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Evaluation of a method to measure long term cortisol levels

Laura Manenschijn*, Jan W. Koper, Steven W.J. Lamberts, Elisabeth F.C. van Rossum

Department of Internal Medicine, Erasmus MC, University Medical Center, Rotterdam, The Netherlands

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ABSTRACT

Introduction: Elevated levels of cortisol are known to induce various symptoms and diseases, e.g. abdominal obesity, type 2 diabetes, osteoporosis and cardiovascular disease. Measuring serum, saliva and urine cortisol is limited to one time point. Measurement of cortisol in scalp hair is a recently developed method to measure long term cortisol levels. The aim of this study was to investigate whether hair cortisol is a feasible parameter to measure cortisol exposure.
Experimental: We collected hair samples of 195 healthy individuals, 9 hypercortisolemic and one hypocortisolemic patient and measured hair cortisol levels. Cortisol was extracted from scalp hair using methanol and cortisol levels were measured using a salivary ELISA kit. Measurement of waist and hip circumferences and blood pressure was performed in 46 healthy subjects.
Results: We found a positive correlation between hair cortisol and both waist circumference ($r = 0.392$, $p = 0.007$) and waist-to-hip ratio (WHR) ($r = 0.425$, $p = 0.003$). No correlations were found between hair cortisol levels and BMI, blood pressure or age. There was no decline in cortisol levels in six consecutive hair segments. Hair cortisol levels were elevated in patients with known hypercortisolism ($p < 0.0001$).
Conclusions: Hair cortisol was positively correlated with WHR, suggesting that hair cortisol reflects cortisol exposure at tissue level, which was also supported by elevated hair cortisol levels in hypercortisolemic patients and concordance between hair cortisol levels and clinical disease course. Cortisol levels in hair are slightly influenced by hair treatment but not by natural hair colour, use of hair products, gender or age.

1. Introduction

The stress hormone cortisol has a wide spectrum of physiological effects throughout the human body and is involved in glucose and lipid metabolism, body composition, immunosuppressive and anti-inflammatory actions [1]. The effects of long term exposure to elevated cortisol levels comprise increased visceral fat mass, redistribution of body fat with accumulation of adipose tissue on abdomen and trunk and muscle atrophy. In addition, high levels of cortisol induce hypertension, insulin resistance and dyslipidemia, leading to an increased cardiovascular risk as is seen in Cushing's syndrome, which is a state of cortisol excess, or in use of high doses of exogenous glucocorticoids [2,3].

Until now, most studies addressed the relationship between total serum cortisol and symptoms or disease. Conflicting data have been published and the exact relationship between cortisol-mediated effects and symptoms or diseases remains to be established. Likely explanations for these conflicting data are the circadian rhythm, the pulsatile secretion of cortisol and the daily variation due to circumstances (e.g. stress or infection). This complicates the use of serum, saliva and urine cortisol in epidemiological studies. Recently, several studies have shown that endogenous cortisol can reliably be measured in scalp hair [4–10]. Scalp hair grows with an average rate of one centimeter (cm) per month, therefore one cm of scalp hair could represent cortisol levels of one month. This suggests that hair cortisol can be used as a method to retrospectively measure cortisol exposure over the past weeks or months. This offers new potential ways to study the effects of, e.g. chronic stress, which is thought to be accompanied by a hyperactive hypothalamic–pituitary–adrenal (HPA) axis. This has already been evaluated in several studies. In chronic pain patients, cortisol levels were elevated compared to healthy persons [9] and in healthy pregnant women hair cortisol levels were positively correlated with perceived stress scale (PSS) scores [5]. Furthermore, unemployment, which is a chronic stressor, was also associated with increased cortisol levels in hair [11]. Recently, Pereg et al. showed elevated hair cortisol levels in patients with acute myocardial infarction [12].

Although the mechanism of cortisol incorporation into hair is not fully understood, measurement of cortisol levels in scalp hair is a very promising technique. Until now relatively small study populations were used and therefore replication is needed to evaluate

* Corresponding author at: Department of Internal Medicine, Room Ee-542, Erasmus MC, P.O. Box 2040, 3000 CA Rotterdam, The Netherlands. Tel.: +31 10 7044197; fax: +31 10 7035430.
E-mail address: l.manenschijn@erasmusmc.nl (L. Manenschijn).

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the use of hair cortisol as a marker of long term endogenous cortisol levels. Hence, we determined cortisol levels in scalp hair of healthy individuals and patients with hyper- and hypocortisolism. The aim of this study was to investigate whether hair cortisol is a feasible parameter to measure cortisol exposure. We studied this by measuring cortisol levels in scalp hair of healthy individuals, patients with hypercortisolism and one patient with hypocortisolism. Factors that could influence hair cortisol concentrations were determined and we studied whether hair cortisol concentrations were associated with cortisol specific tissue effects such as body composition.

2. Experimental

2.1. Study population

In total 195 healthy individuals who did not use glucocorticoids participated in this study. All participants had to fill out a questionnaire concerning hair and medical conditions. In the initial group of 149 healthy individuals hair cortisol levels were determined as well as height and weight. This group was extended by an additional 46 persons, in whom also waist and hip circumferences and blood pressure were measured. BMI was calculated as kg/m^2. To validate the measurement of cortisol in scalp hair, we also collected hair samples from 10 adult patients, nine with hypercortisolism and one with hypocortisolism. Diagnosis of hypercortisolism was made if 24-h urinary cortisol levels >850 nmol/L. Hypocortisolism was diagnosed as serum cortisol levels below 200 nmol/L. Approval was given by the local Medical Ethics Committee and all participants gave written informed consent.

2.2. Hair collection

Around 100 strands of hair were collected from the posterior vertex of the scalp and were cut off as close to the scalp as possible. The hair was taped to a piece of paper and the scalp end was marked. The samples were stored in an envelope at room temperature until analysis.

2.3. Hair preparation

Hair samples were prepared as described previously by Sauvé et al. [7]. A minimum of 10 mg of hair was weighed. In all participants, the three cm closest to the scalp end were used. In 28 women, in addition to the most proximal 3 cm, the remaining hair strands were divided in 5 segments of 3 cm, which resulted in analyses of 18 cm long hair, divided in six consecutive 3 cm segments. The different hair segments were put into separate glass vials and cut into small pieces, using small surgical scissors. 1 mL of methanol was added and the vial was sealed and incubated overnight for 16 h at 52 °C while gently shaking. After incubation, the methanol was removed, put into disposable glass tubes and was evaporated under constant stream of nitrogen. The samples were dissolved in 250 μL phosphate buffered saline (pH 8.0) and samples were vortexed for 1 min. Before analysis, the samples were vortexed again for 30 s.

2.4. Hair analysis

Cortisol levels were measured using a commercially available ELISA Kit for Salivary Cortisol (DRG Instruments GmbH, Marburg, Germany) as per manufacturer's directions with the reagents provided. 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 stated by the manufacturer.

2.5. ELISA recovery

To validate the ELISA we created cortisol standards in PBS with concentrations of 5, 10, 20, 40, 80 and 160 nmol/L and measured the recovery in duplicate. We also spiked two hair samples with 20 nmol/L hydrocortisone, to measure recovery when hydrocortisone was dissolved in hair extract.

2.6. Statistical analysis

Statistical analyses were performed using SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, USA). Differences in baseline characteristics were tested using Chi-square tests, Mann–Whitney U-tests and ANOVA. Variables that were not normally distributed were logarithmically transformed. The effects of the frequency of hair washing, natural hair colour, hair treatment and the use of hair products on cortisol levels were analyzed using univariate analysis of variance (UNIANOVA). Differences in hair cortisol levels between healthy individuals and patients with known hypercortisolism were analyzed with a Mann–Whitney U-test. Correlations of hair cortisol with blood pressure, BMI, waist and hip circumference and waist-to-hip ratio (WHR) were analyzed using Pearson's correlations. An ANOVA for repeated measurements was used for testing segment differences in healthy individuals with long hair.

3. Results

3.1. Assay recovery

The recovery of 5, 10, 20, 40, 80 and 160 nmol/L cortisol standards from PBS was 122.0%, 95.0%, 86.5%, 85.8%, 90.5% and 89.1%, respectively. When hydrocortisone was added to hair extracts, the mean recovery was 84.5%.

3.2. Healthy controls

Baseline characteristics of all healthy individuals are shown in Table 1. We found a significantly higher number of subjects who treated (dyed, bleached or permanent waved/straightened) their hair in women compared to men. Waist circumference, WHR and systolic blood pressure were all significantly lower in women. In the total group hair cortisol levels were normally distributed after log transformation (Kolmogorov–Smirnov $p = 0.200$).

Interestingly, we found positive correlations between cortisol in hair and waist circumference ($r = 0.392$, $p = 0.007$) and WHR ($r = 0.425$, $p = 0.003$) (Fig. 1). We found no correlation with BMI ($p = 0.646$), hip circumference ($p = 0.096$) or systolic and diastolic blood pressure ($p = 0.109$ and $p = 0.365$, respectively). In addition, we found no correlation between hair cortisol levels and age ($p = 0.388$).

In the total group cortisol levels in hair that was treated (dyed, bleached, permanent waved/straightened) were borderline significantly lower than in untreated hair (24.27 pg/mg hair versus 29.38 pg/mg hair, $p = 0.051$). Since there were only female participants in the group with treated hair, we compared mean cortisol levels between women with treated hair and women with untreated hair. We still found lower cortisol levels in treated hair (24.27 versus 29.44 pg/mg hair), although this was not statistically significant ($p = 0.079$). The use of hair products, such as hair spray, mousse, gel and wax, on the day of hair sample collection was not significantly associated with altered cortisol levels after adjusting for age, gender and hair treatment (24.83 pg/mg hair versus