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Short communication

Effects of shampoo and water washing on hair cortisol concentrations

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ABSTRACT

Background: Measurement of cortisol in hair is an emerging biomarker for chronic stress in human and nonhuman primates. Currently unknown, however, is the extent of potential cortisol loss from hair that has been repeatedly exposed to shampoo and/or water.
Methods: Pooled hair samples from 20 rhesus monkeys were subjected to five treatment conditions: 10, 20, or 30 shampoo washes, 20 water-only washes, or a no-wash control. For each wash, hair was exposed to a dilute shampoo solution or tap water for 45 s, rinsed 4 times with tap water, and rapidly dried. Samples were then processed for cortisol extraction and analysis using previously published methods.
Results: Hair cortisol levels were significantly reduced by washing, with an inverse relationship between number of shampoo washes and the cortisol concentration. This effect was mainly due to water exposure, as cortisol levels following 20 water-only washes were similar to those following 20 shampoo treatments.
Conclusions: Repeated exposure to water with or without shampoo appears to leach cortisol from hair, yielding values that underestimate the amount of chronic hormone deposition within the shaft. Collecting samples proximal to the scalp and obtaining hair washing frequency data may be valuable when conducting human hair cortisol studies.

1. Introduction

Assessment of hypothalamic–pituitary–adrenocortical (HPA) axis activity in both humans and animals commonly involves sampling of blood or saliva. Measures from these fluid compartments reflect short-term assessments and are subject to the potentially confounding influences of circadian variation and in some cases, the stress of the sample collection procedure. Although measurement of corticosteroid excretion in urine and feces obviates some of these problems, such approaches still restrict investigators to a time frame of around 24 h or less.

Recent research supports the possibility that measurement of cortisol in hair can provide an integrated assessment of chronic HPA axis activity over a period of months that is not subject to the influences of circadian variation or the stress of sample collection [1,2]. Validation of this approach has been provided by studies in our laboratory conducted using rhesus monkey hair [3]. These studies showed that (1) the material measured in monkey hair reacted identically to authentic cortisol in an enzyme immunoassay (EIA), (2) the large majority of the immunoreactive cortisol in hair resisted removal by brief solvent washes, suggesting that most of the cortisol was embedded in the hair shaft rather than coating the outside of the shaft (e.g., deposited on the surface from dried sweat), (3) cortisol extraction from the interior of the hair was maximized by pulverizing the samples using a ball mill, and (4) cortisol levels did not vary systematically as a function of location on the hair shaft (distal vs. proximal to the scalp) in animals maintained under standard colony conditions (i.e., without the imposition of a stress manipulation).

Additional validation of hair as a suitable matrix for assessing long-term changes in HPA activity comes from the demonstration that hair cortisol levels are elevated after exposure to a stressor or other conditions associated with elevated circulating cortisol concentrations. For example, we recently found that rhesus monkeys subjected to a mandatory relocation had increased hair cortisol following the move [4]. In human subjects, hair cortisol levels have been shown to be sensitive to employment status [5], chronic pain [6], and pregnancy [7].

Although measuring cortisol in hair has great potential for assessing long-term HPA activity noninvasively, the fact that hair shafts are continuously exposed to the external environment raises

Abbreviations: HPA, Hypothalamic–pituitary–adrenal axis; EIA, Enzyme immunoassay; NICHD, National Institute of Child Health and Development; NIH, National Institutes of Health; ANOVA, Analysis of variance; SEM, Standard error of the mean.

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some concern. Indeed, two recent segmental analyses that examined either 1- or 3-cm long segments of human hair both demonstrated that cortisol concentration decreased as a function of distance from the scalp [7,8]. This finding is in contrast to the lack of a segmental effect in rhesus monkeys maintained under constant conditions. It is possible that the segmental effect observed in human studies is, at least in part, caused by repeated washing of the hair and possibly also by the use of styling products and/or dyes. However, there are no previous reports on the effects of hair washing on cortisol concentrations under controlled conditions. Therefore, the present study was designed to investigate the effects on cortisol levels of repeatedly exposing hair samples to a shampoo solution or to water alone.

Because of the obvious difficulty inherent in obtaining adequate lengths of human hair that had never been washed, we chose to perform the study with rhesus monkey hair. Macaques are a reasonable model for demonstrating the effects of washing on cortisol concentrations due to the known similarities between macaque and human hair [9]. For example, both types of hair show a mosaic growth pattern characterized by independence of phase across nearby follicles and the tendency of follicles to grow in groups of 3 (range 2–7). Furthermore, daily hair growth rates are about the same (~0.37 mm in humans vs. 0.33–0.38 mm in macaques). We hypothesized that the findings of the previous segmental analyses [7,8] were, at least in part, due to repeated hair washing that caused cortisol to leach from the hair shaft. Consequently, we predicted that hair cortisol content would decline systematically as a function of the number of washes with a shampoo-containing solution. In addition, we predicted that washing with water alone would also significantly reduce hair cortisol content because of the known ability of water to causing swelling of the hair shaft [10].

2. Materials and methods

2.1. Animals

Hair was obtained during routine health exams from 18 adult female and 2 adult male rhesus monkeys (*Macaca mulatta*) ranging in age from 8 to 27 y (mean = 14.5 y) (Table 1). The monkeys were maintained in a large 5-acre outdoor enclosure containing several corncrib structures and an attached indoor housing environment at the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development Laboratory of Comparative Ethology in Poolesville, Maryland. The monkeys' diet consisted of Purina Monkey Chow supplemented with fresh fruits and vegetables. Water was available *ad libitum*. The research described in this report was approved by the NICHD Animal Care and Use Committee, performed in accordance with the NIH Guide and Use of Laboratory Animals, and complied with the Animal Welfare Act.

2.2. Sample collection and pooling

Hair was gently shaved from the nape of the neck using the collection procedure described by Davenport et al. [3]. Samples were collected in February of 2008, placed in aluminum foil packets, and shipped to the University of Massachusetts Amherst where they were maintained at 4 °C or colder. These particular hair samples were not collected for the purposes of this research; however, there was a sufficient amount of hair left over from previous studies for use in our experiment. The hair sample remnants were combined to create four separate pools, each consisting of hair from five different monkeys (Table 1). Hair pooling was done in order to (1) provide a sufficient sample size for all treatment conditions to be performed on the same material (i.e., pool), and (2) constitute multiple replicates (each hair pool was considered to be one replicate) of each wash condition. The pools were balanced as closely as possible for both age and gender. In order to ensure that each pool was thoroughly mixed, hair from the five monkeys was combined and gently combed to distribute the different samples. Next, each pool was subdivided into five different treatment conditions: a no treatment control condition, a 20 water-only wash condition, and conditions involving 10, 20, or 30 washes with an aqueous shampoo solution.

Table 1
Identification number, age, and sex of monkeys assigned to each pool and mean age of animals in each pool.

	Animal ID	Age (y)	Sex
Pool 1			
	23	27.0	F
	T27	8.8	F
	39C	18.8	F
	R32	9.8	F
	R47	9.7	F
		Mean = 14.8 y	
Pool 2			
	27B	21.8	M
	T19	8.8	F
	43C	17.8	F
	R27	9.8	F
	I31	13.6	F
		Mean = 14.4 y	
Pool 3			
	34C	20.8	F
	R49	9.6	M
	D17	16.8	F
	P11	10.8	F
	K13	12.8	F
		Mean = 14.2 y	
Pool 4			
	37C	18.8	F
	D43	16.6	F
	P02	10.9	F
	G43	14.6	F
	M05	11.8	F
		Mean = 14.5 y	

2.3. Hair treatments

Pooled hair samples weighing approximately 250 mg were placed in disposable 15-ml screw-cap plastic culture tubes. For the shampoo conditions, each treatment began with an addition of 10 ml of a room-temperature solution of Pantene Pro-V®, a popular over-the-counter human shampoo, dissolved in tap water. The shampoo concentration was 10%, which is commonly used in shampoo testing studies (e.g., see http://www.dsm.com/en_US/downloads/dnpsa/D_Panthenol.pdf). The tube was then capped and the sample was gently washed by repeated inversion for 45 s. This exposure time was determined by an informal survey of female acquaintances of one of the authors (AFH) in which the participants were asked to estimate how much time their hair was in contact with the shampoo solution when they washed their hair. The shampoo solution was then decanted, after which 10 ml of room-temperature tap water was added and the tube was recapped and gently inverted for 30 s before being decanted again. This rinse procedure was repeated three additional times for a total of four post-wash rinses. Four rinses were found to be necessary to remove all visual traces of the soapy shampoo solution from the sample. Finally, the samples were thoroughly blow-dried while still in the tube using a standard hair drier (low heat setting) for no longer than 5 min or until dry. Blow drying of the samples was carried out because this method was frequently used by humans, especially women, to dry their hair after washing. This cycle of washing, rinsing, and blow-drying was performed a total of 10, 20, or 30 times, according to the treatment condition to which each sample was assigned. For the water-only condition, the procedures were identical except that 10 ml of tap water was substituted for the 10% shampoo solution at each wash step. The water-only condition was performed with 20 washes in order to provide a direct comparison to the 20 shampoo wash condition.