General and Comparative Endocrinology 176 (2012) 259–264

Contents lists available at SciVerse ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen

Seasonal rhythms of salivary cortisol secretion in captive Asian elephants (*Elephas maximus*)

Asunción Menargues Marcilla [a,*], Vicente Urios [a], Rubén Limiñana [a,b]

[a] Estación Biológica Terra Natura, Instituto Universitario de Investigación CIBIO, Universidad de Alicante, Apdo. correos 99, E-03080 Alicante, Spain
[b] Instituto de Investigación en Recursos Cinegéticos (IREC), CSIC-UCLM-JCCM, Ronda de Toledo, s/n, E-13071 Ciudad Real, Spain

ARTICLE INFO

Article history:
Received 4 August 2011
Revised 5 January 2012
Accepted 3 February 2012
Available online 15 February 2012

Keywords:
Animal welfare
Asian elephant
Non invasive
Salivary cortisol
Seasonality
Stress

ABSTRACT

Salivary cortisol has been recently used to assess welfare of captive and free-ranging animals. However, rhythms of cortisol secretion may vary annually and thus, it is necessary to take into account these rhythms when evaluating the physiological significance of fluctuations of this hormone throughout the year as stress indicator in animals. Here, we analyze monthly differences in cortisol secretion in Asian elephants (*Elephas maximus*) during a year. Saliva samples of eight adult female Asian elephants were collected and analyzed using Radioimmunoassay. Results revealed an overall seasonal pattern of salivary cortisol secretion and significant differences in cortisol concentration among months were found. Overall, the highest cortisol levels were recorded in October, and then decreased until reaching the lowest concentration in April. However, some individual variations were found respect this annual overall trend. The occurrence of this annual pattern of cortisol secretion should be taken into account when using cortisol as a tool to assess animal welfare in captive animal at zoological parks, as well as it opens new questions to further analyze this pattern and its variations, as well as the endogenous mechanisms controlling it.

1. Introduction

Glucocorticoids (GCs) have been recently used to assess welfare in captive and free-ranging animals. Cortisol is a glucocorticoid released from the adrenal cortex as result of the activation of the hypothalamic-pituitary adrenal axis (HPA) during a stressful situation. Therefore, this hormone is considered an indicator of animal welfare, since its level increases during times of distress [20,26]. However, not all these situations of stress are harmful for individuals, as for example, GCs are also released in response to situations that are not normally regarded as stressful such as hunting, courtship or copulation [4]. On the contrary, chronic stress also increases GCs concentration, and this may have deleterious effects such as immunosuppression and atrophy of tissues [22], inhibit bone formation and accelerate bone loss [17], and low reproductive success [9,18]. Circadian and seasonal variations in cortisol levels regulate physiological processes and play an important role in metabolism and homeostasis [24]. Several studies have found an effect of temperature on seasonal cortisol secretion in mammals, generally showing higher values of GCs in the coldest months, although a great variation (even within the same species) has been found [8,14,29,35].

There is a high correlation between salivary glucocorticoids levels and free-unbound serum glucocorticoids levels [30]. Hence, measurement of salivary cortisol concentration is a non-invasive method that reflects free glucocorticoids concentration in blood [13,16,34] and therefore, it can be used to assess animal welfare [3,6,11,20,23,25]. Some studies show that cortisol exhibits variations that fluctuate within or about 24 h (ultradian and circadian rhythms, respectively) [5,7,10,12,15,28] and annual changes in cortisol secretion (seasonal or circannual rhythms) [15,21,31,33]. Nevertheless, these circannual rhythms in cortisol secretion have not yet been described in Asian elephants. Knowledge of seasonal secretory rhythms of cortisol is necessary when evaluating the physiological significance of fluctuations of this hormone throughout the year as stress indicator in animals. The aim of this study is to test whether a pattern of seasonal cortisol secretion exists, to help interpreting data on cortisol concentration for welfare assessment in this species.

2. Material and methods

2.1. Location and animals

The study was conducted at Terra Natura Zoological Park, Alicante, Spain (38°32'N, 0°08'W). Daily salivary cortisol concentration was recorded for eight non-pregnant female Asian elephants (*Elephas maximus*), with ages ranging from 21 to 46 years (Momo:

* Corresponding author. Address: Universidad de Alicante, Campus San Vicente del Raspeig, Edificio Ciencias III, E-03080 Alicante, Spain. Fax: +34 965 90 38 15.
E-mail address: a.menargues@ua.es (A. Menargues Marcilla).

0016-6480/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.ygcen.2012.02.001

21, Grande: 24, Kaisoso: 24, Tania: 31, Petita: 34, Motki: 37, Jasmin: 40 and Baby: 46). Three of the elephants (Tania, Jasmin and Motki) arrived at Terra Natura Zoological Park in August 2004 from Austria (Gänserndorf Safari Park); three other individuals (Petita, Baby and Kaisoso) arrived in December 2004, having previously resided at the Safari Park Vergel (Vergel, Alicante, Spain); and the other two (Grande and Momo) arrived in May 2005 from Austria (Gänserndorf Safari Park). During night-time, individuals were housed in cages; one of the elephants (Baby) was housed individually in a cage measuring $5 \times 6 \times 3.5$ m, due to conflicts with other elephants, while the rest of the individuals were housed in groups in cages measuring $5 \times 8 \times 3.5$ m; two groups consisted of two individuals (Kaisoso and Petita; Momo and Grande), and another group of three elephants (Tania, Modki and Jasmin). During day-time (09:00–19:00 h) elephants stayed in three different dry meadows of 3000, 5000 and 8000 m^2, which were equipped with artificial lakes and waterfalls. Animals were fed on a diet of oats, branches, fruits and vegetables, and were provided with water *ad libitum*. All the animals were trained and managed using free-contact by keepers with whom they became familiar.

2.2. Sample collection

Saliva samples were taken on a daily basis at 07:30 h (local time) during a whole year from first August 2005 to end of July 2006, before feeding and cleaning cares, and previously to release the elephants into the meadows. Samples were always collected at the same time to avoid the possible effect of circadian variations in salivary cortisol concentration, given that significantly large variations in cortisol concentration occur during early morning even in short time intervals. Samples were collected using the Salivette® kit (Sarstedt, AG & Co, Numbrecht, Germany) and centrifuged at 2000 rpm during 2 min at 15 °C. The eluted saliva was stored at −20 °C until assaying.

2.3. Hormone analysis

Cortisol concentration from every saliva sample was measured in duplicate, using a modification of the solid-phase Radioimmunoassay (RIA) (Coat-A-Count®, Siemens Medical Solutions Diagnostics) and the tubes were counted on a Packard Cobra Auto Gamma Counter (Auto-Gamma® 5000 series, Cobra 5005, Packard Instruments Company). A minimum of 0.4 ml of saliva was needed for the duplicate assay [20]. Modifications were made in the form of increasing the volume of the sample to 200 μL in the standard curve, through 1:10 dilutions of the standards and by increasing the incubation period to 24 h to increase assay sensitivity [19]. This assay was previously validated for Asian elephants [20].

2.4. Statistical analyses

Differences in cortisol concentration among months were analyzed using repeated measures analysis of variance (ANOVA), using all data obtained from each individual (random factor) in each month. The Huynh and Feldt correction was used in Mauchly's Test of Sphericity, as the sphericity assumption was violated. The Bonferroni multiple comparison post hoc test was used to test for pairwise differences in mean monthly cortisol concentrations.

The Pearson's correlation coefficient was used to test whether elephant's individual cortisol profiles were correlated with mean monthly cortisol concentrations of all individuals. Pearson's correlation was also used to test for correlation between mean monthly temperatures and mean monthly cortisol concentration of all elephants. Temperature data were provided by Villajoyosa Weather Station (which is located 7 km away from Terra Natura Zoological Park) from the Irrigation Technology Service, Valencian Institute of Agrarian Research. Statistical analyses were conducted using SPSS v.15.0. All values reported here are mean + SD, and statistical significance for tests was set at $p < 0.05$.

3. Results

Fig. 1 shows the individual salivary cortisol profiles of the eight Asian elephants during one year. Overall, mean monthly salivary cortisol concentration of the elephants ranged from 1.55 + 0.67 nmol/l (Tania, January) to 4.93 + 1.97 nmol/l (Grande, October) (Table 1). In general, mean monthly salivary cortisol concentration increased between May and October in all individuals,

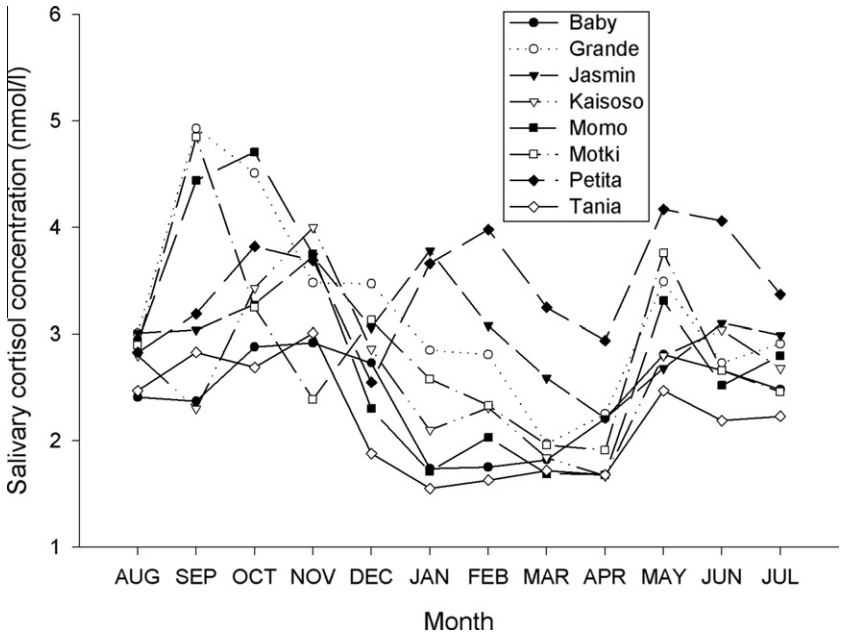

Fig. 1. Mean monthly concentration of salivary cortisol of eight Asian elephants from August 2005 to July 2006. Units of cortisol concentrations are expressed in nanomol/liter (nmol/l).