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Developmental patterns of hair cortisol in male and female nonhuman primates: Lower hair cortisol levels in vervet males emerge at puberty

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Abstract

Studies have yielded inconsistent results with regard to effects of age and sex on short term markers of hypothalamic pituitary adrenal (HPA) activity. Hair cortisol provides a retrospective proxy measure of the cumulative activity of the HPA axis over the preceding 3–4 month period. In order to describe potential developmental trends in this biomarker, we assessed hair cortisol levels between 1–12 years of age in a cross-sectional study of 350 vervets (222 females and 128 males). Monkeys were grouped according to age as 1 (young juvenile), 2 (juvenile), 3 (early adolescent), 4 (late adolescent-young adult), and 5–12 (adult) years of age such that fully mature animals were included in the 5–12 year old age group. We observed that hair cortisol level was higher among the younger monkeys and declined with age ($p < .001$). More importantly the effect of age significantly interacted with sex ($p = .02$), such that hair cortisol was consistently lower in males than females beginning at age 3 ($p < .05$ or better). The developmental decline began one year earlier in females than males suggesting an influence of the earlier maturational processes typical in both human and nonhuman primates. The advantage of lower cortisol levels in the males may be related to social group patterns of male emigration during adolescence in many nonhuman primate species.

Keywords

development; sex differences; hypothalamic adrenal pituitary adrenal axis; allostatic load; *Chlorocebus aethiops sabaeus*

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Contributors Mark L. Laudenslager and Lynn A. Fairbanks designed the study and analyzed the data collaboratively. Matthew J. Jorgensen managed hair collection, maintained the colony data base, and contributed significantly to the manuscript. Mark L. Laudenslager developed the hair cortisol assay in his laboratory and was responsible for overseeing the analysis of hair cortisol for the study. All authors have approved the final manuscript.

Conflict of Interest We have none to report.

1. Introduction

Following a seminal review by Kirschbaum & Hellhammer (1989) pointing to the utility of cortisol measured in saliva as a meaningful and reliable approach to noninvasive assessments of the activity of the hypothalamic pituitary adrenal (HPA) axis, there was an exponential increase in publications applying this approach to acute measurement of HPA activity (Laudenslager, Bettinger, & Sackett, 2005). Salivary cortisol reflects a snapshot of a dynamic, changing system affected by acute stress reactivity as well as by underlying diurnal (Hellhammer, Wust, & Kudielka, 2009) and pulsatile (Young, Carlson, & Brown, 2001) patterns. Current views suggest that cortisol may contribute to disrupted health and well-being via prolonged elevation of cortisol levels which fail to return to basal levels, often referred to as allostatic load (Lupien, McEwen, Gunnar, & Heim, 2009). Salivary measures may fall short when documenting the long term status of the HPA.

Hair cortisol has been hypothesized to provide a retrospective marker of cumulative activation of the HPA in nonhuman primates (Davenport, Tiefenbacher, Lutz, Novak, & Meyer, 2006). We have shown that social group relocation is associated with an increase in hair cortisol levels in adult female vervets (Fairbanks, Jorgensen, Bailey, Breidenthal, Grzywa, & Laudenslager, 2011) and hair cortisol is further related to behavioral phenotypes (Laudenslager, Jorgensen, Grzywa, & Fairbanks, 2011). The present study investigates age and sex differences in hair cortisol under stable conditions in a colony of vervets. While results are mixed, numerous human and animal studies have shown sex differences in HPA regulation appearing at puberty (Kudielka & Kirschbaum, 2005; McCormick & Mathews, 2007; Stroud et al., 2011). Emergence of sex differences in reproductive hormones at puberty may play a role, as testosterone and other androgens have been shown to suppress CRH-stimulated HPA activity in human and nonhuman primate males (Rubinow, Roca, Schmidt, Danaceau, Putnam, & Cizza, 2005; Toufexis & Wilson, 2012).

In this study, we compared female to male hair cortisol in vervets under stable conditions from the early juvenile period to full adulthood in a cross sectional study. As there are developmental and age related changes in HPA activity (Lupien, et al., 2009), we expected that there would be age dependent differences in hair cortisol which were influenced by sex.

2. Methods

2.1 Subjects

Subjects were 350 captive vervet monkeys (*Chlorocebus aethiops sabaeus*) ranging in age from 1 to 12 years of age living in stable social groups as previously described (Laudenslager et al., 2011). Offspring were raised by their mothers and female offspring remained with their mothers and female kin. Males were transferred at adolescence to male peer groups or introduced into breeding groups as adults. The sample included 128 males (mean age = 3.84 ± 2.76 years) and 222 females (mean age = 5.68 ± 3.22 years).

The animal facility was fully accredited by the American Association for the Accreditation of Laboratory Animal Care (AAALAC) in accordance with the Guide for Care and Use of Laboratory Animals (NIH, 1996) and Psychological Well-Being of Nonhuman Primates (ILAR, 1998) (Fairbanks et al., 2011). All relevant procedures were approved by the Institutional Animal Care and Use Committees.

2.2 Hair cortisol collection and assay

Hair collection took place when the animals were anesthetized for their annual veterinary examination between December 2007 and January 2008. Samples were collected during

mating season when most of the post-pubescent females would be cycling. Only 2 females were pregnant and cortisol values did not differ from the rest of the sample. Hair (average length 5.6 ± 0.7 cm) from each subject was collected from the intra-scapular region, taking care not to damage the skin (Laudenslager, et al., 2011). Hair cortisol analysis was performed as previously described (Davenport et al., 2006; Laudenslager, et al., 2011). Hair cortisol assays included a pooled hair control which was extracted and measured as part of each assay. Inter assay CV was 4.23% and intra assay CV was 2.36%.

2.3 Statistical analysis

A Kolmogorov-Smirnov test indicated that hair cortisol levels deviated significantly from normality ($S-K = .064$, $df = 350$, $p = .002$). After performing a natural log transformation, the hair cortisol levels did not deviate from normality ($S-K = .034$, $df = 350$, $p = .20$). Thus natural log transformed values were used in analyses. Untransformed values are presented in the figure for ease of comparison with other published data.

Effects of age and sex were tested using a cross-sectional life stage approach. Subjects were classified into ages 1, 2, 3, 4, and 5-12 years of age. Mean age in years (range) for these groupings were 1.35 (1.08 – 1.49, $n=41$), 2.40 (2.07 – 2.99, $n=55$), 3.42 (3.0 – 3.64, $n=48$), 4.45 (4.22-4.78, $n=32$), and 8.08 (5.06 – 12.76, $n=174$), respectfully. Estrous cycling begins between 2-3 years; females are fully grown by age 4-5 and have adult dentition by age 5-6. Males show an increase in testosterone levels indicating puberty between 3-4 years and are fully grown and sexually mature at age 5. At the time of sample collection, all juveniles and adolescents were living in their natal social group. The adult males (age 5-12) were removed from their natal group and living in all male groups ($n= 27$) or as breeding males in the matrilineal groups ($n=18$).

The between subjects effects of Sex and Age (5 groups) were tested by analysis of variance with post hoc contrasts for sex differences between age groups and for age difference with sex. Effects of housing for adolescent and adult males were assessed using one-way analysis of variance.

3. Results

Figure 1 shows the mean ($\pm$ SEM) hair cortisol levels for males and females across the five age groups. Hair cortisol declined significantly across age groups ($F_{(4, 340)} = 9.983$, $p < .001$). Although the effect of sex was significant ($F_{(1, 340)} = 12.22$, $p = .001$), it interacted significantly with age group ($F_{(4,340)} = 2.877$, $p = .023$). Post hoc contrasts indicated significant sex differences at age groupings 3, 4, and 5-12 but not for 1 or 2 years of age. Since management of males included transfer from natal groups to all male peer groupings or subsequently to breeding groups, we tested differences between the males based on housing. Among the males, hair cortisol did not differ between natal group adolescents (age 3: 43.4 ± 1.5 pg/mg), all male groups (ages 5-7: 45.2 ± 2.9 pg/mg), and breeding adults (ages 6-12: 46.7 ± 2.9 pg/mg) (One Way ANOVA $F_{(2,73)} = .34$, NS).

The decrease in hair cortisol by age group occurred between different age groups based on sex which suggested earlier maturation in females. Females showed a significant decline from the prior year between age groups 1 and 2 (post hoc pairwise comparison, $p < .005$) after which levels did not differ significantly from the prior year. A similar decrease in males occurred a year later between age groups 2 and 3 (post hoc comparison, $p < .001$) after which hair cortisol level did not differ from the previous year.

4. Discussion

The present study identified differences in hair cortisol as a function of sex and age in a large stable population of vervets providing further support for the utility of hair cortisol as a marker of HPA activity. This is the largest nonhuman primate study of a single species (n=350) to date, including males and females from early juvenile period to middle adulthood in which hair cortisol served as a primary measure. The decrease in hair cortisol with age found for immature vervets is consistent with observations from other nonhuman primate studies where glucocorticoid levels are elevated in infancy and decline to puberty, whether measured in plasma, feces or hair (Pryce, Palme & Feldon, 2002; Gesquiere et al., 2005; Fourie & Bernstein, 2011).

This study also revealed significant sex differences that emerge at puberty and continue to adulthood, with vervet females having higher levels of hair cortisol than males when collected in a stable environment. Human studies spanning the period of pre and early puberty, adolescence and early adulthood with regard to stress reactivity and interactions with sex are rare but mixed with regard to sex differences (Kudielka & Kirschbaum, 2005). Several studies have shown that adolescent and young adult males have a greater cortisol response to acute psychosocial stress compared to females (Kudielka & Kirschbaum, 2005), but beginning at puberty, girls and women have higher morning cortisol levels and take longer to return to baseline after challenge (Stroud et al., 2011). One recent study reported *higher* levels of hair cortisol in men, but no sex differences have been found in several other studies of healthy adults (Stalder & Kirschbaum, in press).

There are numerous indications of the influence of the HPA axis on the gonadal axis but only a few human studies have addressed bidirectional influences of the gonadal axis on the HPA (Rubinow, et al., 2005). There is considerable evidence that female mammals have higher basal and stress levels of ACTH and corticosteroids compared to males, and that these differences are related to gonadal hormones (reviewed in McCormick & Mathews, 2007). Experimental studies with humans and non-human primates have demonstrated that replacement of testosterone reduces the sensitivity of the adrenal system to CRH, resulting in lower cortisol release (Rubinow, et al 2005; Toufexis & Wilson, 2012). The emergence of sex differences in hair cortisol at the time of puberty in our results suggests that reproductive hormones may be a mediating factor. However in the absence of hormone challenge with gonadal steroids we cannot be certain regarding a gonadal etiology. As males develop later than females, the decline in hair cortisol is consistent with such an association.

A limitation of the present study is a lack of parallel assessments of cortisol in other compartments such as blood or saliva, as reported in the studies of Davenport et al (2006) or D'Anna-Hernandez, Ross, Natvig, & Laudenslager (2011). Separately we have observed that nonhuman primates show age dependent declines in plasma cortisol cross-sectionally (vervets) as well as longitudinally (rhesus monkey) following acute challenges (in preparation) and in salivary cortisol of pigtail macaques over a limited age range of 1-12 months (Laudenslager et al, 2005). The observed changes in hair cortisol may be representative of underlying changes in HPA regulation with age. An important caveat is that the changes observed as a function of age as well as sex could be due to other yet undetermined factors. These include age or sex differences in hair growth rate, cortisol accumulation in hair, developmental changes in binding proteins affecting entry of free cortisol into the hair, and/or differences in glucocorticoid metabolism. These are critical mechanistic questions to be addressed as the science of hair cortisol progresses.

Hair cortisol is assumed to reflect cumulative activity of the HPA over an extended period of time unlike measurement of cortisol in saliva and plasma which are reflective of acute

changing activity of this system. Although the significance of cortisol measured in hair remains to be fully understood, it is plausible that hair cortisol represents a proxy marker of cumulative HPA responses taking place over the preceding months and is likely to be affected by the magnitude of acute stress response of the individual as well as rate of return to basal levels (e.g., the area under the cortisol response curve) and the diurnal cortisol release pattern of the individual (Stalder & Kirschbaum, in press). The potential for hair cortisol to serve as a simple and noninvasive retrospective marker of total HPA activation with implications for overall health and well-being is substantial.

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5. References

- D'Anna-Hernandez KL, Ross RG, Natvig CL, Laudenslager ML. Hair cortisol levels as a retrospective marker of hypothalamic-pituitary axis activity throughout pregnancy: Comparison to salivary cortisol. *Physiology & Behavior*. 2011; 104(2):348–353. [PubMed: 21397617]
- Davenport MD, Tiefenbacher S, Lutz CK, Novak MA, Meyer JS. Analysis of endogenous cortisol concentrations in the hair of rhesus macaques. *General & Comparative Endocrinology*. 2006; 147(3):255–261. [PubMed: 16483573]
- Fairbanks LA, Jorgensen MJ, Bailey JN, Breidenthal SE, Grzywa R, Laudenslager ML. Heritability and genetic correlation of hair cortisol in vervet monkeys in low and higher stress environments. *Psychoneuroendocrinology*. 2011; 36(8):1201–1208. [PubMed: 21411232]
- Fourie NH, Bernstein RM. Hair cortisol levels track phylogenetic and age related differences in hypothalamic-pituitary-adrenal (HPA) axis activity in non-human primates. *General & Comparative Endocrinology*. 2011; 174(2):150–155. [PubMed: 21893059]
- Gesquiere LR, Altmann J, Khan MZ, Couret J, Yu JC, Endres CS, Wango EO. Coming of age: steroid hormones of wild immature baboons (*Papio cynocephalus*). *American Journal of Primatology*. 2005; 67(1):83–100. [PubMed: 16163714]
- Hellhammer DH, Wust S, Kudielka BM. Salivary cortisol as a biomarker in stress research. *Psychoneuroendocrinology*. 2009; 34(2):163–171. [PubMed: 19095358]
- Kirschbaum C, Hellhammer DH. Salivary cortisol in psychobiological research: An overview. *Neuropsychobiology*. 1989; 22:150–169. [PubMed: 2485862]
- Kudielka BM, Kirschbaum C. Sex differences in HPA axis responses to stress: a review. *Biological Psychology*. 2005; 69(1):113–32. [PubMed: 15740829]
- Laudenslager ML, Bettinger T, Sackett GP. Saliva as a medium for assessing cortisol and other compounds in nonhuman primates: Collection, assays, and examples. In: Sackett, GP, Ruppenthal, GC, editors. *Nursery Rearing of Nonhuman Primates in the 21st Century*. Plenum Publishers; New York: 2005. p. 403–427.
- Laudenslager ML, Jorgensen MJ, Grzywa R, Fairbanks LA. A novelty seeking phenotype is related to chronic hypothalamic-adrenal-pituitary activity as reflected by hair cortisol. *Physiology & Behavior*. 2011; 104(2):291–295. [PubMed: 21396388]
- Lupien SJ, McEwen BS, Gunnar MR, Heim C. Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nature Reviews Neuroscience*. 2009; 10(6):434–445.

- McCormick CM, Mathews IZ. HPA function in adolescence: role of sex hormones in its regulation and the enduring consequences of exposure to stressors. *Pharmacology, Biochemistry & Behavior*. 2007; 86(2):220–233.
- Pryce CR, Palme R, Feldon J. Development of pituitary-adrenal endocrine function in the marmoset monkey: infant hypercortisolism is the norm. *Journal of Clinical Endocrinology & Metabolism*. 2002; 87(2):691–699. [PubMed: 11836307]
- Rubinow DR, Roca CA, Schmidt PJ, Danaceau MA, Putnam K, Cizza G, et al. Testosterone suppression of CRH-stimulated cortisol in men. *Neuropsychopharmacology*. 2005; 30(10):1906–1912. [PubMed: 15841103]
- Stroud LR, Papandonatos GD, Williamson DE, Dahl RE. Sex differences in cortisol response to corticotropin releasing hormone challenge over puberty: Pittsburgh Pediatric Neurobehavioral Studies. *Psychoneuroendocrinology*. 2011; 36(8):1226–1238. [PubMed: 21489699]
- Stalder T, Kirschbaum C. Analysis of cortisol in hair - State of the art and future directions. *Brain Behavior Immunity*. (in press).
- Toufexis DJ, Wilson ME. Dihydrotestosterone differentially modulates the cortisol response of the hypothalamic-pituitary-adrenal axis in male and female rhesus macaques, and restores circadian secretion of cortisol in females. *Brain Research*. 2012; 1429:43–51. [PubMed: 22088823]
- Young EA, Carlson NE, Brown MB. Twenty-four-hour ACTH and cortisol pulsatility in depressed women. *Neuropsychopharmacology*. 2001; 25(2):267–76. [PubMed: 11425510]

Figure 1. Mean untransformed hair cortisol levels (pg/mg $\pm$ SEM) of vervet monkeys as a function of age group and sex (male filled bars and females open bars). Significant post hoc contrasts between sex are indicated by the asterisks (* $<.05$; ** $<.01$) above the bold lines.