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Cortisol levels in hair of East Greenland polar bears

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ABSTRACT

To demonstrate the ability to assess long-term hypothalamic–pituitary–adrenocortical (HPA) axis activity in polar bears (*Ursus maritimus*), a pilot study was conducted in which cortisol concentrations was analyzed in hair from 7 female (3–19 years) and 10 male (6–19 years) East Greenland polar bears sampled in 1994–2006. The hair was chosen as matrix as it is non-invasive, seasonally harmonized, and has been validated as an index of long-term changes in cortisol levels. The samples were categorized according to contamination: eight were clean (2 females, 6 males), 5 had been contaminated with bear blood (2 F, 3 M), and 4 with bear fat (3 F, 1 M). There was no significant difference in cortisol concentration between the three categories after external contamination was removed. However, contaminated hair samples should be cleaned before cortisol determination. Average hair cortisol concentration was 8.90 pg/mg (range: 5.5 to 16.4 pg/mg). There was no significant correlation between cortisol concentration and age ($p = 0.81$) or sampling year ($p = 0.11$). However, females had higher mean cortisol concentration than males (females mean: 11.0 pg/mg, males: 7.3 pg/mg; $p = 0.01$). The study showed that polar bear hair contains measurable amounts of cortisol and that cortisol in hair may be used in studies of long-term stress in polar bears.

1. Introduction

Stress in animals has been measured as catecholamine or corticosteroid hormone concentration in matrices such a blood, feces, urine, feathers, eggs and saliva (Larter and Nagy, 2001; Van der Staay et al., 2007; Bortolotti et al., 2008; Downing and Bryden, 2008; Saco et al., 2008; Lupica and Turner, 2009; Okuliarová et al., 2010; Saeb et al., 2010). More recently, analyses of hair (Koren et al., 2002; Davenport et al., 2006; Dettmer et al., 2009; Gow et al., 2010), and liver and gonad tissue (Flores-Valverde and Hill, 2008) have also been included. Faecal, egg, and especially feather and hair samples have the advantage that they do not show short-term hormonal fluctuations, but rather express chronic stress (Koren et al., 2002; Davenport et al., 2006; Bortolotti et al., 2008; Saco et al., 2008; Okuliarová et al., 2010).

Stress studies involving measurement of hair cortisol have usually been conducted under controlled laboratory conditions (Davenport et al., 2006; Dettmer et al., 2009), meaning easy access to clean and standardized samples. This, however, is not always the case when working with samples obtained from wildlife such as polar bears. For example, East Greenland polar bear hair samples that are collected by the indigenous people during their hunt are often contaminated by polar bear blood and fat (subcutaneous adipose tissue). Recent studies examined cortisol in blood plasma from polar bears that were live captured at Svalbard during 1995–1998 (Tryland et al., 2002; Haave et al., 2003; Oskam et al., 2004). In these studies relationships between cortisol concentrations and a number of organic pollutants in blood plasma were found, although the authors also pointed out that stress induced by the chase, darting, and anesthetization of the polar bears may have influenced cortisol levels.

Acute stress can obviously lead to artificially high baseline cortisol levels in blood plasma samples. Also, blood cortisol samples obtained from sedated animals may vary due to the difficulty in maintaining consistent levels of sedation over time and between individuals (Montfort et al., 1993). As hair samples are not affected by acute stress, but rather reflect long-term hypothalamic–pituitary–adrenocortical (HPA) system activity, measuring hair cortisol in polar bears would provide an important non-invasive and non-biased matrix for studies of long-term stress. Hair samples would also be much easier to work with logistically than blood samples, as they require nothing more than a sharp tool and a plastic bag, with no particular handling or storage requirements, on the way from field to laboratory.

The aim of the present pilot study was to investigate whether it was possible to remove the external source of cortisol (here: bear blood or fat) while retaining hormone tightly bound to the matrix of the hair shaft, and to assess the feasibility of extracting measurable cortisol concentrations from polar bear hair.

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2. Materials and methods

2.1. Samples

Hair from 17 (7 female and 10 male) East Greenland polar bears was included in the present study. Age determination had been done by counting the cementum growth layer groups (GLG) of the lower right incisor (I3) (Hensel and Sorensen, 1980). Samples were collected in East Greenland (app. 61°–82°N, 10°–42°W) during the period 1994–2006. Hair samples are collected routinely for NERI (National Environmental Research Institute, Aarhus University, Denmark) by the subsistence hunters living in Scoresby Sound during their annual catch of polar bears. As the bears are usually flayed in the field, hair samples were often partially cross-contaminated with blood and subcutaneous adipose tissue (fat). Therefore hair samples fell into three categories: clean, blood contaminated, and fat contaminated, depending on their condition. In total there were 8 clean (2 female, 6 male), 5 blood-contaminated (2 female, 3 male), and 4 fat-contaminated (3 female, 1 male) samples. According to our contact person in Scoresby Sound, all hair samples were taken from the chest area of the polar bears. No examination of cortisol content in proximal vs. distal hair was done due to insufficiency of samples and the low levels of cortisol present. However, no differences were found in cortisol concentration of proximal and distal hair segments in rhesus monkeys (Davenport et al., 2006) or across the dogs examined by Bennett and Hayssen (2010).

At NERI, the samples were kept in clear plastic bags in the freezer at −20 °C. However, the hair samples have all been subjected to varying temperatures, also in the plus range, during transportation.

2.2. Cortisol analysis

Hair samples weighing app. 150 to 250 mg were processed and analyzed according to the methods described in Davenport et al. (2006) with modification to assess and remove external sources of cortisol, particularly in the blood- and fat-contaminated samples. Clean samples were subjected to our standard procedure of two 3-min washes, each with 5.0 ml of HPLC-grade isopropanol (Fisher Scientific, Pittsburgh, USA). Contaminated samples were subjected to either two or three additional washes (see Table 1). In all cases, an aliquot of each wash was dried down under a stream of nitrogen gas and reconstituted in assay buffer for subsequent determination of cortisol content of the wash. Washed hair samples were air dried and then ground to a fine powder in an MM 200 ball mill (Retsch, Newtown, USA). Approximately 50 mg of powdered hair was extracted for 24 h with HPLC-grade methanol (Fisher Scientific), dried down, reconstituted in assay buffer, and then analyzed for cortisol using a sensitive and specific enzyme immunoassay (Salimetrics, State College, PA USA). All samples were analyzed in a single assay with an intra-assay coefficient of variation of 3.0%. Inter-assay coefficients of variation averaged less than 7%.

2.3. Statistical analyses

Pearson's correlation tests were applied to test the relationship between cortisol and age or year, respectively. Student's *t*-test was applied to test the difference in hair cortisol concentrations between males and females, and the differences between the three groups (clean, fat-contaminated, and blood-contaminated). Confidence levels were set to $\alpha = 0.05$ and all statistical analyses were conducted using R (version 2.10.0).

3. Results

A single outlier (Table 1: 19.9 pg cortisol/mg hair) fell more than 1.5 times outside the interquartile range above the third quartile in a plot of all cortisol measurements (as determined by R). With sample size being so small, it remains uncertain whether this was in fact an outlier or simply a testimony to natural variation in hair cortisol. Therefore, statistical analyses were conducted both with and without inclusion of the outlier sample.

Cortisol results are shown in Table 1. For individuals with known age ($n = 14$), age range for females was 3–19 years ($n = 6$, mean = 9.5), and for males 6–19 years ($n = 8$, mean = 10.5).There were no statistically significant correlations between age and cortisol ($p = 0.81$) or between year of kill and cortisol ($p = 0.11$, though without removing the outlier a significant $p = 0.02$). Without the outlier, $r = -0.41$ (Fig. 1); with the outlier, $r = -0.57$ (not shown). Females had a significantly higher cortisol concentration than males ($p = 0.01$, though without removing the outlier a non-significant $p = 0.17$). Fig. 2 shows cortisol concentration in female and males (excl. outlier).

Table 1
Results from the analyses of hair cortisol concentration in 17 East Greenland polar bears (*Ursus maritimus*). Columns A–C give the individual sex, age, and year of kill, respectively; column D denotes the physical state of the hair (please see text for further explanation); column E gives pg/cortisol pr. mg. hair; columns E1–5 refers to the content of cortisol in the washing solution after the entire hair sample was washed in it; and column F gives the total cortisol concentration measured in the washed and powdered sample, standardized to be app. 50 mg regardless of the original weight of the hair sample referred to in column E. ND: not detectable; n/a: not available (wash not performed).

A	B	C	D	E1	E2	E3	E4	E5	F
Sex	Age	Year of kill	Hair status	pg cort in wash 1	pg cort in wash 2	pg cort in wash 3	pg cort in wash 4	pg cort in wash 5	pg cort/mg hair
F	16	2000	Clean	108	40	n/a	n/a	n/a	12
F	3	1999	Clean	ND	24	n/a	n/a	n/a	9.7
M	8	2001	Clean	ND	ND	n/a	n/a	n/a	6.5
M	8	2006	Clean	ND	ND	n/a	n/a	n/a	7
M	17	2006	Clean	132	ND	n/a	n/a	n/a	8.6
M	n/a	2006	Clean	128	ND	n/a	n/a	n/a	6.3
M	19	2006	Clean	112	4	n/a	n/a	n/a	5.8
M	n/a	1995	Clean	180	16	n/a	n/a	n/a	19.9
M	8	2000	Fat	180	272	28	ND	n/a	9.8
F	19	2000	Fat	336	380	432	ND	n/a	10.8
F	5	1999	Fat	640	528	148	ND	n/a	8
F	< 5	1994	Fat	460	396	220	ND	n/a	8.2
M	6	2001	Blood	232	20	ND	ND	ND	9
F	6	1999	Blood	1240	296	92	ND	ND	16.4
M	6	2006	Blood	936	612	416	ND	ND	5.5
M	12	2006	Blood	208	72	ND	ND	ND	6.9
F	8	2006	Blood	420	128	28	ND	ND	11.9