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SHORT COMMUNICATIONS

The Effect of Ambient Temperature on Glucocorticoid Level in the Amur Tiger (*Panthera tigris altaica*)

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Glucocorticoid hormones are often used in field studies as a parameter of animal well-being to assess the effect of various anthropogenic and biotic factors [1–6]. At the same time, such an assessment is often performed without taking into account a potentially significant effect of many abiotic factors on the level of glucocorticoids, despite that these hormones play an important role in adaptation of an organism to any environmental changes.

Ambient temperature is one of these environmental factors. Its influence on the level of glucocorticoids can be traced in representatives of the family Felidae. These predatory mammals are distributed almost universally, except the Antarctic and some oceanic islands. However, most species of the family live in southern and tropical regions [7, 8]. Only some of them survive and successfully hunt under conditions of low temperatures and deep snow cover in winter. The tiger (*Panthera tigris*) is of greatest interest in this respect. The greater part of its range lies in Southeast and South Asia [8, 9], while the Amur subspecies *P. t. altaica* lives in the Russian Far East, far north of the main species range.

Ambient temperature at tropical latitudes varies significantly during the day but remains relatively stable over the year, while at temperate latitudes it markedly changes during the year but is less variable during the day [10]. Tigers need numerous adaptations to maintain their body temperature at a fixed level in different climatic zones. Fur performs one of the key functions in adjustment to low temperatures [11, 12]. The fur density of *P. t. altaica* is relatively low, not exceeding 3000 hair follicles per square centimeter of skin, i.e., three times lower than in the Eurasian lynx (*Lynx lynx*), which is best adapted to low temperatures in the family of cats [7]. At the same time, the tiger is the largest of cats and may weigh up to 250 kg. This places it in a more favorable position, compared to small-sized representatives of the family, because large animals lose less heat from the body surface. When morphological adaptations are insufficient for tolerating low temperatures, additional mechanisms of thermogenesis are needed to maintain stable body temperature [13]. In small-sized mammals characterized by significant heat loss from the body surface, brown fat is the main source of thermogenesis [14]. Large mammals have much less brown fat, and additional thermogenesis appears in skeletal muscles by a similar mechanism [13]. These processes require increase in metabolic rate and increased food consumption [15].

The effect of glucocorticoid hormones on facultative thermogenesis remains unclear: on the one hand, an increase of their level intensifies energy metabolism [16, 17], stimulates catabolism of glycogen [18], proteins [19, 20], and fats [21], thereby providing the substrate for thermogenesis; on the other hand, glucocorticoids are known to inhibit the processes of thermogenesis in the brown fat [22].

It has been mentioned in some studies that the level of glucocorticoids in animals depends on weather conditions [23]. In studies focused on the ecology and well-being of wild animals, ambient temperature is commonly not included in the list of factors that deserve consideration, even if they are performed in the temperate zone during the winter period [2, 6, 24].

The purpose of this study was to find out whether ambient temperatures has an effect on the level of glucocorticoids in *P. t. altaica*.

We assessed hormonal status of *P. t. altaica* noninvasively by measuring concentration of glucocorticoid metabolites in feces [25]. Fecal samples were collected from January 2008 to December 2009 from one male and two females in the Center for Reproduction of Endangered Species, Moscow Zoo (Sychevo village, Volokolamsk district, Moscow oblast), as well as from one male and one female in the Novosibirsk Zoo in January–December 2011. On the whole, 104 and 100 fecal samples were taken in Sychevo and Novosibirsk,

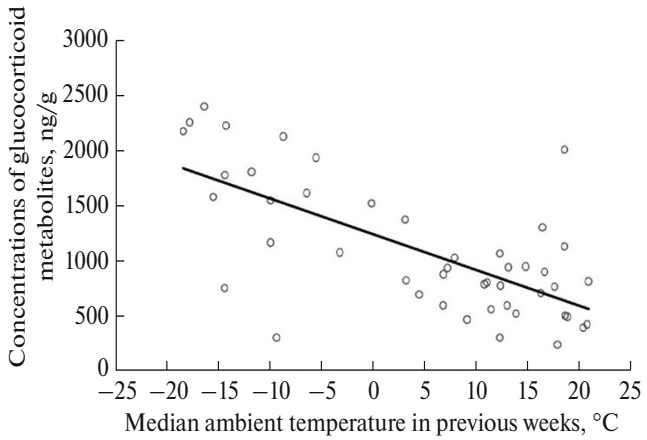

Fig. 1. Correlation between the level of glucocorticoids and air temperature in a female *P. t. altaica*.

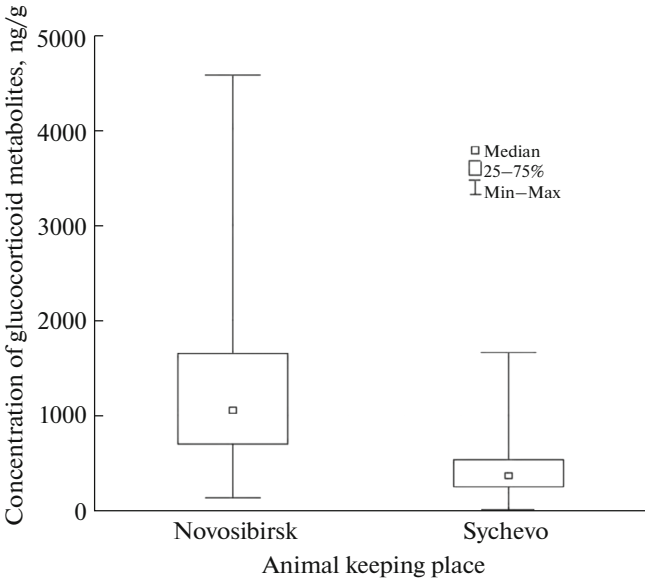

Fig. 2. Average concentrations of glucocorticoid metabolites in tigers from Sychevo and Novosibirsk.

respectively. Animals were fed in the daytime, and fecal samples were taken in the next morning, within 8 h after defecation. Scat samples (5–30 g) were collected in clear plastic bags and labeled (species, sex, animal name, and date of sampling were specified). All samples were frozen, transported to the Chernogolovka research and experimental station (Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences), and stored at –18°C before analysis.

Samples were extracted as described previously [26]. A 100-mg aliquot of each sample was placed in an Eppendorf tube and supplemented with 100 mg of aluminic oxide and 0.9 mL of 90% methanol. The tubes were shaken for 30 min using an Ekros mixer. Then, the tubes were centrifuged for 10 min at 4000 rpm, the supernatant (200–400 μL) was transferred to a new tube and diluted with equal volume of distilled water. The resulting extract was stored at –18°C before analysis.

The concentration of glucocorticoid metabolites in feces was measured by the heterogeneous enzyme-linked immunosorbent assay with the Immuno-FA Kortizol kits produced by the Immunotekh Company (Moscow, Russia). The cross-reactivity of antibodies used in the kit was as follows: 6% with prednisolone; 0.9% with 11-deoxycortisol; 0.6% with corticosterone and deoxycorticosterone; 0.08% with testosterone; and 0.07% with estradiol, estriol, and progesterone. All procedures were carried out as recommended by the manufacturer. This method for measuring the concentration of glucocorticoid metabolites was previously validated for *P. t. altaica* using the ACTH test and animal transportation test [25].

The concentration of cortisol metabolites was converted per gram dry feces. For this purpose, the fecal samples (0.5–2 g) were weighted to an accuracy of 0.1 g using the Ohaus scales (Scout Pro, Ohaus Corporation, United States) and dried at 80°C to constant weight. The moisture content of each sample was determined.

Data on daily average ambient temperature were taken from http://www.rp5.ru. We used archival data of observations at the weather stations nearest to the places where the animals are kept: the Volokolamsk weather station (WMO ID 27502) for the Center for Reproduction of Endangered Species, Moscow Zoo; the Obskaya GMO (WMO ID 29635) and Uchebnaya (WMO ID 29637) weather stations for the Novosibirsk Zoo.

Since data distribution in the series of glucocorticoid metabolite concentrations was lognormal (Kolmogorov-Smirnov test $d = 0.051$; $p > 0.5$), this data set was converted into the logarithmic form for statistical processing.

To assess the effect of ambient temperature on the level of glucocorticoids , we analyzed the following set of continuous variables: the daily average temperature on the day before sampling, and the median value of ambient temperature for previous week, two, three, and four weeks. Due to the high autocorrelation between these values, the method of stepwise linear regression was used. In addition, difference in the daily average temperature between the day before sampling and the next day was calculated. This parameter was also included in the model as a continuous variable. The individuality of animals was included in the model as a categorical variable.

As a result of stepwise analysis, all factors were excluded from the model, except for the median value of ambient temperature for three weeks and the animal individuality. These two factors explain most changes in the level of glucocorticoids ($R^2 = 0.476$; $F = 35.991$; d.f. of model = 5; d.f. of residuals = 198; $p = 0.000$).

Therefore, short-term changes in the ambient temperature had a relatively weak influence on the gluco-

corticoid levels in tigers, i.e., caused no direct stress effect. The greatest impact of median temperature during three weeks shows that tigers gradually adapt to changes in the air temperature.

It may seem strange that the relationship between glucocorticoid level and ambient temperature is well described by the negative linear dependence (Fig. 1), considering that the sample included data collected in the summer period. Thermoregulation in mammals is an active process, and body cooling also requires energy. In the course of this study, the median temperature during three weeks before sampling was no higher than 21°C. Such temperature is likely to be comfortable for *P. t. altaica* and does not require significant efforts for thermoregulation associated with body cooling.

The factor of animal individuality influenced greatly the level of glucocorticoids. We could not directly assess the effect of sampling sites in this model, but it can be assumed that most differences may be related to different conditions of keeping. Thus, the level of glucocorticoids in both animals from Novosibirsk was significantly higher than in those from Sychevo (Fig. 2). A probable explanation is that the animals in the Novosibirsk Zoo were exhibited to the public, while the Center for Reproduction of Endangered Species Moscow Zoo, is closed to the public. The presence of visitors is one of the main factors leading to an increase in the level of glucocorticoids in animals from zoos [27–30].

Therefore, the effect of ambient temperature on the level of glucocorticoids in wild Amur tigers is essential for monitoring the well-being of wild animal populations, especially during the winter period, because this factor has a great impact of the level of glucocorticoids. As shown previously [25], the level of glucocorticoids in *P. t. altaica* is slightly higher in winter than in summer. The assessment of glucocorticoids in the wild has become increasingly popular but is mainly used to estimate the role of individual biotic and anthropogenic factors [2, 24]. Our data show that various abiotic factors, including ambient temperature, must be taken into account for correct interpretation of the results.

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