Sources of variation in hair cortisol in wild and captive non-human primates

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ABSTRACT

Hair cortisol analysis is a potentially powerful tool for evaluating adrenal function and chronic stress. However, the technique has only recently been applied widely to studies of wildlife, including primates, and there are numerous practical and technical factors that should be considered to ensure good quality data and the validity of results and conclusions. Here we report on various intrinsic and extrinsic sources of variation in hair cortisol measurements in wild and captive primates. Hair samples from both wild and captive primates revealed that age and sex can affect hair cortisol concentrations; these effects need to be controlled for when making comparisons between individual animals or populations. Hair growth rates also showed considerable inter-specific variation among a number of primate species. We describe technical limitations of hair analyses and variation in cortisol concentrations as a function of asynchronous hair growth, anatomical site of collection, and the amount and numbers of hair/s used for cortisol extraction. We discuss these sources of variation and their implications for proper study design and interpretation of results.

Keywords: Cortisol; Hair cortisol analysis; Stress; Primates; Chronic stress

1. Introduction

The hypothalamic–pituitary–adrenal (HPA) axis (Nelson, 2005) is the principal endocrine axis responsible for maintaining homeostasis in the face of an external stressor. Cortisol is one of the principal hormones produced by this axis. Both psychosocial and physical stressors can result in elevated cortisol concentrations. The short-term effect of cortisol is adaptive but if chronically maintained at high levels it can have negative health effects (Sapolsky, 1992a, 2004, 2005; de Kloet et al., 1999; Chrousos, 2000). For this reason cortisol is often used as a measure of the physiological costs associated with particular social, biological (e.g. reproductive) and/or environmental conditions (e.g., Sapolsky, 1992b; Saltzman et al., 1994; Gesquiere et al., 2005; Kirschbaum et al., 2009). Short-term or acute fluctuations in cortisol are measured in substrates such as blood, saliva, urine or feces. These substrates reflect HPA activity over a period of minutes to days (Bahr et al., 2000). Measurement of short-term fluctuations in cortisol is useful in the study of the immediate reactions of animals to external stimuli (social/environmental) and the associated physiological consequences or costs (e.g., Sapolsky, 1992a,b, 2004, 2005). For insights into long-term or chronic stress multiple samples need to be collected over a defined period of time. Such approaches are useful in assessing cross-sectional or population level responses to ecological or environmental shifts or assessing the physiological consequences and costs of sustained stress (Pearson et al., 2015). Alternatively, hair is a reliable archive of chronic cortisol secretion (Davenport et al., 2006; Sauve et al., 2007; Accorsi et al., 2008; Thomson et al., 2010). As a means of measuring chronic stress in animals, hair cortisol analyses are finding wider application in both captive- and field-based research (e.g., Davenport et al., 2006; Accorsi et al., 2008; Koren et al., 2008; Bechshøft et al., 2010; Fairbanks et al., 2011; Carlitz et al., 2015; Fourie et al., 2015a,b; also see Russell et al., 2012 for review). The interest in hair as an archive of endocrine activity derives from the various unique features of hair that offer advantages to researchers interested in chronic stress compared to more traditional substrates (e.g., blood, stool, urine, saliva) used to determine cortisol levels. Cortisol is

incorporated into the hair shaft through two principal mechanisms. The free, unbound fraction of cortisol is incorporated during growth of the follicle (Cone, 1996). In addition, sweat and sebum excreted by glands on the skin contain cortisol which can become incorporated into the hair shaft (Anielski, 2008). Consequently, hair samples offer a way to measure the average cortisol levels accumulated over the lifetime of the hair. Evidence suggests that hair can also be analysed serially to reveal temporal changes in cortisol levels (e.g., Kirschbaum et al., 2009; Webb et al., 2010). The time-averaged nature of the cortisol signal in hair reflects the basal hormonal phenotype (long-term time-averaged cortisol secretion) of an individual (Davenport et al., 2006). While other sample types can be used to estimate the basal phenotype and provide high resolution longitudinal insights, multiple sampling over an extended period of time is required. On the other hand, the basal phenotype can be estimated using a single sample when using hair. Another advantage is that hair appears to be a stable environment for steroids, so samples can be shipped and stored at ambient conditions and remain biologically relevant for long periods of time (e.g. >1000 years; Webb et al., 2010, 2015). Hair can also be collected using minimally invasive techniques, such as using a variety of hair traps, from both captive and wild animals (Valderrama et al., 1999). The extraction of steroids, particularly cortisol, from hair is relatively simple and concentrations can be quantified using standard immunosorbent assay techniques (e.g., Koren et al., 2002; Davenport et al., 2006). These properties make it an attractive substrate for assessing chronic/long-term adrenal function in mammals under a variety of conditions.

Various factors may influence the cortisol measured in hair. We know from previous reports that hair colour (Bennet and Hayssen 2010; Yamanashi et al., 2013), hair type (Macbeth et al., 2010), anatomical region from which it was sampled (Sauve et al., 2007; Macbeth et al., 2010; Terwissen et al., 2013; Yamanashi et al., 2013; Carlitz et al., 2014, 2015), as well as preparation techniques (Davenport et al., 2006) can influence hair cortisol concentrations. Here we examine sources of variation in hair cortisol, some of which could be considered sources of error in the accurate

estimation of hair cortisol (e.g., sample preparation, sample weight and number of hairs) and others which reflect real biological variation (variation in hair cortisol by anatomical site, age, sex, hair growth rate) from data accumulated over the course of several studies in wild and captive primates (Fourie and Bernstein, 2011; Fourie et al., 2015a,b). We describe and consider how they impact the interpretation of individual hair cortisol levels, as well as cross-sectional/population level comparisons. These findings have important practical implications for the application of the technique and appropriate study design for both captive and wild animals, and inform the inferences that can be drawn from results given practical constraints of sample collection and study sample composition.

1. Materials and methods

1.1. Sample collection

Hair from wild yellow baboons (*Papio cynocephalus*; n = 5) was collected from animals anesthetized in the field in the course of unrelated research in the Mikumi National Park, Tanzania (Phillips-Conroy et al., 1987). Hair samples from chacma baboons (*Papio ursinus*; n = 14) from Loskop Dam Nature Reserve, Suikerbosrand Nature Reserve and the city of Tshwane (South Africa) were obtained from dead animals found by park rangers, or were provided by wildlife veterinarians attending captured animals. Hair samples from captive Guinea baboons (*Papio papio*; n = 26) collected during annual veterinary exams were provided by the Museum de Besançon, France. Hair samples from other primate taxa, including Allen's swamp monkey (*Allenopithecus nigroviridis*; n = 2), golden-bellied mangabey (*Cercocebus chrysogaster*; n = 3), gelada baboon (*Theropithecus gelada*; n = 1), western lowland gorilla (*Gorilla gorilla*; n = 3), yellow-cheeked crested gibbon (*Hylobates gabriellae*; n = 1), and siamang (*Symphalangus syndactylus*; n = 1) were provided by the San Diego Zoo, also collected during annual veterinary exams. Hair from a white-faced saki

(*Pithecia pithecia*; n = 1) was collected opportunistically from a deceased animal from the Santa Ana Zoo upon necropsy.

Exact ages were not available for the wild animals so age determinations were based on molar dental eruption schedules, based on an age class system devised by Phillips-Conroy and Jolly (1988) for *Papio* baboons: infants – age class 1 = no permanent molars showing (< 2 years); young juveniles – age class 2 = first molar showing (2–4 years); sub-adults – age class 4 = third molar showing (4–8 years) (older juveniles and sub-adults were combined – after Beehner et al., 2009); adults – age class 5 = all teeth in full occlusion (> 8 years). The birth dates of the captive animals were usually known and accurate calendar ages could be calculated for each animal.

Hair from 5 female yellow baboons was collected from three anatomical regions: the base of the tail, thigh and deltoid areas. Hair of all other individuals from both wild and captive populations was sampled from the inter-scapular region. Once received, samples were stored in labelled paper envelopes at ambient laboratory temperature.

Hair from the wild animals (and the captive Guinea baboons) was used to examine the effects of age, sex and the anatomical origin of the hairs on hair cortisol variation. Hair from the captive animals was used to assess hair growth rates and the hair from the saki monkey was used to assess the effect of sonication on extraction efficiency, and the relationship between sample weight and the number of hairs per unit weight on the accuracy with which hair cortisol concentration could be determined.

1.2. Sample preparation and extraction

1.2.1. Cortisol extraction method

Hair samples (10 mg) were weighed into labelled 5 ml borosilicate 12 mm x 75 mm tubes and twice washed in 3 ml isopropanol for 3 min to remove exogenous contaminants, sweat and sebum (Davenport et al., 2006), and dried in a fume hood for 24 h. Once dry, the samples were minced into 1–2 mm pieces with surgical scissors, and 4 ml of methanol was added. The scissors were rinsed into

each tube to ensure that no hair material was lost. The minced samples were then incubated in a sonicating water bath at 50 Hz at 55 °C. Aliquots of saki monkey hair (10 mg) were incubated in the sonicating hot water bath for 0, 60, 120, 240, or 360 min to evaluate the effect of differing incubation times on cortisol extraction from hair. All samples were then incubated on a plate-shaker for the remainder of the 24-h extraction period. The tubes were then centrifuged at $2500 \times g$ for 5 min, and a 3.5 ml aliquot of the supernatant was pipetted into labelled polypropylene tubes and dried under a stream of air in a fume hood. The extract was reconstituted and dried in successively smaller volumes of methanol (2 ml, 1 ml and 500 μ l) leaving the dry extract at the bottom of the tube. The dry extract was reconstituted in 500 μ l of phosphate buffer provided in the assay kits and stored at – 20 °C.

1.2.2. Sample size and composition

In order to assess the effect of the number of hairs of which a sample consists on the accuracy of estimates of the cortisol “phenotype” of an individual, 3 mg, 10 mg and 20 mg hair samples from a saki monkey were processed as described above. The number of hairs in a 1 mg sample was counted and was used as a basis for extrapolating the number of hairs for other weights. The length of five individual hairs was also measured and averaged to get an idea of the relationship between hair length and weight for this species.

1.3. Hormone analysis

Hair cortisol concentrations were quantified using an enzyme-linked immunosorbent assay (ELISA) designed to quantify free unbound cortisol in saliva (ALPCO Diagnostics, Salem, NH, USA). 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%, prednisone = 5.6%, dexamethasone = 1.6%. The sensitivity of this assay was 1

ng/ml. Reconstituted samples (50 µl) were analyzed in duplicate, and results were converted from ng/ml of solute to ng/mg of dry hair weight. Immuno-specificity of the ALPCO salivary cortisol ELISA for endogenous cortisol extracted from non-human primate hair (50 mg sample pools) was demonstrated by showing parallelism between dilutions of cortisol standard (0, 1, 3, 10, 30 and 100 ng/ml) and hair cortisol extracts (neat, 1:2, 1:4, 1:8, 1:16, 1:32) for all the taxa examined. Recovery of cortisol standard added to extracts was greater than 90% for all taxa.

1.4. Hair growth rates

After collection of hair samples from animals housed at the San Diego Zoo (Allen's swamp monkey, golden-bellied mangabey, gelada baboon, western lowland gorilla, yellow-cheeked crested gibbon, Siamang), zoo personnel visually inspected individuals on a regular basis and the date of full hair regrowth was recorded. Zoo personnel reported full regrowth when they could no longer distinguish the shaved area from adjacent unshaved areas. An estimated hair growth rate for each species was determined based on measured mature hair lengths (average of the measurement of 5 individual hairs) and the time that had lapsed between the day of shaving and the day that full regrowth was reported.

1.5. Data analysis

Differences in the amount of cortisol extracted from hair using different sonication times were tested using a Kruskal–Wallis (KW) test, followed by post hoc pairwise comparisons using Gao's non-parametric multiple test procedure (Gao et al., 2008). Inter-aliquot variability was described using basic descriptive statistics, including calculation of the coefficient of variation for each aliquot size category. Hair regrowth rates were similarly examined using descriptive statistics. Variability in hair cortisol from hair sampled across the different body regions in the 5 yellow baboon females was tested using a paired *t*-test. Regression analysis was used to examine the relationship between hair

cortisol and age, and Lowess curves were fitted to the data to visually describe trends in the data. Kruskal–Wallis and post hoc pairwise comparisons were used to assess the variation in hair cortisol across age categories and between sexes in the Guinea and chacma baboon samples. To improve statistical power of group analyses, age classes 3 and 4 were pooled for the Guinea baboon samples. Statistical significance was set to $p < 0.05$ and the software packages Statistica v12 (StatSoft, Tulsa, OK, USA) and the R package nparcomp v2.6 (Konietschke, 2015) in R v3.2 (R Development Core Team, 2015) were used to perform all statistical analyses.

3. Results

3.1. Technical sources of variation

3.1.1. Incubation in sonicating hot water bath

The amount of cortisol extracted from saki monkey hair under the various experimental conditions differed (KW test: $H_{4,44} = 25.17$, $p < 0.0005$). All extractions yielded up to 1.5 times more cortisol when incubated in a sonicating hot water bath than when incubated at ambient temperature (Gao's test: all $p < 0.05$), and the yield of cortisol increased with duration of sonication up to 240 min (Gao's test: 120 min vs. 240 min; $p = 0.01$), after which there was no significant change in yield (Gao's test: 240 min vs. 360 min; $p > 0.05$; Fig. 1).

3.1.2. A model example of hair sample composition: hair of the white-faced saki

Hairs sampled from the inter-scapular region of the white-faced saki were 6.13 ± 0.37 cm (mean $\pm$ standard deviation) long ($n = 5$). 7 of these hairs with an average length of approximately 6 cm weighed approximately 1 mg. Thus, by extension, a 3 mg sample contains approximately 21 hairs, a 10 mg sample contains approximately 71 hairs, and a 20 mg sample contains approximately 144 hairs. The inter-aliquot hair cortisol coefficient of variation decreased from 40% for 3 mg samples ($n = 7$) to 13% in 10 mg samples ($n = 13$) and 9% in 20 mg samples ($n = 5$) (Fig. 2).

3.1.3. Anatomical region

No differences in cortisol concentrations were observed between hair collected from the base of the tail and the shoulder, or the thigh and the shoulder of yellow baboons ($p > 0.05$). However, hair sampled from the thigh yielded significantly higher cortisol concentrations than hair from the base of the tail (paired t-test, $n = 5$, $t(4) = -3.16$, $p = 0.03$; Fig. 3).

3.2. Intrinsic sources of variation

3.2.1. Hair growth rates

Rough estimates of hair growth rates based on observational data recorded by animal keepers at San Diego Zoo suggest considerable inter-specific variation in hair growth rates (Table 1). Hair growth rates varied from 0.7 ± 0.1 cm per month in yellow-bellied mangabeys ($n = 3$) to 3.5 cm per month in the single gelada baboon sample. Time to full regrowth varied from 2.8 months in western lowland gorillas ($n = 3$) to 7.1 months in yellow-bellied mangabeys. Two gorillas had surprisingly short hair (4.3 ± 0.4 cm), which took approximately 3.7 months to regrow to full length. The third individual had much longer hair (13.9 ± 1.3 cm), which appeared to grow more rapidly (5.8 cm per month) and reached full length after only 2.4 months. The long hair and rapid regrowth of one individual accounts for the high standard deviations in hair growth parameters which we reported for this species.

3.2.2. Age and sex effects

Multiple regression analysis confirmed a negative relationship ($\beta = -0.47$, $t(23) = -2.34$, $p = 0.05$) between age and hair cortisol levels in the captive Guinea baboon samples due to the overall higher concentrations in infants (KW test: $H_{1,26} = 8.86$, $p = 0.003$; Fig. 4) compared to other age groups. There were no differences in cortisol concentrations among the older age classes ($p > 0.05$). No

relationship was found between hair cortisol level and sex in Guinea baboons ($p > 0.05$) (note that few males at older age categories were available for this comparison). There was also a negative relationship between age and hair cortisol concentrations in the chacma baboon samples ($\beta = -0.615$, $t(10) = -4.511$, $p < 0.005$). One male (Tsh1 – see Fig. 5) was identified as an outlier (>2 times the outlier coefficient of 1.5), and exhibited hair cortisol concentrations (4.05 ng/mg) that were at least twice as high as those observed in any other chacma baboon for which we had data. This individual was therefore excluded from comparisons between ages and sex classes in the chacma sample. In chacma baboons, hair cortisol levels were higher in females than males ($\beta = 0.524$, $t(10) = 4.847$, $p < 0.005$).

4. Discussion

Using samples collected from wild and captive non-human primates, we explored intrinsic and extrinsic/technical sources of variation in hair cortisol measurements, which have implications for the applicability of this technique for studying adrenal activity and stress. Some of the variability was due to extraction methodology (i.e., effects of sonication). We found that incubating samples in a sonicating hot water bath may present a viable alternative to the use of expensive milling equipment to improve extraction efficiency. Including even 60 min of sonication in a hot water bath significantly increased the extraction rate, compared to shaking incubation at ambient temperature alone. The amount of cortisol extracted did not increase after 240 min of incubation in the sonicating hot water bath, and appeared to decrease at the longer incubation interval (360 min), although this decrease was not statistically significant. We decided to limit the sonicating hot water bath incubation step to 240 min to avoid possible degradation of cortisol due to prolonged exposure to high temperatures and/or sonication. Additional experiments are required to examine whether heat or sonication or the combination of both is the main driver of the increased extraction rate. Published protocols for the extraction of cortisol from hair require the reduction of the substrate to small

particles by cutting (e.g., Sauve et al., 2007) or milling (e.g., Davenport et al., 2006) in order to
 maximize the cortisol extracted. It is known that sonication, specifically sonication in a hot water
 bath, can significantly improve the extraction of endobiotics from substrates with similar
 physicochemical properties to hair (Schinor et al., 2004). It is also used in doping research to extract
 anabolic steroids and their metabolites from hair (e.g., Gaillard et al., 2000; Anielski, 2008). It is
 thought that sonication accelerates the transfer of compounds into solution by disrupting the matrix
 (Melecchi et al., 2002). Some researchers do include sonication as a brief step (~30–45 min), using
 standalone sonicators, in cortisol extractions from hair (Koren et al. 2002; Kalra et al., 2012).

The size of the hair sample aliquots had an effect on measured cortisol, with samples consisting of
 fewer hairs having greater inter-aliquot variability in hair cortisol measured. Our results suggest that
 approximately 10 mg (70 hairs) are needed per aliquot to accurately determine mean hair cortisol
 concentrations for the investigated animal (a white-faced saki), and perhaps for any primate with hair
 of similar length, thickness and growth pattern. Samples consisting of only a few hairs do not yield
 an accurate estimation of mean hair cortisol (“cortisol phenotype” – Davenport et al., 2006) for this
 animal. As more hairs are included in a sample, the accuracy of the estimation of mean hair cortisol
 improves. This pattern of sample composition-dependent variation in hair cortisol is consistent with a
 scenario where different hairs are in different phases of growth (e.g., anagen, catagen or telogen) and
 reflect different periods of endocrine activity (asynchronous growth) (Harkey, 1993). This would not
 be expected if hair growth was synchronous, as all hairs would record an identical period of
 endocrine activity and analysis of individual hairs or aliquots of varying composition (i.e., number of
 hairs) would yield a low and roughly equivalent inter-hair coefficient of variation (CV). Our results
 reflect the former scenario, though the effects of variation in hair thickness, length and local
 anatomical effects (e.g., Sauve et al., 2007) are also likely to affect hair cortisol variation in both
 synchronous and asynchronously growing hair populations. This suggests that as in humans, hair
 growth in the model taxon used for this experiment (white-faced saki monkey) is asynchronous. We

suggest, based on these results, that if the focus of a study is to make inter-individual comparisons, relatively large samples (i.e., greater number of hairs) may be necessary in order to accurately characterize the “cortisol phenotype” of an individual animal. If a study aims to make broad population-based comparisons, a larger error in the estimation of individual hair cortisol may be tolerated and smaller samples (i.e., fewer hairs per sample) could potentially be used. Dimensions and properties of the hair itself act as limiting factors for determining optimal sample size. In large primates with long and thick hair, a single hair may yield enough cortisol to be measured by ELISA, but would yield a poor estimation of the true chronic cortisol for that individual animal because it is not representative of the larger hair population. For a small primate (e.g., a callitrichid), a large number of individual hairs would be needed to make up a sufficient weight to yield enough cortisol for measurement; by default, in this situation the individual’s hair population would be well sampled and able to offer a good reflection of the animal’s true chronic cortisol. In order to strike the appropriate balance it is important for researchers to conduct a preliminary investigation on the properties of the hair of the taxon they are studying to determine the appropriate sampling strategy. For example, 10–20 mg samples (e.g., 50–100 hairs) may be appropriate when studying medium-sized primates, such as saki monkeys or baboons, but individual hairs (~10 mg each) collected from a gorilla nest may not be sufficient to make detailed inter-individual comparisons between animals, although they could be informative as part of a large cross-sectional regional data set comparing different populations.

We found that variation in hair cortisol concentrations may be affected by the anatomical region from which a sample is taken. Variability across body regions in hair cortisol concentrations has been reported in humans (Sharpley et al., 2010a, b), grizzly bears (Macbeth et al., 2010), and chimpanzees and orang-utans (Carlitz et al., 2014, 2015), and emphasizes the importance of controlling for anatomical region when sampling hair. This may not always be possible, especially when hair is collected opportunistically or via remote sampling methods, such as hair traps. In such

cases, anatomical variability in hair cortisol should be examined in the study species and considered in the study design. Depending on the nature of the questions posed it may be possible to accommodate the variation resulting from differences in anatomical region in the study design.

Although sample sizes were small, and completion of hair growth was assessed by observation only, to our knowledge our findings on hair growth rate across several non-human primate species are the first to be reported. However, the estimated growth rates we report should be interpreted with caution as this methodology is imprecise. More precise experiments should be conducted to confirm the observations we report. The importance of these results is that they emphasize that considerable variation in hair growth rates may exist within and between species. A better understanding of this variability is needed to interpret individual hair cortisol levels in the context of seasonal and other environmental effects, as well as when making inter-individual/species comparisons.

We found differences in hair cortisol as a function of age in both chacma and Guinea baboons. Infants and young juveniles had higher hair cortisol levels than other age groups. Similar age-related patterns in hair cortisol have been observed in both olive baboons (*Papio anubis*) and vervet monkeys (*Chlorocebus aethiops*) (Fourie and Bernstein, 2011; Laudenslager et al., 2012; Fourie et al., 2015b). Such negative correlations between cortisol and age reflect ontogenetic patterns of adrenocortical function, and have been found in other studies using cortisol measured in plasma and feces collected from captive and wild *Papio* baboons (Castracane et al., 1981; Gesquiere et al., 2005). We found a significant effect of sex on hair cortisol in chacma baboons, with higher levels in females. Similar sex differences in hair cortisol were observed in olive baboons (Fourie, unpublished data), and although the sample size was too small for statistical comparison, female Guinea baboons also appeared to have higher hair cortisol concentrations than males (Fig. 4). This may be a taxon-specific characteristic of the *Papio* baboons reflecting either or both a genetic physiological difference between the sexes (e.g., differences in corticosteroid-binding globulin binding capacity – Klosterman et al. 1986), perhaps related to the comparatively greater energetic and physiological

demands of female reproduction (cortisol is known to be elevated during pregnancy – Klosterman et al., 1986), or differences in socio-behavioral effects on stress. Further research is needed to explore these intriguing sex differences in cortisol in the *Papio* baboons.

A survey of serum cortisol across several primate species reported a number of cases where there was a significant difference between males and females, but the nature of the differences appeared to be species-specific (Klosterman et al., 1986). Female cortisol levels exceeded male levels in captive vervet monkeys (Laudenslager et al., 2012); this trend was not observed in wild vervet monkeys (Fourie et al., 2015b). Bernstein et al. (2009) also report a non-significant trend of higher fecal cortisol in male langurs. No systematic gender differences in cortisol have been reported in humans (e.g., Kiess et al., 1995; Soldin et al., 2005), although Raul et al. (2004) reported a trend of higher hair cortisol concentration in men compared to women (also see Bechshøft et al., 2010 on polar bears).

Many studies have shown the potential of hair cortisol analysis to track physiological stress in individual animals (Fairbanks et al., 2011; Carlitz et al., 2014) and animal populations (Martin and Réale, 2008; Macbeth et al., 2010; Fourie et al., 2015a,b). Our results reflect similar individual chronic stress effects as evidenced by the wild, young adult dispersing male chacma baboon (Tsh1 in Fig. 4b) who took up residence in the city of Pretoria, Tshwane, South Africa (<http://mg.co.za/article/2006-03-17-east-rand-baboon-still-evades-captors>). Local authorities actively pursued this individual for several months before he was captured, sedated and relocated. Thus, this particular animal was known to have experienced an extended period of intense stress before sampling, and his unusually high cortisol levels reflected this stress.

Hair steroid analysis presents a powerful and still largely underutilized tool in animal research. The technique is especially suited to address questions about chronic and basal stress hormone levels as hair archives uniquely averaged cortisol levels. The nature of the hair matrix makes it an especially stable environment for steroids and means that hair can be transported and stored under non-specific

conditions. This, along with the use of remote techniques for collecting hair in wild and captive animals (Valderrama et al., 1999) makes hair highly suited for use in the field. However, careful project design and measured interpretation of results, based on the issues presented here, is essential. The best collection and sample preparation methods need to be decided on and standardized given the practical constraints of the project (also see Russell et al., 2015). Sampling error should be considered in the context of the research question and practical constraints, and a balance should be found. This can be done by determining the impact of anatomical site and hair characteristics on hair cortisol variation for a given species. Captive populations, if they can be investigated, may be useful for this purpose. Practical constraints may preclude sampling the same anatomical region or always getting large numbers of hairs, especially if remote sampling methods are used. Modifying the research question, or study design (e.g., increasing sample sizes) and effect-size thresholds can successfully adjust for increased variability in the data due to sampling effects. Many kinds of biological information are captured in hair cortisol (e.g., age, sex, individual experience); these can be the focus of interest but can also be confounding factors if not controlled for.

In sum, hair cortisol analysis is a relatively new, exciting and powerful tool in the study of physiology and stress in both wild and captive animals, and like any method has its own advantages, limitations and technical concerns, all of which need to be considered in order for it to be deployed to effectively address research questions.

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Table 1. Estimates of the hair growth rates and time to full regrowth of hair for six zoo-kept primate species.

Species	n	Estimated Average Growth Rate (cm/month)	SD	Average Length (cm)	SD	Estimated Time Archived (months)
<i>A. nigroviridis</i>	2	1.7	0.3	5.8	1.0	3.4
<i>C. chrysogaster</i>	3	0.7	0.1	5	1.0	7.1
<i>T. gelada</i>	1	3.5		12	0.6	3.4
<i>G. gorilla</i>	3	2.7	2.7	7.5	0.7	2.8
<i>H. gabriellae</i>	1	0.8		4.6	6.5	5.8
<i>S. syndactylus</i>	1	1		5.1	0.5	5.1

Figure captions

Fig. 1. Mean and standard error plot showing that incubation in a sonicating hot water bath increases the amount of cortisol extracted from hair. All extractions that were incubated in a sonicating hot water bath yielded more cortisol than samples extracted under ambient conditions.

Fig. 2. Inter-aliquot variance of white-faced saki monkey hair samples consisting of different numbers of hairs (3 mg = 21 hairs, 10 mg = 71 hairs and 20 mg = 144 hairs) per aliquot.

Fig. 3. Plot showing significantly higher cortisol concentrations were sequestered in hair from the thigh region compared to hair from the base of the tail and shoulder regions in five adult female yellow baboons.

Fig. 4. Scatter plot with Lowess regression fitted to the data showing the age-related decline in hair cortisol levels in both male and female Guinea baboons.

Fig. 5. Box plot showing the age-related decline in hair cortisol levels in both male and female chacma baboons. Female chacma baboons had higher hair cortisol levels than males. Note the high hair cortisol level of the adult male Tsh1 that was captured after an extended pursuit over several weeks in the city of Pretoria.

Figures

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.