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METHODS

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## Technical Approaches to a Cortisol Assay in Hair for Retrospective Stress Evaluation

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Received October 13, 2014

**Abstract**—The traditional assessment of the intensity of stress effects includes measurements of the content of the stress hormone cortisol in biological fluids, such as blood and less frequently, saliva or urine. However, various factors that influence the biosynthesis and/or metabolism of cortisol and the importance of measurement of cortisol over a large period of time make group studies and interpretation of data difficult using this biological material. Moreover, detection of the level of cortisol at the time of sampling of blood or saliva does not reflect the total stress load to which the patient was subjected in the period preceding the study. Recently, a quantitative assay of cortisol in hair was considered as an approach to the evaluation of the long-term (months) period of system stress load. Here, we adapted an assay method for cortisol in human hair. The method is based on methanol extraction of cortisol from a hair sample, methanol evaporation, and dilution of the extracted substances in phosphate-buffered saline followed by the enzyme-linked immune-sorbent cortisol assay. We also applied this approach to assessment of the corticosterone level in rodent hair, which may be used for comparative/translational studies.

*Keywords:* cortisol, corticosterone, glucocorticoids, hypothalamic-pituitary-adrenal axis, stress, hair

**DOI:** 10.1134/S1819712415010067

### INTRODUCTION

It is well known that cortisol plays an important role in the stress response of the body and its concentration in the blood rapidly grows after activation of the hypothalamic–pituitary–adrenal axis (HPAA) induced by a stress factor. However, in addition to immediate alteration of the cortisol level, there are some pathological conditions, such as hypo- and hypercorticism, where low or high levels of cortisol are maintained for a long period of time and predetermine stress-induced diseases [1–3].

Until recently, the consequences of stress were evaluated using a cortisol assay in blood, saliva, or urine samples. These approaches are used for the assessment of the short-term consequences of stress and it is impossible to apply them for longitudinal studies, which is a disadvantage. Moreover, various factors, including circadian rhythms [4, 5], food intake [6], and physical training [7], may influence the individual cortisol level and make cortisol assessment at a specific time point incorrect. Thus, individual variations in circadian rhythms and a substantial decrease in the cortisol content in the morning, which is the usual time of blood sampling in hospitals, may explain the significant variability of the data within groups.

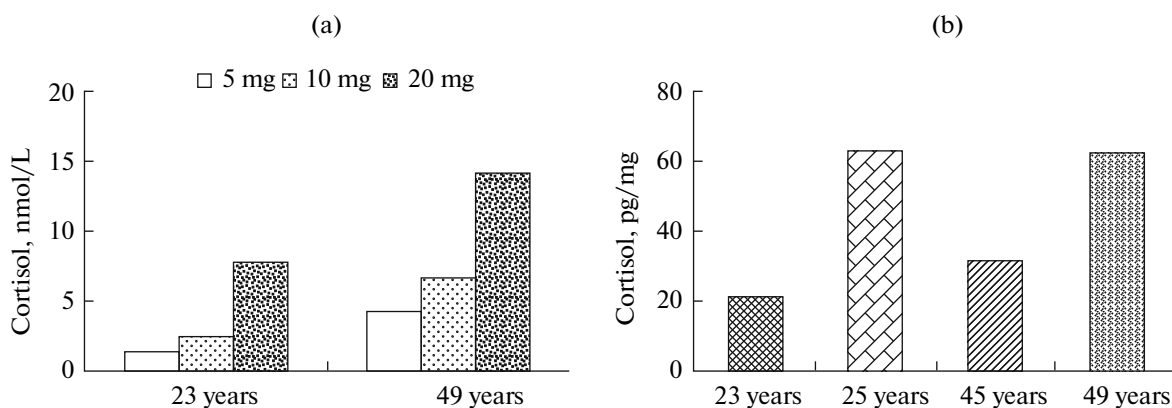
Analysis of the constitution of hair is widely used for the evaluation of the contents of drugs or toxicants [8]. The rate of hair growth is approximately 1 cm/month; therefore, assessment of the drug level may reflect the duration of the consumption of a substance [9]. Measurement of the content of the cortisol stress hormone in hair is considered as an opportunity to perform a retrospective analysis of the systemic level of cortisol that accumulated during a long period, such as months, which may reflect the cumulative activity of HPAA in clinical and experimental studies [10].

In the present study, we adapted a known method for cortisol extraction from hair to its following assessment under the conditions of a clinical laboratory.

### MATERIALS AND METHODS

Hair samples from four 22–49-year-old male volunteers were used for the study. The hair was cut using scissors in close proximity to its roots from the back of the head. The weights of the samples were approximately 30–40 mg. Cortisol was extracted according to the method of Yamada et al. [11] with minor modifications. A hair sample of 5–20 mg was cut with small surgical scissors and put into a 2-mL Eppendorf tube with a screw cap. After the addition of 1 mL of methanol, the samples were incubated for 16 h at 52°C using a thermoshaker (Biosan, Latvia). Methanol extracts were transferred into the other tubes and dried

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**Fig. 1.** (a) Data on the calibration of cortisol extraction from hair samples of various weights and (b) cortisol contents in the hair of subjects of various ages. Abscissa axis, the ages of the subjects.

using a Concentrator plus vacuum concentrator (Eppendorf, Germany). After methanol evaporation, 250  $\mu$ L of phosphate-buffered saline (pH 7.4) was added to the tubes, while the remainder was dissolved for 10 min at 25°C using a thermoshaker. Because not all extracted substances could dissolve in a water buffer solution but could affect the immune-enzyme hormone assay, the samples were filtered through Eppendorf-compatible paper filters with a pore size of 10  $\mu$ m (Thermo Scientific, United States). To our knowledge, this step is not described in the protocols that are recommended for cortisol assessment in hair but it is important for correct hormone measurement. This scheme was also used for corticosterone extraction from the hair of Wistar rats prior to and after moderate pain-emotional stress. For this purpose, the rats were subjected to electric pain stimulation for 10 min every 5 days during 30 days.

Cortisol was measured in hair extracts using an Access cortisol kit (Beckman Coulter, United States) for the immune-enzyme assay with competitive interaction in accordance with the instructions of the manufacturer. Measurements were performed using an Access-2 immunochemical analyzer (Beckman Coulter, United States). Corticosterone was measured in rat hair extracts using a DRG corticosterone ELISA kit (DRG International Inc., United States), which is also based on competitive interaction, in accordance with the instructions of the manufacturer. Data are presented as the mean values that were calculated by the analyzer from several parallel samples.

## RESULTS AND DISCUSSION

The data (Fig. 1a) demonstrate that cortisol extraction increased linearly depending on the increase in the sample weight from 5 to 20 mg. In spite of differences in the initial hormone levels, this relationship was observed in subjects with ages of 23 and 49 years. In four 23–49-year-old subjects, the cortisol content in the hair varied from 21.62 to 62.45 pg/mg (Fig. 1b);

these values were similar to those reported by other researchers who used an immunoassay for the measurement of the cortisol level in hair [12]. Sauv e et al. [12] reported that hair dyeing and the head region that was chosen for hair sampling did not affect the cortisol content; however, cosmetic treatment that led to hair death decreased the cortisol level. In this study, no correlations were observed between the cortisol levels in the hair, blood serum, and saliva, whereas a significant correlation with a low correlation coefficient, viz.,  $r = 0.33$ ,  $P = 0.041$ , was found between the cortisol contents in hair and daily urine.

Cortisol is thought to penetrate the hair shaft from the blood flow via simple diffusion and be related to the pool of free cortisol but not the total cortisol pool [13, 14]. Various diseases, including hypo- and hypercorticism; age; and the local synthesis or metabolism in a hair follicle may influence the rate of hair growth and cortisol intake [15, 16].

In the hair of Wistar rats, the initial level of corticosterone was 189.74 pg/mg; however, the corticosterone content increased to 227.88 pg/mg after 30-day intermittent moderate stress. An increase in the hair cortisol content was observed in male rhesus monkeys after transportation stress [17].

Positive correlations between the cortisol content in the hair and chronic stress have been revealed in pregnant women [18]. Cortisol analysis was performed in the hair of patients who suffered from psychological disease, bipolar affective disorder, in whose etiology HPA deregulation may play a role. In patients with clinical manifestation of this disease after 30 years, the cortisol level was significantly higher compared to healthy subjects [19].

Thus, assessment of the cortisol content in hair is a non-invasive informative approach for longitudinal studies of alterations in the systemic level of this stress hormone that continue for a long periods of time in both healthy subjects and patients with various somatic and psychological pathologies.

## ACKNOWLEDGMENTS

This study was supported by the Russian Science Foundation, project no. 14-25-00136.

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