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Time course of cortisol loss in hair segments under immersion in hot water

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ABSTRACT

Background: Hair cortisol is supposed to be a good biomarker of chronic stress. Major loss of hair cortisol in long-term exposure to environmental factors affected strongly its proper assessment of chronic stress in human. However, there was no research on time course of hair cortisol loss during the long-term exposure.
Methods: Hair samples with longer than 1 cm in the posterior vertex region were cut as close as possible to the scalp. The 1-cm hair samples were treated by ultraviolet irradiation or immersion in shampoo solution or water immersion at 40, 65 and 80 °C. Hair cortisol content was determined with high performance liquid chromatography tandem mass spectrometry.
Results: Ultraviolet irradiation and immersion in shampoo solution and hot water gave rise to the significant cortisol loss in hair. Hair cortisol content was sharply decreased with water immersion duration during initial stage and slowly decreased in the following stage.
Conclusions: The 2-stage loss process with water immersion duration modeled to some extent time course of hair cortisol loss in long-term exposure to external environments. Cortisol from hair samples closest to the scalp in the posterior vertex could represent more accurately central hypothalamo–pituitary–adrenal activity.

1. Introduction

Endogenous cortisol in hair has great potential to be a valid retrospective biomarker for assessing long-term basal activity of central hypothalamo–pituitary–adrenal (HPA) axis and changes of central HPA activity in cumulative exposure to chronic stress. The possibility was strongly demonstrated by the substantial increases of hair cortisol when exposed to chronic stresses [1–5] and by the parallel increases of hair cortisol with systemic cortisol secretion in three hypercortisolism models [6–8]. Hair cortisol as a novel biomarker was based on its biologically endogenous source, i.e., large inter-individual variation in hair cortisol resulted from distinct inter-individual difference in endogenous factors, including biological, psychological and psychiatric characteristics, such as body mass index [7], hair growth rate [9], systemic diseases (hypercortisolism or hypocortisolism) [7,8], perceived stress and depression under long-term high chronic stress [1–5]. Moreover, hair growth rate decreases with age and various diseases and varies among ethnic groups [9]. However, hair shafts were always exposed to external environments since they emerged from the skin in human head and over all animals. Proper assessment of hair cortisol might be readily and strongly affected by a series of human life habits and natural environmental factors, such as washing with shampoo and water, cosmetic treatments (hair dyeing, perming and bleaching), ultraviolet (UV) irradiation in long-time sunshine exposure and exposure to the external cortisol environment etc. For instance, a wash-out effect was suggested to possibly cause inter-segment loss in hair cortisol along hair shaft from the scalp-near segment to distal segment [5,6,10]. Sequentially, Kirschbaum et al. [6] recommended that hair cortisol in the first 1–6 cm segments near the scalp could assess reliably HPA axis activity over past 1–6 months if the hair growth rate is 1 cm/month [11], and that the other segments beyond 6 cm far from the scalp may be impossible to give a valid retrospective record over periods longer than 6 months. Consequently, it was necessary to explore the environmental factors which gave rise to the hair cortisol loss.

There were relatively limited researches about external factors influencing hair cortisol content [12–14]. These factors included hair dyeing [12,14], repeatedly washing with shampoo and/or water [13], hair bleaching, permanent waving or straightening [14] and use of hair products (spray, mousse, gel and wax) [14], all of which reduced hair cortisol content by different extents. Few researches concerned for the effect of ultraviolet irradiation and successive immersion in hot water on hair cortisol content and focused on time course of hair cortisol change under long-term successive exposure to hot water. Additionally, Sauve et al. found that there was large variation in cortisol content of hair samples cut from posterior vertex, nape, temporal, anterior vertex and frontal sections of the head in 14 human participants [12]. Unfortunately, their study found that there was no significant difference in hair cortisol content among the 5 head locations [12]. Therefore, our present study aims to validate the location-dependence of hair cortisol content in human. Then we investigated the effect on hair cortisol of UV irradiation, and successive immersion in shampoo and hot water, which simulated natural environmental factors and human life habits. Finally

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we focused on the time course of hair cortisol loss in long-term immersion in hot water.

In the present study, 30 participants were recruited to examine statistically significant difference in hair cortisol content among 5 head locations because 14 samples in the study of Sauve et al. [12] might be too small. We predicted that there was significant difference among hair cortisol contents in various locations of head. Since Hamel et al. had studied in detail the effect of short-time shampoo washing and water alone on hair cortisol content [13], we designed a long-time shampoo washing to replicate the washing out effect. It was reported that cannabinoids in hair samples were lost in the exposure to sunlight [15], indicating that light or UV irradiation might destroy cuticle structure of hair due to its strong degradation ability. Immersion in water solution damaged hair structure [16], resulting in hair cortisol loss [13]. We predicted that UV irradiation and immersion in hot water would significantly reduce hair cortisol content.

2. Materials and methods

2.1. Chemicals and solution preparation

HPLC grade acetone, hexane and methanol (MeOH) were from Dikma Lake Forest, CA. HPLC grade formic acid (HCOOH) and analytical grade ammonium formate were from Tedia, Fairfield, OH. Analytical grade Cortisol was from National Institutes for Food and Drug Control, China, and deuterated cortisol (cortisol-9, 11, 12, 12-d4) was from Isotec, Sigma Aldrich, St. Louis. MO. Water used throughout the experiments was triple-distilled deionized water. Solid phase extraction (SPE) C18 columns were from Dikma.

Cortisol solutions were prepared in MeOH at a final concentration of 1, 5, 10, 20, 50, 80 and 100 ng/ml. Deuterated cortisol was prepared in MeOH at a final concentration of 50 ng/ml. The mobile phase was methanol and deionized water containing 0.1% formic acid (8:2/v:v) which was filtered through micro porous membrane (0.22 μm) prior to use.

2.2. Study participants and hair collection

30 male healthy adolescents (Group A, mean age ± SD: 16.2 ± 1.2) were recruited for investigating the location-dependence of hair cortisol content and another 28 male healthy participants (Group B, S01–S03, mean age ± SD: 27.3 ± 2.5; S01–S12, 26.1 ± 11.4; S13–S20, 24.3 ± 16.2; S21–S28, 24.9 ± 0.9) for investigating the effect on hair cortisol content of shampoo washing, UV irradiation and immersion duration in hot water and water temperature. Participants with dyed or perm or bleached or shorter (<1 cm) hairs were excluded. All participants provided written informed consent before inclusion. The Health Science Research Ethics Board of Southeast University approved the study.

Hair samples with longer than 1 cm were cut as close as possible to the scalp with iron scissors. Hair samples in Group A were cut from left section, right section, vertex, anterior vertex and posterior vertex of head and in Group B were from the posterior vertex region. As-collected hair strands were stored in dry tubes at −20 °C for cortisol analysis. The hair samples were cut as 1-cm hair segments prior to analysis. The 1-cm hair segment closest to the scalp was used in the present study.

2.3. Hair treatments

After being washed with 5 ml methanol for 2 min at room temperature and dried in N_2, hair sample (250 mg) was treated with shampoo solution, or UV irradiation or hot water, respectively. The methanol washing procedure aimed to remove the contaminations on the outer surface of hair strands.

For the shampoo washing, shampoo (Rejoice™) concentration was 10% by weight in water. The hair samples from three individuals (S01–S03) were immersed in 10% shampoo solution at room temperature for 4 h, and then rinsed with pure water for three times to remove all shampoo solution adsorbed in the surface of hair and dried with nitrogen.

For UV irradiation, wavelength of UV lamp with 680 W was 254 nm. The hair samples from 12 individuals (S01–S12) were located 45 cm far from UV lamp and were irradiated under a luminosity of 300 lx for 9 h in room temperature and then rinsed with water and dried with nitrogen.

For hot water conditions, the hair samples from 8 individuals (S13–S20 or S21–S28) were immersed in hot water at 40, 65 and 80 °C for the desired duration, and then rinsed with water and dried with nitrogen. At each of the specified time points, the portions of the same hair sample were removed from the washing solution, and then rinsed with water and dried with nitrogen.

2.4. Hair cortisol analysis

After the treatments were done, as-treated or untreated hair samples were washed twice with methanol, dried with nitrogen and pulverized in the ball mill. The repeated methanol washing procedures aimed to completely remove the contaminations and non blood-borne cortisol coated on the outer surface of hair strands. A 20-mg amount of the powdered hair was incubated at 40 °C for 24 h in 1 ml methanol in the presence of 2.5 ng cortisol-d_4 as internal standard (I.S.). The incubation medium was separated by centrifugation at 12,000 rpm for 10 min and the supernatant was transferred to a dry tube and then evaporated to dryness by pure nitrogen.

The extraction in the dry tube was resuspended with 50 μl methanol and 1 ml water, and then transferred to SPE C_{18} column which was activated with 3 ml methanol and washed with 3 ml deionized water prior to use. The deposited on the SPE C_{18} column was rinsed with a sequence of 1 ml 2:8 (v/v) acetone/deionized water, 1 ml deionized water, and 1 ml hexane, and then dried for 30 min and eluted successively with 0.8 ml methanol three times. The eluate finally obtained was evaporated to dryness and resuspended in 50 μl mobile phase for LC/MS/MS analysis.

A 5 μl volume of the resuspended extract was injected onto a 150 mm × 4.6 mm 5 μm C18 analytical column (Zorbaxtm Eclipse XDB-C18, Agilent Technologies, Waldbronn Germany) protected by a 10 mm × 4.6 mm 5 μm C18 finger tight guard cartridge (Opti-Guard, Agilent). The chromatographic separation was operated on Agilent 1200 HPLC system (Agilent). The flow-rate was 200 μl/min and the column temperature was 30 °C.

Detection was performed in a 3200 QTRAP liquid chromatography tandem mass spectrometer (ABI, Foster City CA) equipped with an electrospray ionization (ESI) source. Nitrogen (99.999%) was employed as nebulizing gas. The optimum ionization of cortisol and cortisol-d_4 was achieved in negative mode at ion-spray voltage of −4500 V using the parameters as listed in Table 1.

The symmetrical heaters were operated at 400 °C with curtain gas at 10.0 psi, ion source gas (gas 1) at 40.0 psi, gas 2 at 40 psi and collision gas for collision-activated dissociation at Medium. Q1 conditions were optimized for the deprotonated molecular ion ($[M+HCOO]^-$) of both compounds. Ions were examined in multiple reaction-monitoring mode, observing the transitions of the mass-to-charge ratio from 407.20 to 331.2 for cortisol and 411.20 to 335.20 for

Table 1
Optimum ionization and fragmentation conditions for cortisol and cortisol-d_4.

	Precursor ion (Da)	Product ion (Da)	DP (V)	EP(V)	CEP (V)	CE (V)	CXP (V)
Cortisol	407.20	331.20	−32.78	−4.71	−26.72	−27.18	−2.15
Cortisol-d4	411.20	335.20	−39.26	−4.90	−21.33	−25.02	−3.54

DP: declustering potential, EP: entrance potential, CEP: collision cell entrance potential, CE: collision energy and CXP: collision cell exit potential.