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The Effect of Body Region on Hair Cortisol Concentration in Common Marmosets (*Callithrix jacchus*)

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Common marmosets (*Callithrix jacchus*) are a valuable research model for the study of neuroscience and the biologic impact of aging due to their adaptivity, physiologic characteristics, and ease of handling for experimental manipulations. Quantification of cortisol in hair provides a noninvasive, retrospective biomarker of hypothalamics-pituitary-adrenal (HPA) axis activity and information on animal wellbeing, including responses to environmental and social stimuli. To obtain valid and reliable measurements of long-term HPA activity, we investigated the variability of cortisol concentration in the hair depending on the body region of marmosets. Hair was collected from the back and tail of 9 adult common marmosets during annual health screenings (male *n* = 3; female *n* = 6) and these samples were analyzed for cortisol via methanol extraction and enzyme immunoassay. We found that hair cortisol concentration differed between the tail and back regions, with the tail samples having a significantly higher cortisol concentration. These results indicate intraindividual and interindividual comparisons of hair cortisol concentration should use hair obtained from the same body region in marmosets.

Abbreviations: HCC, hair cortisol concentration; HPA, hypothalamic-pituitary-adrenal

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Common marmosets (*Callithrix jacchus*) are small, New World Primates endemic to Northeast Brazil. Marmosets are similar to humans in their physiology, neuroanatomy, cognition, and sociality.[11,16] They have become an important model in behavioral and biomedical research, including studies of the physiologic effects of aging and neurologic disease. Furthermore, their use in studies of aging or development includes their frequent use in longitudinal studies. Therefore, the ability to frequently and noninvasively measure glucocorticoid hormones as a marker of the response to environmental or social stimuli is an important aspect of longitudinal research in this species.

Glucocorticoids are regulated by the hypothalamic-pituitary-adrenal (HPA) axis, a complex neural negative-feedback system that perceives environmental stimuli and regulates hormones accordingly. Cortisol is the dominant glucocorticoid hormone found in most mammals[25] and is involved in several physiologic processes, such as metabolizing sugar, fat, and protein stores into usable energy and the inhibition of swelling and inflammation.[21] Quantification of cortisol concentration can therefore provide valuable insight into how an animal is responding to its environment or to an experimental manipulation and can also provide a valid, retrospective biomarker of HPA axis activity and individual health.[4,5,14,26]

Cortisol can be measured in blood, saliva, urine, feces, and hair. These tissue types differ in terms of the time frame reflected in the sample and the invasiveness of sample collection.[22] Different tissues reflect different intervals with regard to when the cortisol was secreted from the adrenal cortex. Blood and saliva are considered to provide point measures because they indicate a time frame of minutes since the cortisol was secreted.[22] Urine and feces are considered to be state measures; they reflect a time frame from hours to a day.[22] Finally, hair incorporates a chronic time frame of weeks or months.[22] Long-term HPA axis activity can be quantified retrospectively from hair, which accumulates its cortisol concentration over weeks or months, depending upon the length of hair analyzed[22] and the growth dynamics of the hair.[20] Blood-born substances, such as cortisol, diffuse from blood capillaries into hair follicle cells.[7,27] Only cortisol molecules that are not bound to proteins diffuse into blood capillaries; therefore, hair cortisol concentration (HCC) represents unbound cortisol molecules.[7,27] Once cortisol molecules enter the follicle cells, they are deposited into the hair shaft.[7] The cortisol deposition process only occurs at a specific time during hair growth, and cortisol deposition into the hair shaft over time is directly proportional to blood cortisol concentration.[7]

The stage of growth of a hair follicle influences the cortisol deposition process. All hair has 3 stages of growth: the anagen, catagen, and telogen. The anagen is the active stage when growth occurs; in the catagen or intermediate stage, old hair shafts break down and newer shafts are produced in preparation for the next round of growth; lastly, the telogen stage is the resting phase of hair growth. Most cut or plucked hair samples are expected to be in the anagen phase, while shed hair is likely in the telogen phase.[13] Cortisol deposition occurs during the anagen stage as active hair follicles require nutrients via the

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bloodstream to grow. This slow rate of deposition provides information on HPA activity over weeks or months.[22]

While hair cortisol concentration is increasingly used to assess retrospective HPA axis activity in captive and wild animals, a standard body region from which to obtain hair samples has not been determined. Studies report sampling from various regions, including the back of the neck, back, shoulder, chest, the base of tail, thigh, deltoid, and interscapular regions.[1,7-9] In this investigation, we sought to determine if HCC was affected by the body region from which the samples were taken in common marmosets. We compared HCC from 2 common and readily accessible locations of sample collection in common marmosets, the back and the tail.

Materials and Methods

Subjects. We obtained hair samples from 2 juvenile (1 y) and 7 adult (3 to 9 y) common marmosets (*Callithrix jacchus*; male $n = 3$, female $n = 6$) housed at the Southwest National Primate Research Center, TX Biomedical Research Institute, San Antonio, TX, USA. These opportunistic samples were obtained from animals as they underwent health screenings before being assigned to other research protocols at the Center. All animals were deemed healthy. Juveniles were housed with their family groups at the time of sample collection; adults were nonbreeding and either pair-housed or single-housed for behavioral reasons. Animals were housed in one of two types of custom aluminum caging used at the SNPRC, either a group cage (SNPRC rectangular custom) or smaller custom aluminum caging for pairs and singly-housed individuals (SNPRC single custom).[18] All animals were housed in a manner that allowed visual, olfactory, and auditory access to other marmosets. Marmosets were maintained in accordance with the *Guide for the Care and Use of Laboratory Animals*.[15] This facility is AAALAC-accredited, and all work was approved by the Texas Biomedical Research Institute's Institutional Animal Care and Use Committee. Room temperatures maintained at 80 °F (range 76 °F to 84 °F) and a 12-h light-dark cycle. Fresh food was available ad libitum and consisted of a purified diet (Harlan Teklad TD130059 PWD), Mazuri diet (AVP Callitrichid 5LK6), supplemented with fresh fruit, seeds, and dairy. Further husbandry specifics followed methods outlined elsewhere.[19]

Sample collection procedure. Veterinary staff collected hair samples from the back and tail region (approximately 100 g from each location; see Figure 1) of all subjects during a scheduled health screening. All animals were sedated for this exam. An electric shaver was used, with care taken not to nick the skin and contaminate the sample with blood. Hair was collected from the back (between the shoulder blades), then from the tail (8 cm from the base). The electric shaver was cleaned with disinfectant solution after each use. After collection, samples were stored out of direct sunlight in 15 mL screw-cap polypropylene centrifuge tubes until processing.

The procedure for extracting cortisol from hair followed a previously published report[24] that was based on a procedure detailed by others.[23] Processed samples were washed 3 times in HPLC-grade isopropanol, and then air-dried under a hood for 3 to 5 d. Once dry, samples were ground into a fine powder with a ball mill (MM400; Retsch, Newton, PA). Fifty milligrams of each sample was then placed in a 2 mL microcentrifuge tube for methanol extraction. The ground hair was incubated while gently rotating in 1.0 mL of HPLC-grade methanol for 24 h. Samples were immediately centrifuged for 2 min at 14,000 rpm12,000 x g to pellet the powdered hair. Next, we evaporated 600 µL of the sample supernatant for 45 min using a nitrogen evaporator.

Figure 1. Illustration depicting where the hair was sampled from the back and tail from marmosets.

Samples were then reconstituted with 200 µL of assay buffer and diluted 1:40 before being analyzed, in duplicate using a commercially available enzyme immunoassay (EIA) available expanded high range sensitivity for salivary cortisol EIA kit (#1 to 3002; Salimetrics; State College, PA). Resulting values (expressed as µg/dL) were converted into pg/mg for analysis.

Statistical Analysis. Intraassay coefficient of variation (CV) was 4%, whereas the interassay CV was 8.2%. Details of the parallelism tests for validating the procedure for common marmosets were described in detail previously by others.[24] We performed the Shapiro–Wilk test of normality for HCC values obtained from the back and tail before statistical analysis. We conducted a paired-samples *t* test, comparing HCC from the back and tail regions in marmosets. We also performed correlational analysis on the HCC values from the back and the tail. Alpha was set at 0.05 and all analyses were performed using RStudio.[28]

Results

The range of obtained HCC in this study is shown in Table 1. All subjects had a cortisol concentration that was lower for hair obtained from the back than from the tail. The Shapiro–Wilk test indicated HCC values for both back and tail were normally distributed ($HCC_{BACK,}$ D(9) =0.958, $P = 0.78$; $HCC_{TAIL,}$ D(9) =0.962, $P = 0.816$). Visual inspection of the Q-Q plots confirmed

Table 1. Hair cortisol concentration in the back and tail regions, from 9 common marmosets.

Sex	Age (yrs)	Back (pg/mg)	Tail (pg/mg)
F	1	3894	7937
F	1	3228	7974
F	3	2714	7655
F	5	5425	7289
F	5	4304	7766
F	8	5206	8826
M	4	3092	6773
M	4	4719	6908
M	7	4120	6505

normality. A paired-samples *t* test confirmed HCC was significantly different between the back and tail regions ($t(8) = 9.43$, $P < 0.001$), with the cortisol concentration determined from back hair being significantly lower than cortisol concentration obtained from tail hair ($M_{BACK} = 4078 \pm SE_{BACK} = 315$ pg/mg; $M_{TAIL} = 7515 \pm 241$ pg/mg). In addition, there was no overlap between HCC from the back and tail; the highest HCC from the back was still lower than the lowest tail HCC score (Figure 2). HCC values from the back and tail were not significantly correlated ($r(7) = 0.16$, $P = 0.68$).

We did not conduct statistical tests to determine whether HCC differed in male and female marmosets as a function of body location, as the sample of males ($n = 3$) was too small to fulfill requirements for nonparametric testing. We did also not conduct statistical tests to determine if HCC varies as a function of age (juvenile or adult), as the sample had only 2 juveniles.

Discussion

HCC varied by body region in common marmosets, with samples obtained from the back providing significantly lower values than samples obtained from the tail. These results are consistent with previous research on chimpanzees (*Pan troglodytes*), yellow baboons (*Papio cynocephalus*), marmots (*Marmota vancouverensis*), caribou (*Rangifer tarandus granti*), reindeer (*R. t. tarandus*), and grizzly bears (*Usrus arctos*), which also reported significant differences in HCC as a function of body location.[2,3,9,20,29]

Two possible reasons for cortisol variability by body location are regional variation in hair growth and regional differences in vascularization. One group[20] considered how timing and patterns of hair growth may lead to varying HCC between body regions in grizzly bears. In this species, molting patterns likely played a role in HCC variation by body region, with higher HCC in regions that were preparing to molt.[20] The authors suggested that new hair grew in the neck region before the grizzly bear actually molted in the neck.[20] However, seasonal shedding is not the only determinant of hair growth. While marmosets do not molt like grizzly bears, the molting in grizzly bears highlights that elevated HCC levels are likely linked to increased hair growth. We theorize the differences found in the present study are due to the timing and pattern of hair growth in marmosets. Hair growth only occurs in the anagen stage, and this is the only hair stage in which cortisol deposition occurs.[22] Therefore, our data indicate differences in hair stages by body region. Furthermore, we speculate that the tail hair has a slower rate of growth, thereby providing a longer opportunity for cortisol deposition into the hair. Along with hair growth patterns, researchers have theorized that blood flow differences also contribute to varied HCC by body region.[1,3] Cortisol is deposited into the hair shaft in proportion to the unbound cortisol in the blood.[7] If blood flow varies by body region, then unbound cortisol levels in the blood will vary as a result, affecting HCC.

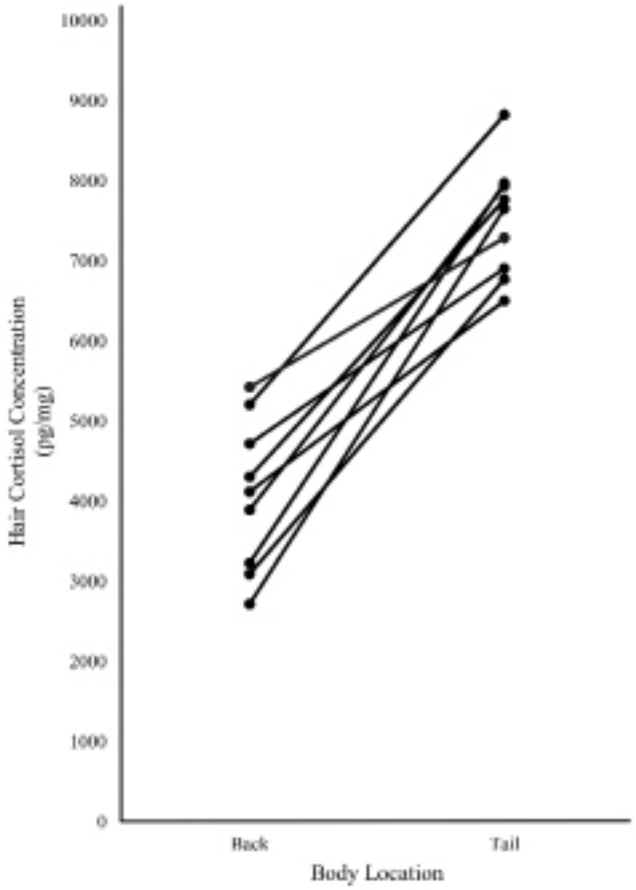

Figure 2. Hair cortisol concentration (HCC) from the back and tail regions in common marmosets. ($M_{BACK} = 4078 \pm 315$ pg/mg; $M_{TAIL} = 7515 \pm 241$ pg/mg)

Cortisol levels in New World monkeys are 2 to 10 times higher than Old World monkeys.[6] This difference is also reflected in hair cortisol and may be related to the phenomenon of glucocorticoid resistance found in some New World monkeys.[6] Glucocorticoid resistance is the result of species-variation in glucocorticoid receptor binding affinity and receptor signaling.[6] The HCC values obtained in this study are similar to those found in other studies that used the same assay protocol.[10,24] One group[24] compared marmoset HCC values across published studies and found that variation in assay procedure corresponded to differences in obtained values.

Our results, combined with previous work,[23,30] highlight the importance of standardizing assay methodology and sample collection procedures, both within and across laboratories. Specifically, we found that intraindividual and interindividual comparisons of hair cortisol must use hair obtained from the same body region. Doing so will allow for greater reliability of any comparison between HCC data for longitudinal investigations or when comparing HCC in marmosets across treatment conditions.

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