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Hair Cortisol in the Evaluation of Cushing Syndrome

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Abstract

Purpose—Hair cortisol evaluation has been used to help detect patients with suspected Cushing Syndrome. Our goal was to correlate segmental hair cortisol with biochemical testing in patients with Cushing syndrome and controls. This study was a prospective analysis of hair cortisol in confirmed Cushing syndrome cases over 16 months.

Methods—Thirty six subjects (26.5±18.9 years, 75% female, and 75% Caucasian) were analyzed by diurnal serum cortisol, 24 h urinary free cortisol corrected for body surface area (UFC/BSA), and 24 h urinary 17-hydroxysteroids corrected for creatinine (17OHS/Cr). Thirty patients were diagnosed with Cushing syndrome, and six were defined as controls. 3-cm hair samples nearest to the scalp, cut into 1-cm segments (proximal, medial, and distal), were analyzed for cortisol by enzyme immunoassay and measured as pmol cortisol/g dry hair. Hair cortisol levels were compared with laboratory testing done within previous 2 months of the evaluation.

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Compliance with Ethical Standards

Conflict of Interest: The authors declare that they have no conflict of interest.

Informed Consent: Informed consent was obtained from all individual participants included in the study.

Ethical Approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This article does not contain any studies with animals performed by any of the authors.

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Results—Proximal hair cortisol was higher in Cushing syndrome patients (266.6 ± 738.4 pmol/g) than control patients (38.9 ± 25.3 pmol/g) (p = 0.003). Proximal hair cortisol was highest of all segments in 25/36 (69%) patients. Among all subjects, proximal hair cortisol was strongly correlated with UFC/BSA (r=0.5, p=0.005), midnight serum cortisol (r=0.4, p=0.03), and 17OHS/Cr, which trended towards significance (r=0.3, p=0.06).

Conclusions—Among the three examined hair segments, proximal hair contained the highest cortisol levels and correlated the most with the initial biochemical tests for Cushing syndrome in our study. Further studies are needed to validate proximal hair cortisol in the diagnostic workup for Cushing syndrome.

Keywords

Hair cortisol; Hair; Cortisol; Cushing syndrome; Cushing disease; Children; Adolescence

Introduction

The diagnosis of Cushing Syndrome (CS) is often challenging and inconclusive, despite numerous tests used for the detection of hypercortisolemia and its origin, and is associated with high morbidity and high risk for mortality, if undiagnosed and untreated. The Endocrine Society Clinical Practice Guideline for the diagnosis of this rare disease recommends the following initial tests for CS: 24 h urine free cortisol (UFC), 1-mg overnight or 2-mg 48 h dexamethasone suppression test, late night salivary cortisol, or combination of any of the above (1). Diurnal serum cortisol and 24 h urinary 17-hydroxysteroids (17OHS) have been used in the research setting in the workup of CS.

In some cases, biochemical hypercortisolemia may change daily, requiring repetition of tests to confirm or exclude disease, particularly in patients with so-called “cyclical CS” [1–3]. The selection of diagnostic cutoff values can dramatically alter the sensitivity and specificity of UFC, late night salivary, and serum cortisol tests [1–6]. Furthermore, collecting multiple samples of UFC, 17OHS, diurnal serum cortisol, or salivary cortisol with dexamethasone suppression testing is more reliable than using only one isolated sample to diagnose hypercortisolism [2,7]. Regardless of the cutoff parameters, current testing for hypercortisolemia limits detection to a 24 h period [8,9].

As a potential solution to the limitations of these tests, hair cortisol has been increasingly studied as an additional means to diagnose patients with CS [10]. Much like hemoglobin A1C is a longitudinal marker of blood glucose levels, hair cortisol can be a measure of the body’s glucocorticoid levels over the previous several weeks to months. Hair cortisol has aided in the diagnosis of cyclical CS [11], following post-treatment progress of various presentations of CS [10,11] and monitoring hydrocortisone dosing or patient compliance in adrenal insufficiency [12–15]. Furthermore, moderate correlations of hair cortisol with either serum and salivary cortisol levels or both have been found in elementary school girls, pregnant patients, and non-pregnant participants [16–18]. However, no study to date has compared the serum or urinary cortisol levels for CS patients with segmental hair cortisol data. Comparing hair cortisol with the standard workup for CS should allow further research

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to investigate the applicability of hair cortisol as an initial or supportive diagnostic test for CS.

In this study, we investigated the reliability of measuring segmental hair cortisol levels of the 3 cm hair samples closest to the scalp in the evaluation for CS. This clinical study was conducted at the National Institutes of Health (NIH) Clinical Research Center (CRC).

Materials and Methods

Study population and biochemical testing

We recruited 49 patients referred to the NIH CRC for the evaluation of CS from September 2013 to January 2015. History and physical examination was performed, and exposure to exogenous steroids was evaluated. CS was diagnosed using clinical assessment, biochemical data, and radiological studies, and confirmed with histopathology (when surgery was performed). Biochemical evidence of CS was evaluated with serial UFC, serial 17OHS corrected for creatinine excretion (17OHS/Cr), serum cortisol and adrenocorticotropic hormone (ACTH) levels, and 48 h low-dose dexamethasone suppression testing. Diurnal serum cortisol and ACTH were collected via a previously placed intravenous catheter at 2330h and 0000h (midnight), repeated only if the patient was awake or in discomfort, and at 0730h and 0800h (early morning) the following day, as previously described [19]. In the final analysis using both diurnal cortisol and ACTH, the average of 2330h and 0000h values represented midnight values, and average of 0730h and 0800h values represented the early morning values. Body mass index (BMI) was calculated in kg/m^2, and body surface area (BSA) was calculated using the formula from Dubois and Dubois [20]. For the final analysis, UFC was corrected for BSA (UFC/BSA) to standardize for height among all ages and sizes, as previously described [21]. For comparison to hair cortisol values, we only selected pre-operative laboratory values that occurred within the prior 2 months of hair collection.

Of the 49 patients initially recruited for the study, five patients were excluded because of diagnosis of primary aldosteronism without evidence of cortisol co-secretion, and eight did not have CS but were excluded for midnight serum cortisol levels>49.7 nmol/L. A total of 36 patients were included in the analysis.

All research subjects signed an informed consent. The Institutional Review Boards of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD) (until 2010) and National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) (2010-present) at NIH approved the research protocols (Clinical Trial Registration Numbers: NCT00005927 and NCT00001595).

Hair sample collection

Hair was collected as previously described [22,23]. In brief, a pencil-width selection of strands of hair was cut with sterile scissors at the vertex of the scalp, capturing at least 3 cm closest to the scalp, without plucking the hair roots to avoid contamination from serum. The 3 cm of hair closest to the scalp was isolated and then further cut into 1-cm lengths (proximal, medial, and distal). The 1 cm samples were individually wrapped in aluminum

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foil and stored at −20ºC, until transferred to room temperature for shipping to the reference laboratory for processing.

Hair cortisol analysis

Hair samples were processed and analyzed for cortisol according to the methods previously described [23]. Briefly, samples were washed in isopropanol to remove external contamination, dried, and then milled to a fine powder using a Mini-Beadbeater-16 (BioSpec, Bartlesville, OK). Cortisol was extracted overnight into methanol, extracts were dried, and the cortisol was analyzed using a sensitive and specific enzyme immunoassay (Salimetrics, Carlsbad, CA). Assay readouts were converted to picomoles cortisol per gram dry hair weight, and the intra-assay coefficient of variation was <2%. For this assay, estimated cortisol recovery ranges from 97% to 113%. Cross-reactivity with other endogenous steroid hormones is extremely low as reported by the manufacturer, ranging from 0.006% for testosterone to 0.21% for corticosterone. Cross-reactivity for synthetic glucocorticoids is as follows: prednisolone = 0.56%, triamcinolone = 0.08%, and dexamethasone = 19.2%.

Statistical analysis

Calculations were performed with SPSS 20.0 software (SPSS Inc., Chicago, IL, USA). Based on a standard deviation (SD) of 25 pmol/g for hair cortisol levels and expected difference of at least 35 pmol/g between cases and controls, a sample size of 35 subjects in a 1:6 ratio was required in order to reach a statistically significant difference with a p-value cutoff of 0.05 and statistical power of 0.8. Results are expressed as mean ± SD unless otherwise indicated. Hair cortisol measurements were logarithmically transformed to induce approximate normality. For group comparisons, the independent Student's t-test was used to analyze differences in numerical variables; the χ^2 test was employed to analyze differences in categorical variables. The Pearson product was used for analysis of correlations between variables. Non-parametric tests were used as appropriate. The p-value for significance was set at <0.05.

Results

Demographics

The mean subject age of the 36 patients in the final analysis was 26.5 ± 18.9 years (CS: 26.2 ± 18.8 years and controls: 27.8 ± 21.2 years) at the time of presentation to the NIH CRC, and 21/36 patients in our cohort were less than 18 years. Thirty patients were diagnosed with CS. The etiology of hypercortisolemia among the CS patients included Cushing Disease (CD; n=20), ectopic CS (n=2), and adrenal (ACTH-independent) CS (n=8). Most subjects in both groups were Caucasian (CS: 70.0%, n=21 and controls: 100%, n=6) and female (CS: 73.3%, n=22 and controls: 83.3%, n=5). Patients were similar in weight, height, BMI, and BSA (Table 1). Complete demographics, laboratory data, and imaging studies and diagnoses for each patient are presented in detail in the Supplemental Table.

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Biochemical evaluation

Patients diagnosed with CS had higher serum and urinary values than the control group. The CS group, compared to the control group, had significantly greater midnight serum cortisol (435.5±237.5 vs. 33.3±7.7 nmol/L, p<0.001) and early morning cortisol (468.7±192.6 vs. 259.8±176.4 nmol/L, p=0.02), respectively. In the urine studies, the CS group had significantly greater UFC/BSA (599.0±631.8 vs. 24.6±20.6 nmol/24 h/m^2, p<0.001) and 17OHS/Cr (5.6±2.9 vs. 1.7±0.5 mmol/24 h/mol Cr, p<0.001) than the control group, respectively.

Hair cortisol evaluation

Proximal hair cortisol values were higher in the CS than control group (266.6±738.4 vs. 38.9±25.3 pmol/g, p=0.003). However, the differences in hair cortisol values in the medial, distal, and average segments between the CS and control groups did not reach statistical significance. The proximal 1 cm hair had the highest median cortisol levels of all segments in all patients (Fig. 1) and controls (Supplemental Fig.) and represented the highest hair cortisol level in 69.4% (25/36) of all patients and 73.3% (22/30) of CS patients.

Correlation analysis

Correlation analysis of the full study population (Table 2) revealed a positive correlation between proximal hair cortisol levels and UFC/BSA (r=0.5, p=0.005, Fig. 2a), midnight serum cortisol (r=0.4, p=0.03, Fig. 2b), and 17OHS/Cr (r=0.3, p=0.06, Fig. 2c), which trended towards significance. Similarly, in all CS patients (Table 3), there was a positive correlation between proximal hair cortisol and UFC/BSA (r=0.5, p=0.009), early morning serum cortisol (r=0.4, p=0.03), and midnight serum cortisol (r=0.3, p=0.07), which trended towards significance. For the medial hair segment, there were mild correlations between medial hair cortisol and UFC/BSA in the entire patient cohort (r=0.3, p=0.05; Table 2) and the CS group (r=0.4, p=0.05; Table 3). For the distal hair segment, there was no statistically significant correlation between distal hair cortisol and any plasma or urinary assessment of hypercortisolemia. Average hair cortisol was mildly correlated with UFC/BSA in all patients (r=0.4, p=0.03; Table 2) and in the CS group (r=0.4, p=0.02; Table 3). In a subgroup analysis of only patients with CD, no correlation of statistical significance was found between any hair cortisol measurement and any of the initial biochemical tests, including diurnal serum ACTH levels (Table 4).

Discussion

We found that proximal hair cortisol directly correlates with midnight serum cortisol and UFC in patients with and without CS. The most proximal 1 cm of hair was the best section of hair for stratifying the two groups of patients in our cohort. Additionally, the proximal hair cortisol correlated best with UFC/BSA among our cohort of CS patients. Other studies reported moderate correlations of hair cortisol with UFC, either as one-time UFC measurement in 39 non-obese subjects (r=0.333, p=0.041) [16] or measuring UFC daily over 63 days in ten healthy participants (r=0.422, p=0.089) with hair collected at the end of 9 weeks [24].

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While the growth rate of hair on the vertex is assumed constant, about 1 cm/month [22], hair cortisol concentration is not always consistent. Assuming the rate of hair growth is approximately 1 cm/month at the vertex of the head [25], the most proximal 3 cm of hair may represent the previous 3 months of cortisol exposure. Depending on the cutting technique, at least 0.5 cm of hair, which may represent 2 weeks of potential cortisol exposure, may remain [22]. We chose the initial 3 cm of hair for its reliability compared to distal aspects [26], and our initial goal with segmentation into 1 cm portions was to hopefully identify suspected cases of cyclical CS, as done previously [11].

Both in our study and in the Manenschijn, *et al.* study with 14 patients with CS [11], segmental hair cortisol stratified CS patients from controls; ectopic CS patients had the highest biochemical measurements of hypercortisolemia than all other CS patients. In our study, with a higher proportion of CD and adrenal CS cases, the differences in hair cortisol levels among all patients and CS subgroups were not as stark. Because of the slight differences between our findings and the study on suspected cyclical CS patients [11], we speculate several confounding factors that can alter hair cortisol measurement in CS patients.

For the measurement of hair cortisol in CS, there are several exogenous and endogenous factors on hair cortisol levels to consider. In several studies [11,14,27], overall hair cortisol values may be overestimated due to lack of isopropanol washings to remove sweat cortisol contamination, and mincing, instead of grinding, may have reduced the yield of actual cortisol from inside the individual hairs. Excessive shampoo washings and hair dyeing [28,29] and prolonged exposure to UV light [30] can diminish quantification in hair samples (Fig. 3a), as supported by findings in a large cohort without psychiatric disease [31]. Even though sweat cortisol has not been found to alter *in vivo* analysis of hair cortisol levels after short-term exposure to physiological stressors [32], it remains unclear how long term exposure of hair to cortisol from sweat may affect measurements.

Cutaneous production of cortisol *de novo*, activation of cortisone to cortisol, and deactivation of cortisol to cortisone might have significant roles in the discrepancies between hair cortisol levels in CD, adrenal CS, and ectopic CS. In the skin, there are similar synthetic enzymes and feedback mechanisms, which may function like the hypothalamic-pituitary-adrenal (HPA) axis [33] but with additional capabilities for paracrine regulation [34]. Hair follicles and nearby structures have the ability to produce cortisol via 11-beta-hydroxysteroid dehydrogenase (11BDH) type 1 (11BDH1, Fig. 3b) and under ACTH stimulation (Fig. 3c) [33,35,36] and deactivate cutaneous cortisol via 11BDH type 2 (11BDH2), which is expressed in the arterioles supplying the hair bulb (Fig 3d) [37].

With the similar synthetic machinery and feedback mechanisms like the HPA axis, hypercortisolemic states might affect the measured hair cortisol while possibly having minimal to no systemic effect. Similar to ectopic CS and CD, excess systemic ACTH might be expected to hijack the HPA-like axis leading to increased cortisol in hair. Consequently, due to the additive effect of adrenal and cutaneous cortisol synthesis from elevated systemic ACTH, ectopic CS and CD might be expected to have a significantly higher hair cortisol value than in adrenal CS, where this cutaneous axis should suppressed (Fig. 3c). However, a direct linear relationship between ACTH and hair cortisol was not seen in our cohort, and

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even among the CD patients, there was no significant correlation with any non-hair tests (Table 4). We speculate other factors, like the balance of cortisol activation and deactivation by local 11BDH1 (Fig. 3b) and 11BDH2 (Fig. 3d) might have a significant impact in CS patients, skewing hair measurements. Further studies in hair cortisol evaluation in CS patients should address the effects of 11BDH1, 11BDH2, and response to systemic ACTH on hair cortisol measurements as well as the *in vivo* glucocorticoid synthetic activity of the hair follicle cells in ACTH-dependent and ACTH-independent CS.

Limitations

Study results should be interpreted with caution: our study was limited by the small control group (n=6), exclusion of patients with midnight serum cortisol > 49.7 nmol/L or hyperaldosteronism, and the majority age of patients less than 18 years. The low number of control patients may confound the interpretation of the hair cortisol cut-off values in the clinical setting. Pseudo-CS patients can have elevated hair cortisol, as in alcoholism [38], obesity [39,40], psychiatric illnesses and chronic stress [41,42], and our exclusion of patients with midnight cortisol > 49.7 nmol/L limited applicability of our study to cases of pseudo-CS and primary aldosteronism without evidence of cortisol co-secretion.

In the current analysis, we did not correct for several covariates, including hair heterogeneity and biometrics, due to sample size limitations. Hence, the results should be taken in the relevant context. Furthermore, most of our patients were able to be evaluated on only one occasion in the 2 months prior to hair sampling before surgery, which limits the correlation of segmental hair cortisol to monthly cortisol levels.

As 58% of our cohort age was less than 18 years, pubertal status on cortisol metabolism may be a factor in hair cortisol measurement. The half-life of cortisol is not significantly different amongst all pubertal stages, even though pubertal status greatly affects clearance and distribution of total and free cortisol [43]. Additionally, puberty is impaired or suppressed in adolescents with CS [44], which could diminish appreciation of differences from various pubertal stages.

However, our study's strengths are that it is the largest sample so far to analyze segmental hair cortisol in CS and that it is the largest study to compare hair cortisol to any biochemical test for hypercortisolemia in patients with CS. Our study also included a large cohort of CD patients, which has been under-represented in prior studies on hair cortisol. We demonstrated hair cortisol correlates well with some biochemical measures of hypercortisolemia in CS patients, and these findings support further research on the use of this modality in the workup for CS.

Conclusions

We found that, compared with the distal aspects and average of the hair shaft, the most proximal 1-cm hair cortisol is the best marker for reflecting hypercortisolemia in the workup for the rare and diagnostically challenging disease, CS, which if undiagnosed and untreated, has a high associated morbidity and mortality. Our findings support further investigation into the use of the most proximal 1-cm of hair, and hair cortisol in general, as an initial or supportive diagnostic test for CS.

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Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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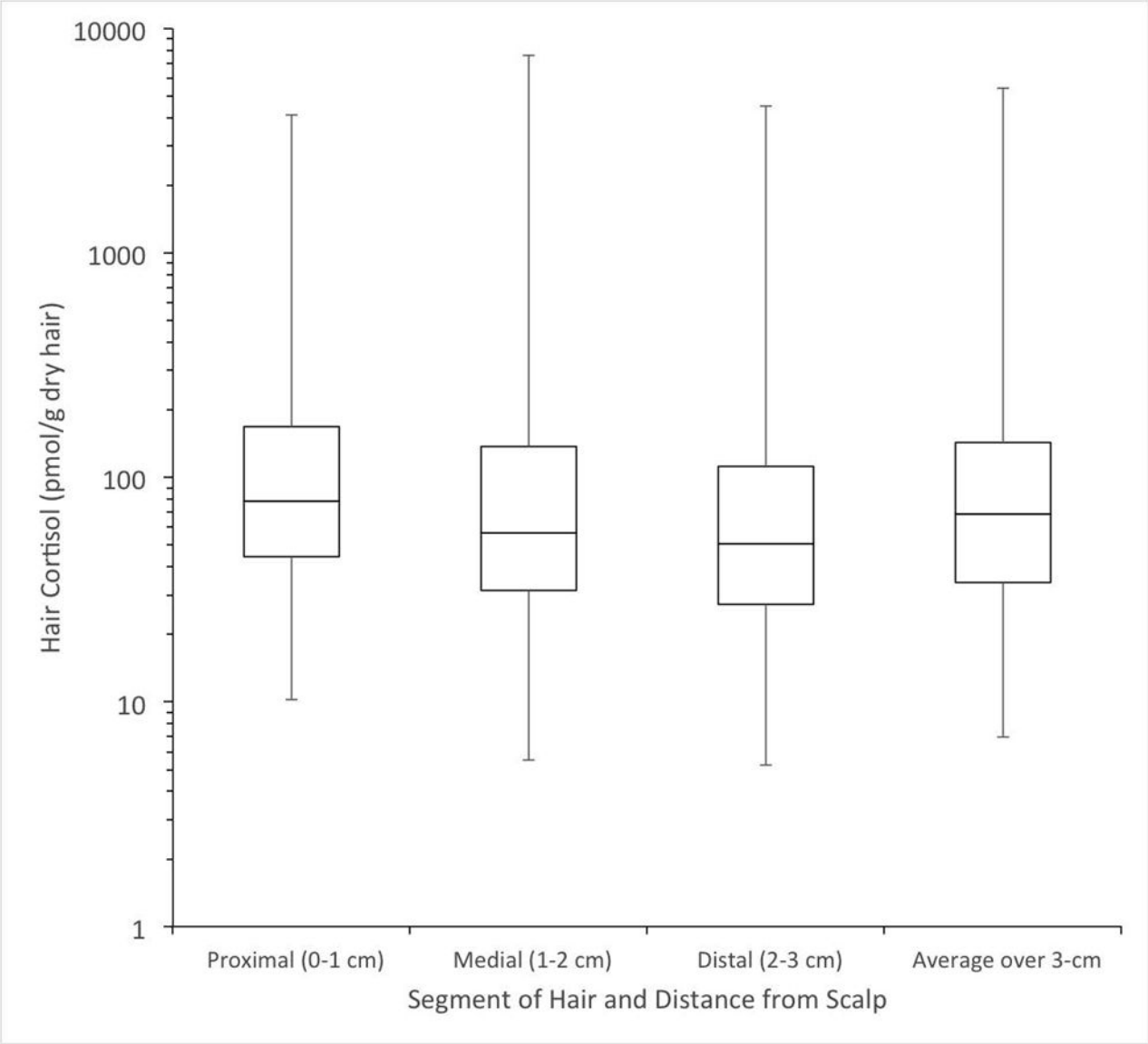

Fig. 1.

Box plot of hair cortisol segments in the proximal 3 cm of hair of all 36 patients. All 36 patients' hair cortisol measurements for the proximal, medial, distal, and average hair segments are presented as median and interquartile ranges, with whiskers representing the maximum and minimum.

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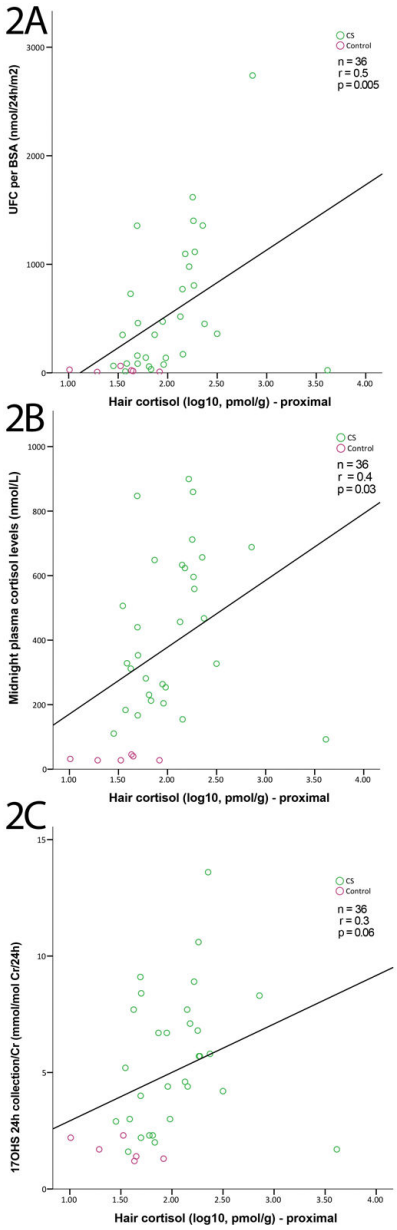

Fig. 2.

Correlations between proximal hair cortisol and UFC/BSA (A), midnight serum cortisol (B), and 17OHS/Cr (C) among CS patients (green circles) and controls (purple circles). For all graphs, proximal hair cortisol values were $\log_{10}$-transformed for normality. (A) UFC/BSA was strongly and positively correlated with proximal hair cortisol (r=0.5, p=0.005). (B) Midnight cortisol was moderately and positively correlated with proximal hair values (r=0.4, p=0.03). (C) 17OHS/Cr was moderately and positively correlated with proximal hair cortisol but trending toward significance (r=0.3, p=0.06).

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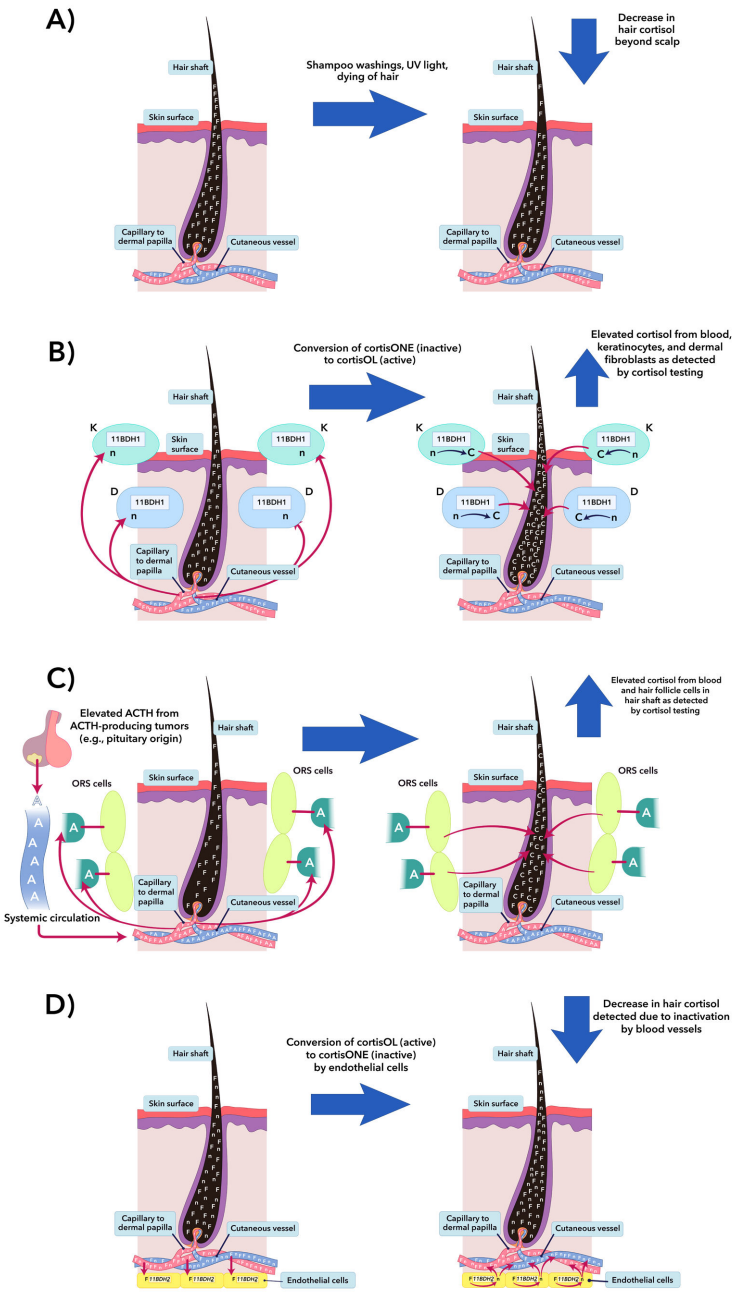

Fig. 3.

Potential confounding factors in hair cortisol of CS patients. For simplicity, it is assumed that lipophilic cortisol diffuses from vessel to hair shaft via simple diffusion, similar to how illicit drugs are suspected of being incorporated into hair [45,46]. (A) Excessive shampoo washings and hair dyeing [28,29] can leach cortisol from hair, and prolonged exposure to UV light causes photo-degradation or cross-linking of glucocorticoid molecules [30], decreasing the quantification of cortisol measured in hair. (B) Assuming that skin has local HPA-like activity and appropriate negative feedback loops [33,36], the production and dynamics of locally-synthesized CRH and ACTH should be negligible under systemic

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suppression in chronic hypercortisolism as in murine models [47]. As may be seen in adrenal tissue in ACTH-dependent CS, *in vitro* studies of cultured skin sections have shown doubling to tripling of cortisol production after exposure to ACTH [48]. Human epidermal keratinocytes (K) and dermal fibroblasts (D) contain 11-beta-hydroxysteroid dehydrogenase (11BDH) type 1 (11BDH1), which can convert cortisone (inactive) to cortisol (active). In the K and D cells, 11BDH1 is site-dependent and is up-regulated with age, exposure to sunlight, and exposure to glucocorticoids [35,49], and the mechanisms causing increases in cutaneous cortisol in aging persons, such as increased 11BDH1 activity and expression, [49] might also occur in CS patients. The actual change in total hair cortisol by the increase of 11BDH1 kinetics from glucocorticoids in hypercortisolism has not been evaluated. (C) Of the hair follicle components in humans, the keratinocytes of the outer root sheath (ORS) cells possess the ability to produce cortisol after *in vitro* exposure to CRH and ACTH [33]. Under stimulation from excess systemic ACTH from any cause, ORS cells may locally synthesize cortisol, potentially causing an elevation of total cortisol detected in the hair shaft beyond expected from adrenal-synthesized cortisol. (D) Arterioles feeding the hair bulb express 11BDH type 2 (11BDH2) [37], which may deactivate systemic cortisol to cortisone and potentially lowering the cortisol detected in hair. In hypercortisolemic states, homeostasis is maintained by increased glucocorticoid catabolism, which is probably higher in ectopic CS than CD, likely due to higher cortisol levels in ectopic CS than CD [50]. Therefore, similarly in the skin, cutaneous 11BDH2 might also significantly reduce systemically- and locally-produced cortisol, and this enzyme might have a large role in the expected results in our CS group. This figure was designed using the program, Adobe Illustrator® CS6. F=systemically-produced hydrocortisol, n=hydrocortisone, C=locally-produced hydrocortisol, and A=systemically-produced ACTH.

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Table 1

Demographic characteristics.

Variable	All N=36	CS n=30 (83.33%)	Controls n=6 (16.67%)	p value CS vs. Controls*
Age (years)	26.5±18.9	26.2±18.8	27.8±21.2	0.8
Female, n(%)	27 (75.0)	22 (73.3)	5 (83.3)	0.6
Ethnicity, n(%)				0.5
Asian	3 (8.3)	3 (10.0)	0	
African-American	3 (8.3)	3 (10.0)	0	
Unknown	3 (8.3)	3 (10.0)	0	
Caucasian	27 (75.0)	21 (70.0)	6 (100.0)	
Height (cm)	151.2±18.2	148.9±18.5	162.8±11.9	0.08
Weight (Kg)	74.7±26.5	74.4±27.8	76.7±21.0	0.7
BMI (Kg/m^2)	31.9±7.7	32.6±7.9	28.7±6.3	0.3
BSA (m^2)	1.7±0.4	1.7±0.4	1.8±0.3	0.7

Data are presented as number (percentage) or mean ± SD.

*Mann Whitney U test was performed for continuous variables, and χ^2 test was performed for categorical variables.

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Table 2

Correlations and levels of significance of biochemical data in all patients (n=36). Hair cortisol values (measured in pmol/g dry hair weight) were $\log_{10}$-transformed for normality when undergoing statistical analysis. All tests represented lab values from all 36 patients, except for the ACTH values, which excluded one patient due to lack of collection. Diurnal ACTH values (n=35) are not presented, because the r-value for all was ≤0.2 with a p-value ≥0.3.

	Hair cortisol segment (distance in cm from scalp)	Average UFC/BSA over D1+D2 (nmol/24h/m^2)		Average 17-OHS/Cr over D1+D2 (mmol/24h/mol Cr)		Midnight cortisol (nmol/L)		Early morning cortisol (nmol/L)	
		r	p	r	p	r	p	r	p
1	**Proximal**	0.5	0.005	0.3	0.06	0.4	0.03	0.3	0.09
2	**Medial**	0.3	0.05	0.2	0.2	0.2	0.3	0.2	0.3
3	**Distal**	0.3	0.1	0.2	0.3	0.1	0.5	0.1	0.5
	Average over 3 cm	0.4	0.03	0.3	0.1	0.2	0.2	0.2	0.2

Statistical significance was considered for p's<0.05. D1+D2=Day 1 and Day 2.

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Table 3

Correlations and levels of significance of biochemical data in 30 patients with Cushing Syndrome. Hair cortisol values (measured in pmol/g dry hair weight) were $\log_{10}$-transformed for normality when undergoing statistical analysis.

	Hair cortisol segment (distance in cm from scalp)	Average UFC/BSA over D1+D2 (nmol/24h/m^2)		Average 17OHS/Cr over D1+D2 (mmol/24h/mol Cr)		Midnight serum cortisol (nmol/L)		Early morning serum cortisol (nmol/L)	
		r	p	r	p	r	p	r	p
1	**Proximal**	0.5	0.009	0.3	0.09	0.3	0.07	0.4	0.03
2	**Medial**	0.4	0.05	0.2	0.2	0.2	0.3	0.3	0.1
3	**Distal**	0.3	0.2	0.2	0.4	0.2	0.4	0.2	0.3
	Average over 3 cm	0.4	0.02	0.3	0.1	0.3	0.1	0.4	0.06

Statistical significance was considered for p's<0.05. D1+D2=Day 1 and Day 2.

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Table 4

Correlations and levels of significance of biochemical data in 20 patients with Cushing Disease. Hair cortisol values (measured in pmol/g dry hair weight) were $\log_{10}$-transformed for normality when undergoing statistical analysis.

	Hair cortisol segment (distance in cm from scalp)	Average UFC/BSA over D1+D2 (nmol/24h/m^2)		Average 17OHS/Cr over D1+D2 (mmol/24h/mol Cr)		Midnight serum cortisol (nmol/L)		Early morning serum cortisol (nmol/L)		Midnight serum ACTH (pmol/L)		Early morning serum ACTH (pmol/L)	
		r	p	r	p	r	p	r	P	r	p	r	p
1	**Proximal**	0.2	0.3	−0.05	0.8	0.2	0.5	0.2	0.4	−0.1	0.6	−0.08	0.8
2	**Medial**	0.02	0.9	−0.1	0.6	0.01	1.0	0.04	0.9	−0.05	0.9	−0.1	0.7
3	**Distal**	−0.05	0.8	−0.2	0.5	−0.04	0.9	−0.2	0.5	−0.05	0.8	−0.1	0.7
	Average over 3 cm	0.06	0.8	−0.1	0.6	0.04	0.9	0.03	0.9	−0.08	0.8	−0.1	0.7

Statistical significance was considered for p's<0.05. D1+D2=Day 1 and Day 2.