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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.

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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

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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 (Bennett 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.

2. Materials and methods

2.1. 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.

2.2. Sample preparation and extraction

2.2.1. 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 × 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 μl) leaving the dry extract at the bottom of the tube. The dry extract was reconstituted in 500 μl of phosphate buffer provided in the assay kits and stored at –20 °C.

2.2.2. 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 "phe-