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Hair cortisol levels track phylogenetic and age related differences in hypothalamic–pituitary–adrenal (HPA) axis activity in non-human primates

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ABSTRACT

Hair has been shown to archive a uniquely time averaged signal of endocrine activity, and holds attractive advantages for both laboratory and field research. Prior research has explored the potential of hair hormone analysis to examine hormone–behavior relationships. To date, no research has focused on the potential of the technique to investigate age-related changes or taxon differences in endocrine function. It is known that non-human primate infants of many taxa exhibit high cortisol levels after parturition, which rapidly decline with age. It has also been shown that hypercortisolism generally characterizes platyrrhine (New World monkey) endocrine function. These endocrine trends have been characterized using cortisol levels determined from serum, plasma, and feces. Here we test whether cortisol levels determined from hair recover similar phylogenetic and age related patterns in endocrine function in non-human primates. In order to test whether hair cortisol reflect infant hypercortisolism with significant age-related decline, hair cortisol levels are measured in samples from wild vervet monkeys (*Chlorocebus aethiops*) and captive Guinea baboons (*Papio hamadryas papio*), ranging in age from infants through juveniles. Further, in order to test whether platyrrhines exhibit significantly higher hair cortisol levels compared to strepsirrhines and catarrhines, and therefore faithfully recover similar signals as more traditionally used substrates (e.g. serum), hair cortisol levels are quantified in adult female hair samples collected from a broad range of non-human primate taxa. Results confirm that hair cortisol levels accurately reflect known phylogenetic and age related patterns of circulating cortisol levels. Therefore, these results suggest that hair may be an ideal hormone bearing substrate for research focused on the examination of population endocrine profiles, cross-sectional studies of endocrine function and taxon variation in hormone levels, as well as stable behavioral trends.

1. Introduction

Over the past few decades, a number of studies have reported the extraction and measurement of endogenous glucocorticoids and androgens from hair (e.g. [18–20]). Refined protocols for use in enzyme linked immunosorbent assay (ELISA) procedures have also recently been published (e.g. [12]). Lipid solubility and low protein binding favor the incorporation of the unbound (free) fraction of steroids into the hair shaft over the protein-bound fraction [28]. It is thought that the free fraction of the hormones in circulation diffuses into the hair follicle from neighboring capillaries and is incorporated into the hair shaft during growth [3,11,39]. Therefore, hormone levels quantified from the hair shaft reflect averaged circulating levels accumulated over the growth period of the hair. Various studies have confirmed that the concentration of hormone (progesterone – [43]; testosterone and estradiol – [20,43]; and cortisol – [1,12]) measured in hair is significantly correlated with averaged concentrations of the same hormone calculated from multiple samples from more traditionally-used substrates, collected over the corresponding period of hair growth (plasma – [18]; serum – [43]; saliva – [12]; and feces – [1]).

One of the principal advantages of using hair is that only a single sample is needed to provide an averaged measure of endocrine activity. Traditional substrates (e.g. serum, saliva, and feces) are acutely sensitive to changes in endocrine function, which may be induced through trapping, handling, or environmental disturbance associated with sample collection. Additionally, circadian patterns of endocrine function may also affect the measured hormone levels. Therefore, multiple samples may be required to establish basal hormone profiles. Hair is therefore not suited to

Abbreviations: HPA, hypothalamic–pituitary–adrenal; LOWESS, locally weighted scatter-plot smoothing; KW-ANOVA, Kruskal–Wallis analysis of variance; CI, Conover–Inman; ELISA, enzyme linked immunosorbent assay.

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studies of short-term hormonal fluctuations or responses but is particularly suited for tracking chronic hormone levels. Another major advantage of hair as a substrate, especially to field researchers, is that hair can be stored and transported under ambient non-specific conditions. It appears that steroid hormones (at least cortisol) may be relatively stable in the hair matrix under a range of conditions for a long period of time (~1000 years – [40]).

These properties of hair are of special interest to researchers interested in understanding the hormonal correlates of stable behavioral patterns [10,22] and of life history, developmental and biological differences among individuals and populations.

Here we report novel hair hormone data, derived from a diverse non-human primate sample. These data are used to examine hair as a potential tool to be employed in investigations of developmental and biological differences in endocrine function. Specifically, we test whether hair cortisol levels faithfully reflect known age-related and phylogenetic variation in endogenous cortisol levels in non-human primates in order to demonstrate the utility of hair as a stable, durable (e.g. [40]), and potentially non-invasively collected (e.g. [16,31,38]) substrate to examine several aspects of development and biology in both wild and captive animals. It is well known that hormone levels change during ontogeny. In particular, among non-human primates, the infant and juvenile period is characterized by higher circulating levels of glucocorticoids (marmosets – [26]; baboons – [8,17]; western lowland gorillas – [35]; mountain gorillas – [29]; and chimpanzees –[2]). Glucocorticoid profiles that span infancy through juvenile phases are particularly well described in baboons and marmosets (marmosets – [26]; baboons – [8,17]). These studies show that infant baboons and marmosets have higher glucocorticoid levels than all older age classes, which rapidly decline with age. Elevated serum cortisol and fecal glucocorticoid metabolite levels in infant yellow baboons (*Papio hamadryas cynocephalus*) have been reported in both wild and captive populations [8,17]. These data show that glucocorticoid metabolite and cortisol levels of yellow baboons decline from early infancy through the early juvenile phases of development. If hair archives circulating levels of cortisol, then similar age-related variation in hair cortisol levels is expected. Therefore, the first aim of this paper is to measure cortisol levels in hair samples derived from a captive population of Guinea baboons (*P. hamadryas papio*) and wild populations of vervet monkeys (*Chlorocebus aethiops*) spanning young infants to juveniles. These data are used to examine whether hair cortisol levels vary predictably with age. Specifically, it is hypothesized that infants have significantly higher hair cortisol levels than older age classes and that hair cortisol levels show a decline with age.

Other biological differences in hypothalamic–pituitary–adrenal (HPA) axis function also exist among primates. Circulating cortisol levels are known to differ among several higher taxonomic groups of primates: the strepsirrhines (i.e. lemurs), the catarrhines (i.e. Old World monkeys, apes, and humans), and platyrrhines (i.e. New World monkeys) [9,27]. Specifically, platyrrhine taxa are generally characterized by hypercortisolism relative to strepsirrhine and catarrhine taxa. Data also suggests that strepsirrhines have somewhat lower cortisol levels than catarrhines, although this difference has not been reported as statistically significant [9]. It appears that platyrrhine cortisol receptors are relatively insensitive to cortisol, and therefore they maintain exceptionally high circulating levels of the hormone [9]. Variation among taxa has also been noted, although the basis for these differences have not been extensively investigated (e.g. [9]). Chrousos et al. [9] showed that humans have low plasma cortisol levels relative to chimpanzees and Old World monkeys, and that northern owl monkeys (*Aotus trivirgatus*) have plasma cortisol levels that are nearly six times lower than those of other platyrrhines [9]. Therefore, the second aim of this paper is to measure cortisol levels in the hair of adult female animals representing several strepsirrhine, catarrhine, and platyrrhine taxa. These data are used to test the hypothesis that platyrrhines have higher hair cortisol levels than strepsirrhines and catarrhines. If hair cortisol recovers similar signals as circulating cortisol, then these data should mirror taxonomic variation in HPA axis activity and elevated cortisol levels in platyrrhines relative to strepsirrhines and catarrhines.

2. Materials and methods

2.1. Animals and collection of hair samples

Approximately 50–100 mg of hair was shaved (using hair clippers) or cut (using scissors) from the back or shoulder of each individual. Hair was cut as close to the skin as possible without causing injury. All hair samples were stored and shipped in labeled paper envelopes under ambient non-specific conditions.

Hair samples used to test for age-related changes in cortisol levels were obtained from male and female wild caught vervet monkeys (*C. aethiops*) and captive Guinea baboons (*P. hamadryas papio*) (Table 1). The vervet samples derive from an unrelated research initiative during which individuals from several free-ranging populations across South Africa were trapped and anesthetized, and various biometric data and biological samples were collected (see [7,36,37] for details pertaining to trapping, anesthetization, and data collection procedures). Animals were aged based on dental eruption and dental wear data and assigned to one of nine age categories (see [37]). Hair from zoo kept Guinea baboons was supplied by the Museum de Besancon, France. Animals were sampled opportunistically during annual veterinary exams. Exact ages were available for all individuals. Only infant and juvenile phases (i.e. vervets: age class 1–5, and Guinea baboons: 0–72 months) are used for the present analysis. Guinea baboons are divided into three age categories for statistical comparison (infants – 0–24 months, young juveniles – 24–48 months, and older juveniles – 48–72 months, see [25,24]).

Hair samples used to compare hair cortisol levels among platyrrhine, strepsirrhine, and catarrhine taxa were derived from a number of sources (Table 2). Although few data exists showing systematic differences in male and female primate cortisol levels, preliminary analyses of a larger data set suggest that significant sex differences in HPA axis activity may exist in some taxa (Fourie, unpublished data). In order to control for the effect of possible sex differences only adult females are used in the comparison of hair cortisol levels among higher taxonomic groups. Hair samples of Nancy Ma's night monkeys (*Aotus nancymaae*), ring-tailed lemurs (*Lemur catta*), white-faced sakis (*Pithecia pithecia*), Coquerel's sifakas (*Propithecus verreauxi coquereli*), and cotton-top tamarins (*Saguinus oedipus*) were clipped from cadavers originating from the Duke Lemur Center and Santa Ana Zoo. Hair samples for golden-bellied mangabeys (*Cercocebus chrysogaster*) and western lowland gorillas (*Gorilla gorilla gorilla*) were opportunistically collected during annual veterinary exams at the San Diego Zoo. Collaborating researchers facilitated the opportunistic collection of hair samples during the course of their research involving common marmosets (*Callithrix jacchus*), tufted capuchins (*Cebus apella*), and patas monkeys (*Erythrocebus patas*) housed at the NIH Primate Center in Poolesville, Maryland. Specifically, marmosets and capuchin monkeys are used in behavioral studies and have identifying patches shaved onto their backs; whenever these identifying marks were made the hair was collected. Patas monkey hair samples were collected during bi-annual tuberculosis tests and veterinary examinations. Wild chacma baboon (*P. hamadryas ursinus*) samples were provided by the National Zoological Gardens of South Africa. These hair samples were collected opportunistically during the course of an unrelated field study. Animals were trapped and anesthetized using procedures similar to those outlined in Rogers and Kidd [30].

Table 1
Table showing the number of individuals for Guinea baboons and vervet monkeys by sex and age class and the mean ± one standard deviation (STD) hair cortisol levels for each age sex group. All hair cortisol values are reported ng/mg.

Guinea baboons	Age class									
	1 (12–24 months)		Hair Cortisol (ng/mg)		2 (24–48 months)		Hair Cortisol (ng/mg)		3 (48–72 months)	Hair cortisol (ng/mg)
Male	5		3.1 ± 1.23		4		1.94 ± 0.27		2	1.56 ± 0.45
Female	3		3.34 ± 1.82		2		1.38 ± 0.16		3	1.64 ± 0.37
Total	8		3.19 ± 1.35		6		1.75 ± 0.37		5	1.61 ± 0.35
Vervets	Age Class									
	1 (<0.5 years)	Hair Cortisol (ng/mg)	2 (0.5–1 year)	Hair Cortisol (ng/mg)	3 (1–1.5 years)	Hair Cortisol (ng/mg)	4 (1.5–2 years)	Hair Cortisol (ng/mg)	5 (2–2.5 years)	Hair Cortisol (ng/mg)
Female	3	3.60 ± 2.04	7	1.50 ± 0.43	4	0.90 ± 0.14	8	1.34 ± 0.48	7	1.28 ± 0.48
Male	4	2.71 ± 1.22	6	1.12 ± 0.18	1	0.9	7	1.00 ± 0.17	2	0.88 ± 0.18
Total	7	3.09 ± 1.54	13	1.33 ± 0.38	5	0.91 ± 0.13	15	1.18 ± 0.40	9	1.19 ± 0.45

Table 2
Table showing the number of individuals and origins of all strepsirrhine, catarrhine, and platyrrhine taxa used in analyses and mean ± one standard deviation (STD) hair cortisol levels for each taxon. All hair cortisol values are reported ng/mg.

Taxa	*n*	Hair cortisol (ng/mg)	Wild/captive	Origin
Strepsirrhines				
Lemur catta	3	0.84 ± 0.57	Captive	Duke Lemur Center
Propithecus verreauxi coquereli	2	0.63 ± 0.30	Captive	Duke Lemur Center
Total	5	7.6 ± 4.40		
Cattarrhines				
Chlorocebus aethiops	23	1.26 ± 0.39	Captive	multiple locations, South Africa
Cercocebus chrysogaster	3	0.72 ± 0.11	Wild	San Diego Zoo
Erythrocebus patas	8	1.58 ± 0.32	Captive	NIH Primate Center
Papio hamadryas papio	12	1.93 ± 0.32	Captive	Museum de Besancon, France
Papio hamadryas ursinus	7	1.10 ± 0.12	Wild	Suikerbos Rand, South Africa
Gorilla gorilla gorilla	5	0.22 ± 0.07	Captive	San Diego Zoo
Total	68	1.31 ± 0.56		
Plattyrrhines				
Aotus nancymaae	2	1.82 ± 1.44	Captive	Santa Ana Zoo
Callithrix jacchus	12	62.30 ± 50.48	Captive	NIH Primate Center
Cebus apella	4	26.04 ± 11.19	Captive	NIH Primate Center
Pithecia pithecia	1	3.26	Captive	Santa Ana Zoo
Saguinus oedipus	1	29.14	Captive	Santa Ana Zoo
Total	18	44.39 ± 45.39		

2.2. Extraction of cortisol from hair

Ten milligram aliquotes were weighed out into labeled 5-ml borosilicate culture tubes. Samples were twice washed in 3 ml isopropanol (C_3H_8O) for 3 min (after [12]) and let dry in a fume hood for 24 h. The samples were then minced into 1–2 mm pieces using a pair of surgical scissors. The minced samples were then extracted in 4 ml of HPLC grade methanol (CH_4O). Samples were incubated in a sonic bath at 50 Hz at 55 °C for 240 min, removed, and incubated on a reciprocating shaker at 100 rpm at room temperature for a further 20 h.

At the end of extraction, the tubes were centrifuged at 4000 rpm at 4 °C for 5 min. 3.5 ml of the extract was then aliquoted into labeled polypropylene tubes and allowed to dry under a stream of air in a fume hood. The dry extract was then reconstituted in successively smaller volumes of methanol (2 ml, 1 ml, and 500 μl) and dried down under air in order to concentrate the dry extract at the bottom of the tube. Finally, the dry extract was reconstituted in 500 μl of ELISA phosphate buffer solution (pH 7) and stored at −20 °C. The mean extraction efficiency for all taxa was 95 ± 5%.

2.3. Cortisol assay

Hair cortisol concentrations were quantified using commercially produced ELISA kits designed to measure the free cortisol in human saliva (ALPCO Diagnostics, Salem, NH). Fifty microliters of reconstituted sample was run in duplicate as directed by the assay protocol. When needed, samples were diluted (either 1:8 or 1:16 dilutions were used for common marmoset, tufted capuchin and cotton-top tamarin samples) so they could be read in the optimal range on the standard curve. Sample results were converted from ng/ml to ng/mg of hair. Mean intra-aliquot variance in cortisol levels for all taxa was 11 ± 7% and had a range of 2–21%.

The manufacturer reported cross-reactivity as follows: cortisol = 100%, prednisolone = 13.6%, corticosterone = 7.6%, deoxycorticosterone = 7.2%, progesterone = 7.2%, cortisone = 6.2%, deoxy cortisol = 5.6%, pednisone = 5.6%, and dexamethasone = 1.6%. The range of detection for this kit is 1–100 ng/ml. Mean intra- and inter-assay coefficients of variation were 2.25% and 4.31%, respectively. The assay produced a mean recovery value for all taxa of 88 ± 6%. The immunospecificity of the assay for hair cortisol for each of the sample taxa was demonstrated by showing significant parallelism between sample dilution curves (neat, 1:2, 1:4, 1:8, 1:16) and a standard dilution curve (modified *t*-test for comparing linear regressions, $p < 0.05$ [44]).

2.4. Statistical analysis

Cortisol values were not normally distributed. Therefore, nonparametric techniques are employed in the analysis of the data. When necessary, cortisol data were log transformed in order to facilitate graphical representation. Statistical comparisons between age classes and taxon groups were made using the untrans-

formed cortisol data. Although Castracane et al. [8] describe some differences in male and female yellow baboon glucocorticoid levels with age, they do not find that these differences are significant. Their data also suggest that both sexes show broadly similar age-related variation in glucocorticoid levels. In order to allow greater statistical power for testing the hypothesis predicting elevated hair cortisol levels in infants and an age-related decline in cortisol irrespective of sex, male and female values were pooled.

To graphically assess the relationship between age and hair cortisol levels, locally weighted scatter-plot smoothing (LOWESS) is applied. LOWESS smoothing is a non-parametric model that fits a smoothed non-linear regression model to localized subsets of the data. In order to test the hypothesis that infants have higher hair cortisol levels than older age classes, the relationship between age and hair cortisol concentrations are analyzed using a linear regression analysis and age categories are statistically compared using a Kruskal–Wallis analysis of variance (KW-ANOVA). Due to the nature of the sample set, the Conover–Inman test (CI) is used to make post hoc pairwise comparisons [14,32]. In order to test the hypothesis that platyrrhines have higher hair cortisol levels than strepsirrhines and catarrhines, the three groups are compared using a KW-ANOVA; pairwise comparisons are made using a CI test. An alpha level of 0.05 is set for all statistical tests, which were run on the SYSTAT 13 statistical software package (Systat Inc., San Francisco, CA).

3. Results

3.1. Hair cortisol in non-human primates

The LOWESS fit shows that both male and female Guinea baboons experience a dramatic decline in hair cortisol levels from infancy through the early juvenile stages (0 to ~30 months), after which hair cortisol levels plateau (Fig 1). Similarly a LOWESS fit to the vervet data shows that both males and females show a steep decline in hair cortisol levels from age class 1 to 3, after which males, but not females, appear to show an increase in hair cortisol levels during age classes 4 and 5 (Fig 2). Mean and standard deviation values for hair cortisol levels for all sex, age, and taxon categories are presented in Tables 1 and 2. Linear regression analyses find a significant negative relationship between age and hair cortisol concentrations for vervets ($F_{1,47} = 14.593$, adjusted $r^2 = 0.221$, $p < 0.001$) and Guinea baboons ($F_{1,17} = 24.759$, adjusted $r^2 = 0.569$, $p < 0.001$; see Fig. 1 and 2). Both vervet and Guinea baboon age classes differed significantly from one another (vervet – KW-ANOVA: $H_{4,49} = 15.9$, $p < 0.005$; Guinea baboon – KW-ANOVA: $H_{2,19} = 9.1$, $p < 0.05$). The post hoc CI test showed that age class 1 vervets (i.e. <6 months old; $n = 7$, 3.09 ± 1.54 ng/mg) had significantly higher hair cortisol levels than age classes 2 ($n = 13$, 1.33 ± 0.38 ng/mg, $p < 0.05$), 3 ($n = 5$, 0.91 ± 0.13 ng/mg, $p < 0.001$), 4 ($n = 15$, 1.18 ± 0.40 ng/mg, $p < 0.005$), and 5 ($n = 9$, 1.19 ± 0.45 ng/mg, $p < 0.005$). The CI test also showed that age class 2 had significantly higher hair cortisol levels than age class 3 ($p < 0.05$). No significant differences in hair cortisol levels were found for any other pairwise comparisons of vervet age classes. A CI test found that age class 1 ($n = 8$, 3.19 ± 1.35 ng/mg) Guinea baboons had significantly elevated hair cortisol levels compared to both age classes 2 ($n = 6$, 1.75 ± 0.37 ng/mg, $p < 0.01$) and 3 ($n = 5$, 0.61 ± 0.35, $p < 0.01$). The CI test found no significant difference between age class 2 and age class 3 ($p = 0.4$).

A KW-ANOVA showed that significant differences existed between strepsirrhines, catarrhines, and platyrrhines (KW-ANOVA: $H_{2,83} = 40.5$ $p < 0.005$). A CI test showed that platyrrhines ($n = 20$, 44.39 ± 45.39 ng/mg) had significantly higher hair cortisol levels than strepsirrhines ($n = 5$, 0.76 ± 0.44 ng/mg, $p < 0.001$) and catarrhines ($n = 68$, 1.31 ± 0.56 ng/mg, $p < 0.001$). The CI test also showed that strepsirrhines had significantly lower hair cortisol levels than catarrhines ($p < 0.05$). Hair cortisol levels reflect a great range of variation among the non-human primate taxa analyzed (see Fig 3).

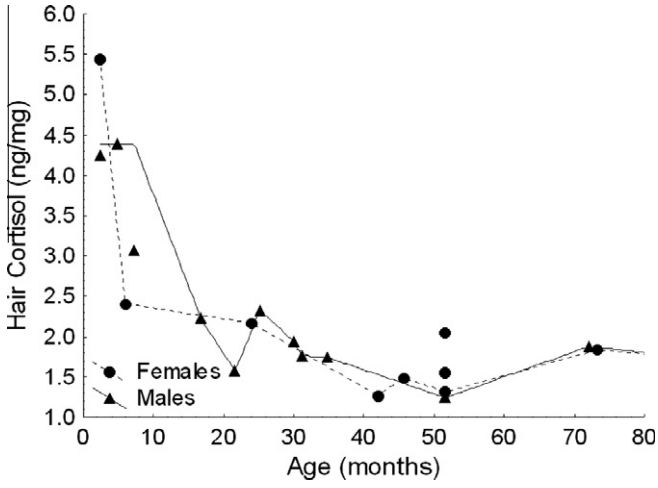

Fig. 1. Guinea baboon hair cortisol plotted against age, showing the age-related decline in male and female hair cortisol from infancy through the early juvenile phases. LOWESS curves have been fitted to the data.

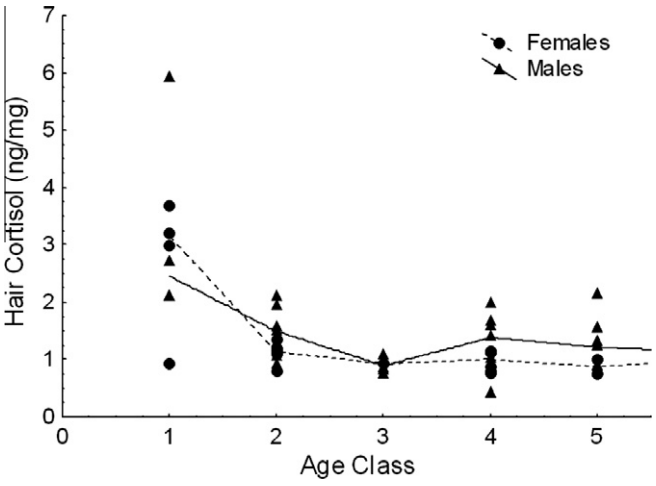

Fig. 2. Vervet hair cortisol plotted against age class, showing the age-related decline in male and female hair cortisol from infancy through the early juvenile phases. LOWESS curves have been fitted to the data.

4. Discussion

The results of our analyses show that age-related cortisol profiles recovered from the hair of Guinea baboons and vervets are similar to those reported for feces and plasma in yellow baboons and marmosets [8,17,26]. Infants in both the vervet and Guinea baboon samples had significantly higher hair cortisol levels than older age classes and hair cortisol levels declined with increasing age. Although hair cortisol results are broadly similar to those reported for yellow baboons and marmosets, some differences can be noted. An increase in male vervet hair cortisol levels is apparent in age classes 4 and 5, roughly corresponding to 1.5–2.5 years of age. Although hair cortisol levels for age class 4 and 5 individuals are not significantly higher than age classes 2 and 3, it may signal the start of a trend associated with male sexual maturation. Whitten and Turner [41] report that male vervets as young as 2 years old can have fully descended testes and can start reproducing by

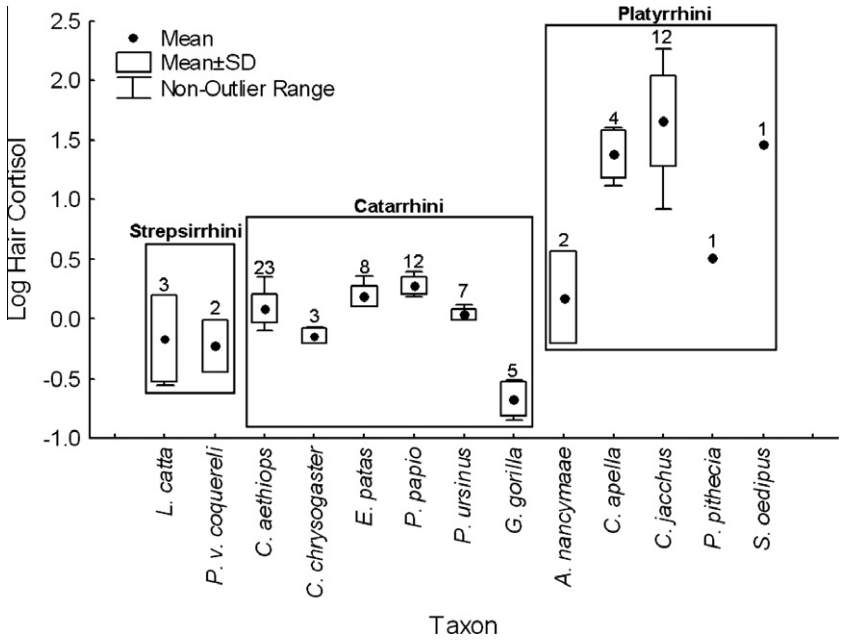

Fig. 3. Box and whisker plot comparing the hair cortisol levels of adult female strepsirrhine, catarrhine, and platyrrhine taxa. The solid circles represent mean hair cortisol values, the boxes represent one standard deviation and the whiskers represent the non-outlier range. The number of samples for each taxon is indicated by the numbers above the whiskers.

3.5 years of age. Male reproductive maturation is associated with increasing testosterone levels [23], which in turn may be associated with the onset of reproductively relevant behaviors, such as dominance competition and male-dispersal (e.g. [5,4,42]). These behaviors can be experienced as stressful events, and therefore are likely to be associated with an elevation in cortisol levels (e.g. reproductive competition and male-dispersal – [6]). The onset of such behaviors may therefore explain a gradual increase in male cortisol levels. Further investigation overlaying the timing of life history milestones onto patterns of endocrine activity may be useful in assessing the reproductive and behavioral ontogenetic correlates of the observed elevation in male cortisol levels in this population and may also be useful in comparing patterns among different populations (e.g. [5,37,41].

Known differences in endocrine function between platyrrhines, strepsirrhines, and catarrhines are reflected in hair cortisol levels and are consistent with previous reports based on serum and fecal cortisol levels [9,27]. Although previous comparisons show that strepsirrhines tend to have lower cortisol levels than catarrhines [9], the data presented here suggest that strepsirrhines have significantly lower hair cortisol levels. Further research is needed to confirm this result. Additionally, Chrousos et al. [9] report relatively low cortisol levels for northern night monkeys compared to other platyrrhines, these platyrrhines have plasma cortisol levels more similar to those typically seen in catarrhines. Cortisol levels measured in Nancy Ma's night monkey hair samples suggest comparable results.

Platyrrhine hypercortisolism is thought to be due to a general tissue resistance to cortisol, specific to this clade. Additionally, Pugeat et al. [27] have shown that cortisol-binding-globulin (CBG) in New World primates have a much lower binding capacity compared to that of Old World primates resulting in higher circulating levels of free-, bio-available cortisol. It should also be noted that differences in CBG excretion, metabolism and clearance among taxa, sexes and age cohorts may also account for differences in the free cortisol measured in hair. CBG levels can respond dynamically to stress, which may differ in temporal span to that of cortisol. Davenport et al. [13] have shown that decreased levels of free cortisol may not necessarily reflect decreased adrenocortical function, but may be as a result of increased CBG levels. These caveats are important to note for all studies focusing on variation in free cortisol levels and need to be further explored to examine the sources of higher level variation (e.g. taxon) in cortisol levels that can not be directly related to stress.

5. Conclusions

A recent resurgence in interest in hair as a hormone bearing substrate has resulted in protocols being validated in a number of mammalian species, including macaques [12], humans (e.g. [15]), hyraxes [19], cats, dogs (e.g. [1]), marmosets [10], ground squirrels [22], and bears (e.g. [21]). These studies have also shown that hair can be used to study the endocrine correlates of a range of behavioral characteristics (e.g. [10,15,22]) and pathological conditions [33,34]. The data presented here further validates the technique in a taxonomically broad range of non-human primates from both wild and captive populations. Importantly, this study demonstrates the potential of biomaterials collections (e.g. cadavers, hair collections – museums/universities) as potential sources of endocrine information. Our data show that besides behavior and pathology, the technique is also suited for examinations of physiological differences among taxa and changes in endocrine function with age. Traditionally, such studies require repeated sedation of animals to collect biological samples or repeat collection of fecal or urine samples in order to determine time averaged estimates of hormone levels. Such basal hormone levels can be determined from a single hair sample, circumventing the often logistically and financially demanding nature of repeat sampling. When research is focused on stable behavioral trends, long term effects or cross-sectional examination of population endocrine profiles, hair may be an ideal candidate substrate from which to determine cortisol or other steroid hormones.

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