chronic systemic cortisol exposure as measured by hair cortisol. This suggests that QoL impairments in patients with AI are not per se the effect of prolonged exposure to elevated systemic cortisol levels.

INTRODUCTION

Adrenal insufficiency (AI) is treated with glucocorticoid replacement therapy, usually 20 to 30 mg of hydrocortisone daily, divided into three dosages (10-15 mg in the morning, 5-10 mg in the afternoon, 4-5 mg in the evening), in order to mimic the natural circadian secretion of cortisol (1). However, even when patients with primary AI are in a stable medical condition, they report impaired quality of life (QoL) (2-6). In addition, in patients with secondary AI due to pituitary disease, hypopituitarism was found to be an important predictor of QoL impairments (7-9). It has been suggested that these QoL impairments are associated with intrinsic imperfections in glucocorticoid replacement therapy, and therefore, it is advised that hydrocortisone replacement should be individualized (10). For instance, there is large individual variation in sensitivity to cortisol, which is partly explained by polymorphisms of the glucocorticoid receptor gene (11). However, determining an optimal hydrocortisone replacement dose is complicated by the lack of reliable chronic parameters, and as a result many patients may be chronically under- or overtreated with potential paramount consequences for well-being and health.

Until now, it is not well established whether QoL is affected by the degree of cortisol exposure (i.e. adequacy of hydrocortisone replacement) in patients with AI. In a single study, authors investigated plasma cortisol day curves and well-being in a small sample of seven patients with AI and demonstrated that subphysiological cortisol levels correlated with lower well-being (12). Other studies examined the relation between the dosage and intake scheme of glucocorticoid replacement therapy and QoL, and demonstrated that in patients with AI, QoL was inversely correlated with the hydrocortisone dose (5;13). Importantly, associations between hydrocortisone intake and QoL do not provide any information about causality, since it might be that high cortisol levels cause QoL impairments, but it might also be that patients with worse QoL need more hydrocortisone.

Addressing this relationship is further complicated by the difficulty of adequately measuring cortisol levels throughout the day, since cortisol levels vary depending on different treatment regimens (i.e. varying hydrocortisone doses, as well as differences in timing, absorption, and metabolism of hydrocortisone), and currently available cortisol measurements (i.e. plasma, urinary, salivary) are limited to short-term assessments.

A promising method to assess cortisol for prolonged periods of time is the analysis of cortisol levels in scalp hair ($CORT_{hair}$) (14;15). We (and others) recently assessed the use of this measure in AI patients treated with exogenous hydrocortisone. Patients with AI have increased levels and hydrocortisone intake has been found to correlate with $CORT_{hair}$ (16;17). A significant gender effect has been reported in $CORT_{hair}$ in patients with AI treated with glucocorticoid replacement therapy, with male patients demonstrating higher $CORT_{hair}$ than females while using the same dose of hydrocortisone (16;17).

In the present study, we aimed to explore whether $CORT_{hair}$ is correlated with QoL. We first compared QoL in patients with stable treatment for AI with QoL in healthy controls. Second,

we examined potential correlations between QoL, $CORT_{hair}$, and daily hydrocortisone intake as another parameter to assess cortisol exposure.

PATIENTS AND METHODS

Patients

Scalp hair samples were collected of 132 patients with primary or secondary AI on hydrocortisone replacement from the Endocrinology out-patient clinic of the Leiden University Medical Center (cohort previously described in (17)). Of this group, nine patients did not fill out QoL questionnaires and three patients filled out less than 75% of the questionnaires and were therefore excluded from the analysis. Thus, 120 patients with longstanding AI on a stable dose were included in the present study. 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, 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 per day divided into three dosages, adjusted at the discretion of the treating physicians) together with the advice to increase the hydrocortisone dose in case of exposure to severe somatic and psychological stressors.

Comparison QoL data of 437 healthy controls were derived from a previous study from our department (18).

The local ethics committee approved this study. All patients gave written informed consent.

QoL assessment

QoL was assessed with the following four validated questionnaires:

The *Short-Form 36 (SF-36)* assesses functional status and general well-being and consists of 36 items covering nine health concepts: 1) physical functioning, 2) social functioning, 3) role limitation (physical), 4) role limitation (emotional), 5) mental health, 6) vitality, 7) pain, 8) general health perception, and 9) general perception of change in health. Scores are expressed on a 0–100 scale, and higher scores indicate better QoL (19).

The *EuroQoL-5D (EQ-5D)* assesses the current health status reflected in five health dimensions; 1) mobility, 2) self-care, 3) usual activities, 4) pain/discomfort, and 5) anxiety/depression. Scores are expressed on a 1-3 scale per dimension, with higher scores indicating worse QoL. Also a visual analogue scale is included ranging from 0 to 100 for recording an individual's rating for their current health-related well-being, with higher scores indicating a better health status (20).

The *Hospital Anxiety and Depression Scale (HADS)* assesses both anxiety and depressive symptoms and consists of 14 items on a 4-point scale. Higher scores indicate more severe anxiety and depressive symptoms (21;22).

The *Multidimensional Fatigue Inventory (MFI-20)* consists of 20 statements assessing fatigue on a five-point scale covering five dimensions; 1) general fatigue, 2) physical fatigue, 3) reduced activity, 4) reduced motivation, and 5) mental fatigue. Scores vary from 0-20; with higher scores indicating greater fatigue (23).

QoL of healthy controls

QoL data of healthy controls were previously collected at our department (18). The EuroQoL-5D and two subscales of the Short-Form 36 (i.e. mental health, vitality) were not assessed in this group of healthy controls. QoL data of 437 healthy controls (136 males) with a mean age of 50.9 ± 13.6 years were available and the total group was used for comparison.

Hair collection, preparation, and analysis

A lock of approximately 150 hairs from the posterior vertex was cut as close to the scalp as possible. The hair samples were taped to paper and stored in the dark at room temperature until further analysis. One cm represents the average cortisol concentrations of one month (15), since it is assumed that hair grows one cm per month, with a range of 0.6 – 1.4 cm/month (24).

Hair samples are specifically taken from the vertex region of the scalp because its most uniform growth pattern and phase (25;26), and importantly, has been specifically been validated for cortisol with the lowest mean coefficient of intra-individual variation (27). For analyses, the most proximal 3 cm of hair was used, corresponding to the most recent 3 months. A minimum of 10 mg of hair was weighed and cut into small pieces. For extraction, 1 mL of methanol was added and the samples were incubated for 16h at 52^{0}C. 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). A commercially available ELISA Kit for salivary cortisol (DRG GmbH, Marburg, Germany) was used to measure cortisol levels. The procedure has been described in detail elsewhere (14). Our laboratory internal upper limit of normal is 52 pg/mg.

Statistical analyses

Data were analyzed using PASW Statistics version 20.0 (SPSS Inc., Chicago, IL). $CORT_{hair}$ were reported as median and interquartile ranges (IQR). Other data were presented as mean±SD, unless mentioned otherwise. After logarithmic transformation, $CORT_{hair}$ were normally distributed. The primary analysis comprised the comparison of QoL of patients with AI to healthy controls by using independent sample t-tests when data were normally distributed and Mann-Whitney U tests when data were not normally distributed. In order to evaluate

whether the previously found gender effect in $CORT_{hair}$ is reflected in QoL, this analysis was also performed after stratification for gender.

The secondary analysis comprised the assessment of the potential association between QoL, $CORT_{hair}$, and daily hydrocortisone intake. Partial correlations were calculated between QoL and $CORT_{hair}$ and daily hydrocortisone intake, adjusted for age and gender. Subsequently, groups were stratified for gender and partial correlations were calculated between QoL and $CORT_{hair}$ and daily hydrocortisone intake, adjusted for age. Regression analyses including linear and quadratic terms were used to examine possible u-shaped associations. Furthermore, regression analyses including both $CORT_{hair}$ and daily hydrocortisone intake were used to differentiate between the contributions of these two factors. Because of the exploratory nature of these analyses, adjustment of the level of significance for multiple testing was not performed, and the level of significance was set at $P < .05$.

RESULTS

Patient characteristics

A total of 120 patients with longstanding AI (46 males) with a mean age of 55.0 ± 14.7 years were included in the analyses. The duration of follow-up was on average 18.5±13.3 years, with a median of 15.8 years (IQR: 8.1-28.9). Patients used a mean daily dose of 21.1 ± 4.5 mg. In the whole group of patients, 34% presented with $CORT_{hair}$ above our lab-internal cut-off for normal (52 pg/mg). Of the males, 59% demonstrated $CORT_{hair}$ higher than the lab-internal cut-off, in contrast to 19% of the females ($P < .001$). As previously reported (17), also in the present study male patients demonstrated higher $CORT_{hair}$ than female patients (75.3 (26.2 - 150.1) vs. 19.7 (11.6 - 38.5), $P < .001$). Furthermore, female patients dyed or bleached their hair more and used hair products more frequently than male patients (all $P \leq .03$) (Table 1). Daily hydrocortisone intake and $CORT_{hair}$ showed a significant, but modest correlation ($r = 0.185$, $P = .047$).

To evaluate whether there were differences in $CORT_{hair}$ between different etiologies of AI, five groups were formed: 1) AI due to previous treatment for Cushing's disease (n = 18), 2) other functioning pituitary adenomas (n = 14, including acromegaly (n = 5), prolactinoma (n = 8), and FSH producing adenoma (n = 1)), 3) nonfunctioning pituitary adenoma+craniopharyngioma (n = 48, nonfunctioning pituitary adenoma (n = 35) and craniopharyngioma (n = 13)), 4) primary AI (n = 18), and 5) other causes of hypopituitarism (n=22, including congenital hypopituitarism (n = 6), hypopituitarism after radiotherapy/surgery/traumatic brain injury (n = 7), and other causes such as pituitary inflammation or Sheehan's syndrome (n = 9)). $CORT_{hair}$ was lowest in patients with CD, but group differences did not reach statistical significance ($P = .126$) (Figure 1). The self-reported hydrocortisone dose was significantly different between groups ($P = .003$), with patients with primary AI using a higher dose (24.7 ± 4.5 mg) compared

to patients with CD (20.0 ± 4.8), NFA+CP (20.9 ± 3.8) or other causes of hypopituitarism (19.2 ± 5.4).

Table 1. Clinical characteristics of AI patients (males vs. females)

	Patients with AI (n = 120)	Males with AI (n = 46)	Females with AI (n = 74)	P value
Age (years)	55.0 ± 14.7	57.2 ± 14.8	53.6 ± 14.6	.167[b]
BMI (kg/m^2)	28.0 ± 5.1	27.5 ± 3.5	28.3 ± 5.9	.799[b]
Duration of follow-up (years)	18.5 ± 13.3	18.2 ± 12.6	18.7 ± 13.8	.995[b]
Use of external glucocorticoids #	15 (13%)	5 (11%)	10 (14%)	.702[c]
Hair cortisol levels*	23.7 (14.0 – 84.7)	75.3 (26.2 – 150.1)	19.7 (11.6 - 38.5)	**<.001[a]**
Hair cortisol above our lab-internal cut-off (52 pg/mg)	41 (34%)	27 (59%)	14 (19%)	**<.001[c]**
Daily hydrocortisone dose (mg)	21.1 ± 4.5	21.5 ± 5.1	20.8 ± 4.2	.334[b]
Daily hydrocortisone dose (mg/kg)	0.26 ± 0.07	0.2 ± 0.07	0.3 ± 0.07	.065[b]
Daily hydrocortisone dose (mg/BSA)	10.7 ± 2.4	10.3 ± 2.5	11.0 ± 2.3	.077[b]
Hair dyed	44 (37%)	0 (0%)	44 (60%)	**<.001[c]**
Hair bleached	18 (15%)	0 (0%)	18 (24%)	**<.001[c]**
Hair permed	3 (3%)	1 (2%)	2 (3%)	.848[c]
Use of hair product	60 (50%)	17 (37%)	43 (58%)	**.024[c]**
Frequency hair wash > 3 times/week	42 (35%)	21 (46%)	21 (28%)	.061[c]

Data are presented as mean (standard deviation), and as n (valid percentage). a Independent samples t-test, b Mann-Whitney U-test, c Chi-square test. AI, adrenal insufficiency; BMI, body mass index; BSA, body surface area; #: use of other external glucocorticoids (in addition to their regular hydrocortisone substitution). P value: AI males vs. AI females.

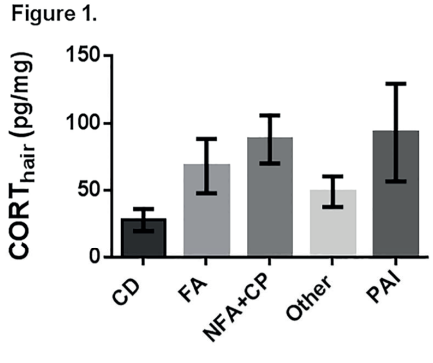

Figure 1. Comparison of CORThair between patient groups.

Mean hair cortisol levels ($CORT_{hair}$) +/- standard error to the mean, stratified per patient category as follows: 1. CD: AI due to previous treatment for Cushing's disease (n = 18); 2. FA: other functioning pituitary adenomas (n = 14, including acromegaly (n = 5), prolactinoma (n = 8), FSH producing adenoma (n = 1)); 3. NFA+CP: non-functioning pituitary adenomas (n = 48, including craniopharyngeoma (n = 13) and NFA (n = 35)); 4. PAI: primary adrenal insufficiency (n = 18); 5. Other: other causes of hypopituitarism (n = 22, including congenital hypopituitarism (n = 6), hypopituitarism after radiotherapy/surgery/traumatic brain injury (n = 7), other causes such as pituitary inflammation or Sheehan's syndrome (n = 9)). The figure shows a difference in $CORT_{hair}$ between patients with AI due to previous treatment for CD and the other groups, but this difference was not found to be statistically significant (P = .126).

QoL

Compared to healthy controls, patients with AI reported worse QoL on all subscales (except general health perception, SF-36) (P < .05). After stratifying for gender, male patients reported worse QoL on 12 of the 15 subscales (P < .05) and female patients reported worse QoL on 14 of the 15 subscales (P < .001) in comparison to controls (Table 2).

Comparing QoL between the different etiology groups revealed significantly more depressive symptoms (HADS) in patients with CD (7.3 ± 4.0) relative to patients with NFA+CP (4.3 ± 3.5) (P = .022) (Figure 2). Furthermore, patients with CD reported more physical fatigue (14.7 ± 2.0), more reduced activity (12.9 ± 1.2) (MFI-20), and worse mental health (58.9 ± 20.2) (SF-36) compared to patients with PAI (11.7 ± 4.7; 10.7 ± 4.2; 77.7 ± 18.2) (P = .004, P = .009, P = .044, respectively). Considering that only patients with CD differed from the other groups, QoL analyses were corrected for etiology of CD.

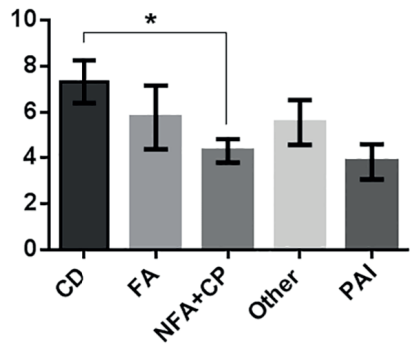

Figure 2. Comparison of depressive symptoms (HADS) between patient groups.
Mean Depressive and Anxiety symptoms as measured by the Hospital Anxiety and Depression Scale (HADS) +/- standard error to the mean, stratified by the following patient categories: 1. CD: AI due to previous treatment for Cushing's disease (n = 18); 2. FA: functioning pituitary adenomas (n = 14, including acromegaly (n = 5), prolactinoma (n = 8), FSH producing adenoma (n = 1)); 3. NFA+CP: non-functioning pituitary adenomas (n=48, including craniopharyngeoma (n = 13) and NFA (n = 35)); 4. PAI: primary adrenal insufficiency (n = 18) 5. Other: other causes of hypopituitarism (n = 22, including congenital hypopituitarism (n = 6), hypopituitarism after radiotherapy/surgery/traumatic brain injury (n = 7), other causes such as pituitary inflammation or Sheehan's syndrome (n = 9).

Relations between $CORT_{hair}$ and QoL (Table 3)

Correlations between $CORT_{hair}$ and QoL, adjusted for age, gender, and etiology CD revealed that in the whole group, higher $CORT_{hair}$ correlated at trend level with more limitations in daily activities (EQ-5D) (r = 0.180, P = .059). After stratification for gender, it was observed that in male patients higher $CORT_{hair}$ was associated with more physical fatigue (r = .355, P = .018). In female patients higher $CORT_{hair}$ was associated with more limitations in daily activities (r = 0.239, P = .046) and more pain (r = 0.269, P = .024) (EQ-5D).

In the whole group, QoL of patients with $CORT_{hair}$ above the lab-internal cut-off for normal was not different from patients with $CORT_{hair}$ in the normal range (P > .05). However, female patients with $CORT_{hair}$ levels above the lab-internal cut-off (n = 14 (19%)) reported lower physical functioning (52.9 ± 28.7, P = .025) and more pain on the SF-36 (52.8 ± 29.7, P = .033), as well as on the EQ-5D (2.2 ± 0.8, P = .049) relative to females with $CORT_{hair}$ within the

Table 2. Quality of life scores in patients with AI vs. healthy controls

		Patients with AI (n=120)	Healthy controls (n=437)	P value[1]	Males with AI (n=46)	Healthy males (n =136)	P value[2]	Females with AI (n=74)	Healthy females (n=301)	P value[3]
SF-36	Physical functioning	73.3±24.7	88.2±16.6	**<.001**	82.6±20.3	90.1±15.9	**.003**	67.4±25.5	87.3±16.9	**<.001**
	Social functioning	67.4±28.8	88.4±18.7	**<.001**	75.3±24.4	91.7±15.4	**<.001**	62.5±30.4	86.9±19.8	**<.001**
	Role limitation (physical)	53.4±44.5	84.5±31.3	**<.001**	66.3±41.2	87.8±26.2	**<.001**	45.4±44.8	83.1±33.0	**<.001**
	Role limitation (emotional)	72.8±40.5	86.5±29.5	**<.001**	82.6±32.0	90.1±24.4	.102	66.7±44.1	84.8±31.4	**<.001**
	Pain	73.0±26.8	85.8±18.5	**<.001**	82.0±21.1	88.0±16.4	.106	67.4±28.5	84.8±19.3	**<.001**
	General health perception	46.8±22.2	71.6±18.7	**<.001**	50.7±22.7	74.5±17.1	**<.001**	44.4±21.7	70.4±19.3	**<.001**
	General perception of change in health	50.3±22.7	53.6±17.9	.140	49.4±18.8	54.4±18.0	.210	50.9±24.9	53.2±17.9	.344
HADS	Anxiety	5.5±3.9	4.1±3.2	**<.001**	3.9±2.7	3.0±2.7	**.036**	6.4±4.2	4.5±3.3	**<.001**
	Depression	5.1±4.1	2.8±2.8	**<.001**	4.2±3.9	2.7±2.5	**.015**	5.6±4.1	2.8±3.0	**<.001**
	Total score	10.5±7.3	6.8±5.3	**<.001**	8.2±6.0	5.7±4.4	**.016**	12.0±7.6	7.3±5.6	**<.001**
MFI-20	General fatigue	11.7±2.2	8.5±4.0	**<.001**	11.0±2.2	7.5±3.5	**<.001**	12.1±2.1	8.9±4.2	**<.001**
	Physical fatigue	13.1±2.6	7.6±3.7	**<.001**	12.3±2.6	7.3±3.5	**<.001**	13.5±2.3	7.7±3.8	**<.001**
	Reduced activity	12.2±2.3	7.2±3.5	**<.001**	11.8±2.5	7.1±3.3	**<.001**	12.4±2.2	7.2±3.5	**<.001**
	Reduced motivation	11.4±2.6	7.3±3.4	**<.001**	11.6±2.9	7.2±3.3	**<.001**	11.3±2.5	7.3±3.4	**<.001**
	Mental fatigue	11.3±2.3	7.8±3.9	**<.001**	10.9±2.2	6.9±3.4	**<.001**	11.5±2.3	8.2±4.0	**<.001**

Mann-Whitney U-test s. Data are presented as mean (standard deviation). AI: adrenal insufficiency. P value[1]: patients with PAI vs. Healthy controls; P value [2]: AI males vs. healthy males; P value[3]: AI females vs. healthy females.

normal range. No differences in QoL were found between male patients with $CORT_{hair}$ above the lab-internal cut-off (n = 27 (59%)), and male patients with $CORT_{hair}$ within the normal range (P > .05). Regression analyses including age, gender, and etiology CD, as well as $CORT_{hair}$ as a quadratic term did not render significant results.

Table 3. Correlations between QoL, $CORT_{hair}$ and hydrocortisone dose

		Patients with AI[a]		Males with AI[b]		Females with AI[b]	
		$CORT_{hair}$	HC dose	$CORT_{hair}$	HC dose	$CORT_{hair}$	HC dose
SF-36	Physical functioning	-.097	-.208*	-.019	-.200	-.122	-.164
	Vitality	-.068	-.251**	.067	-.085	-.164	-.334***
	Change in health	.015	-.317***	.011	-.258	.031	-.349***
MFI-20	Physical fatigue	.120	.136	.335*	.203	-.028	.066
EQ-5D	Activity	.180	.206*	.016	.153	.239*	.264*
	Pain	.131	.134	-.054	.013	.269*	.215
	VAS	-.071	-.297***	.171	-.319*	-.164	-.307*

[a] partial correlations correcting for age and gender; [b] partial correlations correcting for age. *P < .05; **P< .01; *** P< .005. AI: adrenal insufficiency. $CORT_{hair}$ (pg/mg); hydrocortisone dose (mg). Only significant correlations are shown.

Relationships between daily hydrocortisone intake and QoL (Table 3)

Correlations between hydrocortisone intake and QoL, adjusted for age, gender, and etiology CD, revealed that in the whole group, higher hydrocortisone intake was associated with more impairments in physical functioning (r = -0.208, P = .027), less vitality (r = -0.251, P = .007), a greater decrease in perceived health (change in health) (r = -0.317, P = .001) (SF-36), more limitations in daily activities (r = 0.206, P = .032), and a worse perceived health status (r = -0.297, P = .002) (EQ-5D). After stratification for gender, it was observed that higher hydrocortisone intake was associated with a worse perceived health status in male patients (r = -0.319, P = .048). In female patients, higher hydrocortisone intake was associated with less vitality (r = -0.334, P = .005), a greater change in health (r = -0.349, P = .003) (SF-36), more limitations in daily activities (r = 0.264, P = .028), and a worse perceived health status (r = -0.307, P = .012) (EQ-5D). Regression analyses including age, gender, and etiology CD, as well as daily hydrocortisone dose as a quadratic term revealed a significant quadratic contribution of hydrocortisone dose to depressive symptoms (HADS) (β = 1.150, P = .019), mental fatigue (MFI-20) (β = -1.079, P = .033), physical functioning (β = -0.946, P = .046), social functioning (β = -1.232, P = .012), change in health (SF-36) (β = -1.031, P = .039), pain (β = 1.413, P = .004) and perceived health status (EQ-5D) (β = -1.022, P = .036), indicating that relatively low, as well as relatively high hydrocortisone intake was associated with more depressive symptoms, more limitations in physical functioning and social functioning, more pain, and lower perceived health, but less mental fatigue.

Regression analysis including $CORT_{hair}$ and hydrocortisone intake

Regression analysis including both $CORT_{hair}$ and daily hydrocortisone dose, as well as age, gender, and etiology CD, revealed a significant contribution of daily hydrocortisone dose to physical functioning (β = -0.182, P = .046) change in health (SF-36) (β = -0.254, P = .008), limitations in physical activities (β = 0.204, P = .034) perceived health status (EQ-5D) (β = -0.277, P = .004). No significant contribution of $CORT_{hair}$ was found in this by using this regression model. Post-hoc analyses on these significant results using the same regression analyses, but without $CORT_{hair}$, resulted in slightly increased beta's (increases ranging from .008 to .022), indicating that part of the variation of $CORT_{hair}$ was explained by hydrocortisone intake, which is not surprising considering the association between daily hydrocortisone intake and $CORT_{hair}$ (r = 0.185, P = .047).

DISCUSSION

The present exploratory study confirmed that patients with AI report more impairments in QoL compared to healthy controls (2-5), which is dependent on the cause of AI and demonstrated that daily hydrocortisone intake was inversely correlated with QoL (physical aspects). This is in accordance with some (5;13;28), but not all studies (2;4;29). Interestingly, this association was not found with systemic cortisol exposure, since only a few aspects of QoL were associated with $CORT_{hair}$, suggesting that QoL impairments are not per se due to chronic overtreatment with hydrocortisone. Nevertheless, $CORT_{hair}$ did explain a part of the variation of the observed associations between daily hydrocortisone intake and QoL, indicating that the actual cumulative cortisol exposure should also be taken into account.

Previous QoL studies in patients with AI identified several influencing factors, such as autoimmune co-morbidity (30), delay of diagnosis (30), higher age at manifestation, and female gender (30). Furthermore, it is suggested that intrinsic imperfections in replacement therapy also play a role (10). The present study is the first to examine the relation between actual chronic cortisol tissue exposure and QoL in patients with AI as measured by $CORT_{hair}$. Based on this first explorative study it seems that associations between hydrocortisone intake and QoL are not (directly) influenced by cortisol exposure. This suggests that QoL impairments in patients with AI are not per se related to higher cortisol exposure, but it might be more obvious that the relation between hydrocortisone intake and QoL is (at least partly) explained by that patients who take more hydrocortisone basically need more hydrocortisone. Furthermore, assessing the potential effect of occasionally taking higher hydrocortisone doses did not reveal a significant effect (data not shown). Cortisol acts in the central nervous system by binding to mineralocorticoid- and glucocorticoid receptors. The current notion is that the effects of cortisol binding to mineralocorticoid and glucocorticoid receptors follow an inverted u-shaped dose response curve, with both pathological low and high cortisol levels negatively affecting the mediating function of these receptors (31). This mechanism might underlie the

observed impairments in QoL in female patients with $CORT_{hair}$ above the lab-internal cut-off. Since there is no explicit lower limit of $CORT_{hair}$, there is no evidence that underreplacement negatively affects QoL. Interestingly, this inverted u-shaped dose response curve was identified in the quadratic associations found between hydrocortisone intake and physical, mental and social aspects of QoL.

Despite the heterogeneous origin of AI in this cross-sectional analysis, we found that $CORT_{hair}$ correlated with two physical aspects of QoL (physical activities and pain) in female patients, and one physical aspect in male patients (physical fatigue). In addition, female patients with $CORT_{hair}$ above the lab-internal cut-off reported more impairment in QoL relative to females with $CORT_{hair}$ below the lab-internal cut-off. This difference was not found for male patients. The found gender difference in $CORT_{hair}$ in the present sample with males demonstrating higher $CORT_{hair}$ than females, was previously described (17) and may be due to, among other factors, sex-specific differences in levels of circulating cortisol binding globulin. Therefore, analyses of the present study were stratified for gender. Furthermore, in male patients higher $CORT_{hair}$ was associated with higher BMI (17), suggesting a metabolic effect of overexposure. Recently, Quinkler et al. demonstrated in patients with AI using conventional hydrocortisone replacement that switching to once-daily hydrocortisone dual release tablets did not ameliorate QoL, although BMI and HbA1c improved (32). Together with the results of the present study, this would suggest that more adequate cortisol exposure predominantly affect somatic outcome, and to a lesser extent patient-perceived well-being, in particular in males. Furthermore, it was observed that patients with CD showed lower $CORT_{hair}$ relative to the other groups (although not significant). We postulate that this observation is also related to the gender effect since ninety-four percent of the patients with CD were females, and comparing $CORT_{hair}$ between female patients with CD and other female patients revealed no significant results (data not shown). Furthermore, it can be speculated that low $CORT_{hair}$ found in patients with CD might be explained by irreversible changes in cortisol metabolism (e.g. more efficient breakdown of cortisol) related to the previous exposure to elevated cortisol levels.

In addition, the observation that patients with AI due to previous treatment for CD reported more QoL impairments compared to the other diagnostic groups, while also having the lowest $CORT_{hair}$ (not significant), could potentially be explained by the fact that these patients have been exposed to excessive cortisol levels in the past. Previous literature reported that potential damage caused by this excessive exposure to cortisol might only be party reversible (33;34). Therefore, it might be that QoL impairments in the CD group are to a larger extent explained by the previous hypercortisolism, than due to current cortisol levels as measured by $CORT_{hair}$.

As previous studies show, assessment of $CORT_{hair}$ is a useful tool in the diagnosis of Cushing's syndrome and potentially also for AI (16;35) or as indicator of somatic disease and distress (36-38). Furthermore, the assessment of $CORT_{hair}$ in the present study enabled us to

discriminate between cause and consequences, since impairments in QoL were associated with a higher hydrocortisone intake, but were not reflected by higher $CORT_{hair}$. Several small studies on the relation between $CORT_{hair}$ and depressive symptoms, anxiety or general well-being in subjects without AI have been published, however at present no other study primarily focused to this extent on QoL in relation to $CORT_{hair}$ in AI (38-41). Younge and colleagues assessed $CORT_{hair}$, as well as QoL and psychological parameters (i.e., SF-36, HADS) in patients with structural heart disease. They demonstrated that higher $CORT_{hair}$ was correlated with lower self-reported physical functioning, which remained significant after adjustment for age, gender and BMI. No significant correlations were found on other aspects (42). Similarly, in the present study, physical aspects of QoL were associated with hair cortisol levels, while no correlations were found with other aspects of QoL.

In the present study, $CORT_{hair}$ and some physical aspects of QoL were associated with each other in a heterogeneous group of patients with AI. It is important to acknowledge that we studied correlations within a group of patients with AI with impairments in QoL (2-6). This group is potentially yielding a relatively small variation of QoL, thereby impeding finding associations between $CORT_{hair}$ and QoL. Other aspects that should be taken into account while interpreting the results are the multidimensional character of QoL (43), and the possibility that the used generic and domain-specific QoL questionnaires might have been not sensitive enough. Although a disease-specific QoL questionnaire for primary adrenal insufficiency (i.e. AddiQoL (44;45)) could have been more sensitive and suitable, it was not used because it has not yet been translated and validated into the Dutch language. In addition, it should be acknowledged that although the present sample was heterogeneous regarding etiology of AI and that both patients with primary and secondary AI were included, it provides a representative sample of everyday clinical practice. Finally, no conclusions can be drawn about causality due to the cross-sectional design of this study. Future studies using a longitudinal design could provide more information about the time course of QoL impairments, as well as the contribution of $CORT_{hair}$.

In conclusion, this is the first report that further explored the relation between QoL, hydrocortisone intake and actual cortisol exposure in AI patients by measuring hair cortisol, a marker of long-term systemic cortisol exposure. Patients with AI demonstrated several impairments in QoL which were sex-specifically associated with hydrocortisone intake, but were to a lesser extent reflected by chronic cortisol exposure as measured by hair cortisol, suggesting that QoL impairments in patients with AI are not explained by the effect of prolonged exposure to elevated systemic cortisol levels.

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