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Excreted corticosterone metabolites co-vary with ambient temperature and air pressure in male Greylag geese (*Anser anser*)

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Abstract

In many species, seasonal activities such as reproduction or migration need to be fine-tuned with weather conditions. Air pressure and temperature changes are the best parameters for such conditions. Adapting to climatic changes invariably involves physiological and behavioral reactions associated with the adrenals. In the present study, we investigated the effects of ambient temperature and air pressure on excreted immuno-reactive metabolites of corticosterone (BM) and androgens (AM). Focal individuals were 14 paired male greylag geese (*Anser anser*) from a semi-tame, unrestrained flock. BM and AM were measured in individual fecal samples over 25 days in November and December. Two different ACTH-validated assays were used for the assessment of BM: the first one cross-reacting with 11β,21-diol-20-one structures (''old assay'') and the second one with 5β,3α,11β-diol structures (''new assay''). With the ''new assay,'' BM correlated negatively with the minimum ambient temperature of the night before, which may reflect corticosterone involvement in thermoregulation. BM also correlated positively with the minimum air pressure of the previous afternoon, which supports the value of air pressure for predicting weather conditions. Together, these reactions suggest a role of the adrenals in responding behaviorally and physiologically to changes in weather. Preliminary analysis indicated a higher sensitivity to the excreted glucocorticosteroid metabolites in the ''new assay.'' As expected for outside the mating season, no relationships were found between excreted AM and the weather parameters considered. The gradual changes in BM excretion in parallel with weather conditions may be part of the fine-tuning of physiology and behavior by environmental clues.

Keywords: Greylag goose; *Anser anser*; Air temperature; Air pressure; Non-invasive endocrine monitoring; Feces; Corticosterone; Androgens; EIA

1. Introduction

Weather conditions are important environmental modulatory clues in the timing of circadian and circ-annual life-history events. They interact with other classic Zeitgeber such as light–dark cycles to fine-tune behavior and physiology (e.g., Nelson, 1995). In this way, animals are able to cope with a range of conditions in their physical environment and to adjust their seasonal activities correspondingly (Elkins, 1988). Examples have been described for migration (e.g., Blokpoel, 1978), feeding, reproduction or molt (e.g., Wingfield and Kenagy, 1991). Therefore animals have to be able to translate perceived climatic (physical) stimuli into physiological and behavioral responses.

Over the past decades, several studies have indicated the importance of rising air temperature and falling air pressure for the initiation of migratory bouts (e.g., in geese, Blokpoel, 1978; Richardson, 1978; for a review, see Alerstam, 1993). More recently, several field studies on birds have considered the interactions among adrenal and gonadal steroids and weather. Unexpected severe weather conditions have been shown to interrupt breeding by increasing corticosterone and depressing circulating levels of gonadal steroids (e.g., Astheimer et al., 1995; Romero et al., 2000; Wingfield, 1985;

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[1] Key chemicals for the new assay are available in limited amounts free of charge from E. Möstl.

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Wingfield et al., 1983; see also Wingfield et al., 1997a,b). However, elevated corticosterone levels in response to storms or inclement weather have also been observed during the non-breeding season (e.g., Rogers et al., 1993; Romero et al., 2000; Smith et al., 1994; see also Wingfield et al., 1997b). Overall, these results point to a role of the adrenals in seasonally specific physiological and perhaps behavioral adaptations to climate. This is not surprising in light of the classic metabolic role attributed to corticosterone in mobilizing energy reserves, affecting energy allocation (Norris, 1997), and regulating food uptake (Silverin, 1986; see also Silverin, 1998).

Strongly elevated systemic corticosterone may trigger a state of physiological emergency, suppressing physiological and behavioral functions not immediately relevant for survival, such as reproductive behavior (reviewed by Wingfield et al., 1997a). In turn, behavioral and physiological patterns that promote survival such as activity levels or foraging behavior may be affected (Silverin, 1986; Wingfield and Silverin, 1986). Therefore, short-term elevations of corticosterone in response to predictors of adverse weather may serve to maintain body condition and avoid the typical deleterious, long-term effects of chronic stress (e.g., suppression of growth and reproductive physiology; for a review, see Wingfield et al., 1997a). However, to maximize their fitness, organisms should also be able to adjust physiology and behavior appropriately to the normal, non-catastrophic environmental variation (i.e., day–night, temperature fluctuations, seasons, etc.; e.g., Wingfield et al., 1997b).

In the present study we examined whether and how steroid hormones excretion responds to ambient temperature and air pressure. Therefore, we monitored weather conditions and the amounts of excreted metabolites of corticosterone as well as androgens of individuals in a semi-tame flock of greylag geese (*Anser anser*). Immuno-reactive metabolites of corticosterone (BM) and androgens (AM) were measured according to methods established and validated for geese (e.g., Kotrschal et al., 2000; Krawany, 1996). Air temperature and air pressure were measured by an in situ meteorological station. As both climatic factors have a high diagnostic and predictive value for inclement weather, we expected changes in ambient temperature and air pressure to affect adrenal activity related to thermoregulation (Bentley, 1998; Deavers and Musacchia, 1979) or other physiological and behavioral responses to weather conditions (see Wingfield et al., 1997a). Since temperature needs immediate coping whereas air pressure is rather a predictor of conditions to come, a more immediate effect of temperature as compared to air pressure was expected. The study was designed to test whether and how the preceding changes in the two climatic factors mentioned affected the levels of excreted corticosterone metabolites thereafter. Levels of AM were also monitored, although we did not expect them to be particularly modulated by weather conditions, as we sampled outside the breeding season, when levels of excreted androgens in geese are low (Hirschenhauser et al., 1999).

2. Methods

2.1. Study area: weather conditions

The Konrad Lorenz Research Station is located at 550 m above sea level in a valley in the Northern part of the Austrian Alps. Annual temperature and rainfall means are between 7.0 and 7.5 °C and between 1500 and 1600 mm, respectively (M. Bogner, personal communication). Due to the surrounding mountains and the opening of the valley towards North, the temperature means are lower and the rainfall values higher than in neighboring areas of similar altitude. In November 1998, mean temperature values were approximately 3.5 °C beneath the long-term recorded annual November mean (M. Bogner, personal communication).

2.2. Animals

The semi-tame flock of greylag geese was introduced into the Upper-Austrian valley of the river Alm by Konrad Lorenz and co-workers in 1973 (Lorenz, 1988). Geese are unrestrained and generally spend the day on meadows and ponds close to the research station where they are provided with supplemental food twice a day, year-round. At night, the birds roost on a lake approximately 10 km to the South. Animals are subject to natural selection: losses to natural predators (mainly red foxes in spring and golden eagles in winter) may account for up to 10% of the flock per year (Hemetsberger, 2002). All individuals are marked with colored leg rings and are habituated to the presence of humans. Social behavior and individual life-histories have been monitored since 1973.

In the present study, 14 adult males of different ages ($\overline{X} \pm \mathrm{SD} = 6.2 \pm 3.2$ years) were monitored for weather-dependent changes in fecal steroid metabolites. All the focal individuals were paired males without offspring, in order to avoid the potentially confounding effects of social or reproductive status on excreted immuno-reactive metabolites of corticosterone (BM) and androgens (AM) (Hirschenhauser et al., 1999; Kotrschal et al., 1998).

2.3. Data collection

2.3.1. Weather parameters

A solar-energy powered weather station (Bogner & Lehner OEG) was used to monitor ambient temperature

(°C) and air pressure (Pa) in 1-min intervals. Values were transferred to a data-logger, where arithmetic means were calculated over 10-min periods.

2.3.2. Physiological parameters

For the assessment of corticosterone and androgens immuno-reactive metabolites (BM, AM), individual fecal samples were collected for 25 days, between the 9th of November 1998 and the 14th December 1998. Feces were sampled from resting individuals between 9:30 and 11:00 a.m. Sampling was timed to avoid the early morning peaks of adrenal activity (Carere et al., 2003; Rich and Romero, 2001; Schütz et al., 1997) and to obtain an estimate of the daytime baseline levels. Samples were stored at −20 °C within an hour after collection (Hirschenhauser, 1998). It was not always possible to obtain one sample per individual per day, as planned. On average 24.3 ± 1.3 ($\overline{X} \pm \mathrm{SD}$) samples were collected per individual over the entire period (total $N = 340$). Since repeated and regular collection of plasma was neither feasible, nor advisable because of the potential stress induction (Miller et al., 1991) plasma levels of the hormones were not measured. However, in agreement with other studies (e.g., Cockrem and Rounce, 1994; Palme et al., 1996), experiments with domestic geese showed deterministic relationships between plasma steroids and their metabolites in the feces (Hirschenhauser et al., 2000; Kotrschal et al., 2000). Two of the assays presently used were validated in the latter two studies (androgens: Hirschenhauser et al., 2000; corticosterone: Kotrschal et al., 2000). Recent experiments (unpublished data) showed that also the 3α,11β-diol glucocorticoid metabolites detected by the "new assay" (see below for details) are representative for plasma corticosterone levels.

2.4. Assay of fecal steroids

Fecal samples (0.5 g) were extracted in methanol and hydrolyzed as described elsewhere (corticosterone, Kotrschal et al., 1998; androgens, Hirschenhauser et al., 1999). Excreted levels of immuno-reactive metabolites of corticosterone (BM) and androgens (AM) were determined by enzyme immunoassay (EIA). Details about the procedure and the cross-reactivities of the assay were published elsewhere (BM, Kotrschal et al., 1998; AM, Hirschenhauser et al., 1999). Concentration limits for reliable measurements ranged from 0.45 to 112.2 ng/g for BM and from 0.12 to 45.8 ng/g for AM. Intra- and inter-assay coefficients of variations were determined from homogenized pool samples. Mean intra-assay coefficient of variation was 13.8% for BM and 9.1% for AM; mean inter-assay coefficient of variation was 15.7% for BM and 13.1% for AM. These values are in the typical range for EIA procedures on feces, because the number of steps involved increases total variation.

Furthermore, an EIA with an antibody for a new group of corticosterone metabolites was developed and applied in this study. First, 5β-androstane-3α, 11β-diol-17-one (i.e., 11β-hydroxyetiocholanolone, Sterealoids, Wilton, NH, USA) was converted into a carboxymethyl derivative and linked to bovine serum albumin (BSA) as described by Kohen et al. (1975). Antibodies were raised in a rabbit. The biotinylated label was synthesized using a mixed anhydride reaction as described by Möstl et al. (2002) for 11-oxoetiocholanolone. To determine the working dilution of antibody and label, a checkerboard titration (Meyer et al., 1990) was performed. A dilution of the antibody of 1:25,000 and of 1:10,000 for the label showed best results and was used for the EIA. 11β-Hydroxyetiocholanolone was used as standard. The standard curve ranged from 2 to 500 pg/well; the 50% intercept was about 30 pg. The cross-reactivity of the antiserum is shown in Table 1. The intra-assay coefficient of variation was 10.3%; the inter-assay coefficient 13.2% as determined by homogenized pool samples. To distinguish the two assays used for BM, we will indicate the new one as "new assay," and the one described in Kotrschal et al. (1998) as "old assay."

2.5. Data analysis

2.5.1. Weather parameters

Daily temperature variation includes a minimum early in the morning (between 3 and 5 a.m.) and a maximum in the afternoon, at about 2 or 3 p.m. Air pressure patterns show a bimodal daily pattern, with the maximum in the morning and the minimum in the afternoon. As the present data were collected at the beginning of the Alpine winter, we focussed on parameters which could challenge the ability of the animals to cope with it. Fecal samples were collected only during late morning hours, therefore we empirically chose the minimum of the ambient temperature of the previous night and the minimum of the air pressure of the previous afternoon as meteorological parameters to be

Table 1
Specifications of the cross-reactivity in the "new assay"

Metabolite	Percentages
11β-Hydroxyetiocholanolone	100
5β-Pregnane-3α,11β,21-triol-20-one	20.0
5β-Pregnane-3α,11β-diol-20-one	14.6
11-Ketoetiocholanolone	3.5
11-Ketoandrosterone	<1
Etiocholanolone	<1
Pregnanediol	<1
Tetrahydrocortisol	<1
5β-Dihydrocortisol	<1
5β-Pregnane-3α,11β,17α,20α,21-pentol	<1
5β-Pregnane-3β-ol	<1
11,20-Dione	<1
5β-Pregnane-3α,11β-diol-20-one	<1

considered. Together, they represent classical predictors for inclement weather, even though their relative changes are not always temporally related. These two parameters did not correlate with each other during the study (Spearman, $r_s = -0.36$, $n = 24$, $P > 0.05$) and, therefore, they were treated independently. For technical reasons, the weather station failed to provide data in two non-consecutive days during the period of data collection.

2.5.2. Physiological parameters

For comparing results between the two assays for corticosterone (i.e., the new and the old assay), original individual values of fecal metabolites were considered. For the analysis of correlations among weather parameters and levels of excreted steroids, the minimum of the ambient temperature of the previous night and the minimum of the air pressure of the previous afternoon were considered, whereas the daily arithmetic mean of BM and AM was calculated. This way, 23 data points were obtained along the time axis. Daily rates of change of air pressure and ambient temperature of up to 3 days before the fecal samples were also considered post hoc. However, none of them was found to co-vary with AM or BM concentrations (unpublished data).

2.5.3. Statistics

Data were analyzed using the SPSS statistical package (Pfeifer, 1991). Non-parametric tests were used and correlations between hormone levels and weather parameters were scanned for using Spearman's correlation coefficients (Zöfel, 1992). Results of all tests are given two-tailed; levels of significance are corrected according to Bonferroni post hoc test (Rice, 1989).

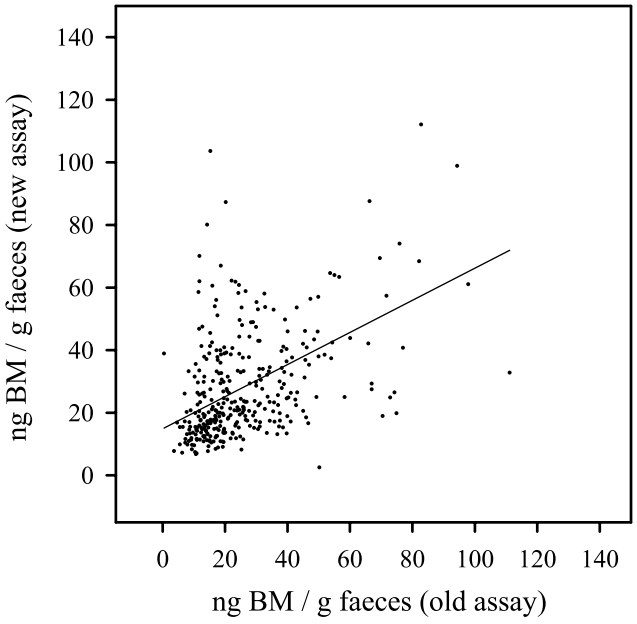

Fig. 1. A significant positive correlation (Spearman, $r_s = 0.45$, $P < 0.007$; $r^2 = 0.23$) was found between levels of excreted corticosterone immuno-reactive metabolites as determined by the two assays, the "old assay" (Kotrschal et al., 1998) and the "new assay." Original individual values are plotted.

3. Results

3.1. Comparison between the two assays

The results obtained by the "new assay" varied in parallel with those obtained by the "old assay" ($r_s = 0.45$, $n = 340$, $P < 0.007$; Fig. 1) and no significant difference was observed between either the medians (Wilcoxon test: $Z = -1.729$, $n = 340$, $P = 0.073$) or the variances (F test: $F_{339,339} = 1.15$, $P > 0.05$) of the two samples. The "new assay" not only revealed more significant results (see below), it was also shown to produce higher peak values within the same sample (ACTH-validation, unpublished data; see also Fig. 1) and it is therefore considerably more sensitive.

3.2. Weather and physiological parameters

The morning minimum of the air temperature correlated negatively with the amounts of excreted immuno-reactive metabolites of corticosterone (BM), as determined by the "new assay" (Table 2; Figs. 2A and 3A) but not by the "old assay" (Table 2 and Fig. 3B). Furthermore, a significantly positive correlation was

Table 2
Analysis of the effects of weather parameters on levels of BM and AM as determined by the "old assay" (Kotrschal et al., 1998) and by the "new assay" (Spearman's correlation coefficients)

	BM (old assay)	BM (new assay)	AM
Air temperature (morning minimum)	$r_s = 0.362$ $n = 23$ $P > 0.0125$	$\mathbf{r_s = -0.536}$ $\mathbf{n = 23}$ $\mathbf{P < 0.01}$	$r_s = -0.265$ $n = 23$ $P > 0.017$
Air pressure (afternoon minimum)	$r_s = -0.049$ $n = 23$ $P > 0.05$	$\mathbf{r_s = 0.62}$ $\mathbf{n = 23}$ $\mathbf{P < 0.008}$	$r_s = 0.222$ $n = 23$ $P > 0.025$

Levels of significance are corrected according to Bonferroni post hoc test (the test may be judged significant at a minimum significance level of 0.01), significant results are marked bold.

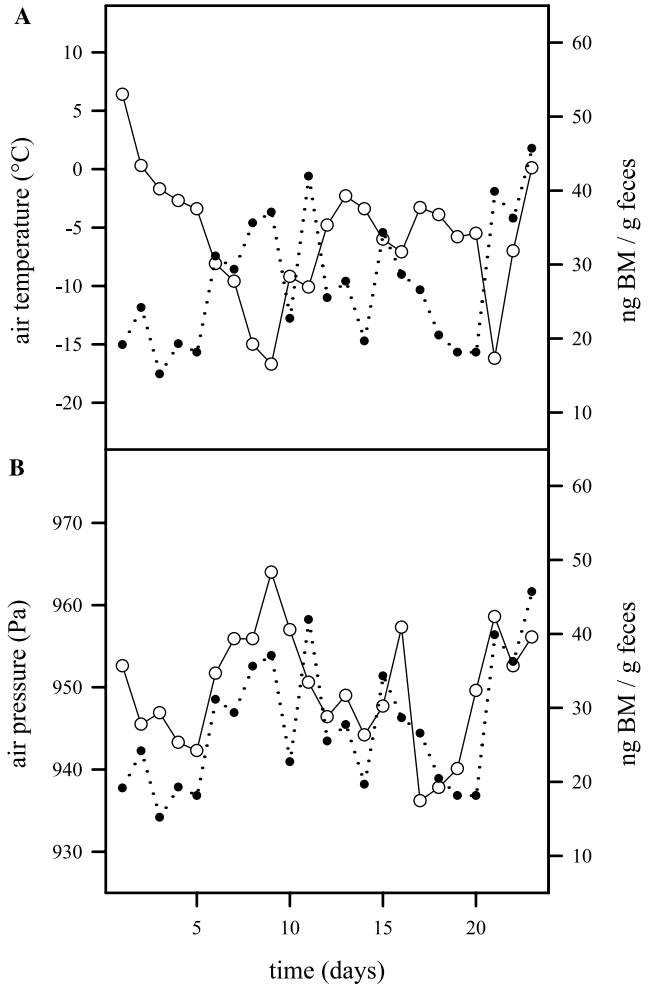

Fig. 2. Covariation of the mean levels of excreted BM (full circles, dotted line) as determined by the "new assay" and (A) the minimum morning temperature (open circles, full line) and (B) the minimum daily air pressure of the foregoing day (open circles, full line).

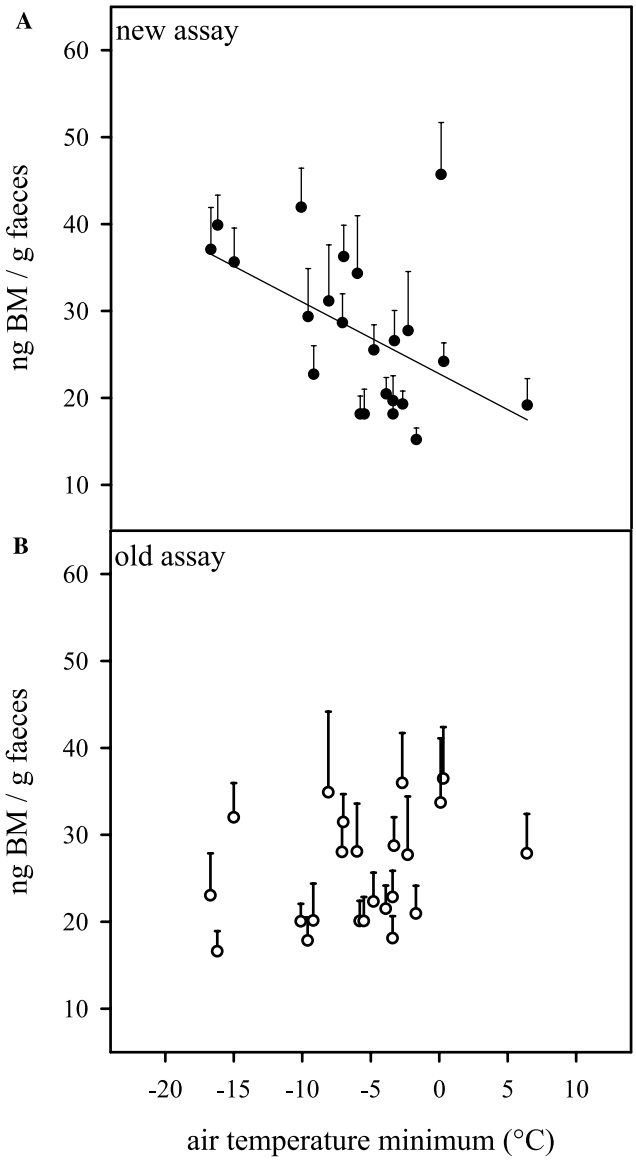

Fig. 3. Minimum values of the morning temperature (°C) and mean levels of excreted corticosterone immuno-reactive metabolites as determined by (A) the "new assay" (+SE; full circles; Spearman, $r_s = -0.536$, $P < 0.01$; $r^2 = 0.26$) and (B) the "old assay" (+SE; open circles; $r_s = 0.362$, $P > 0.0125$).

observed between amounts of excreted BM and the minimum of the air pressure of the afternoon before, with the "new assay" but not with the "old assay" (Table 2; Figs. 2B and 4A and B). None of the weather parameters considered showed significant correlations with excreted immuno-reactive metabolites of androgens (AM; Table 2).

4. Discussion

The linear relationship among atmospheric parameters and corticosterone excretion suggests a continuous and gradual effect of changes in weather conditions on the physiological status of male greylag geese rather than a threshold-function (Fig. 2). The colder the night was, the higher the amounts of excreted corticosterone immuno-reactive metabolites (BM) in the late morning of the following day (Figs. 2A and 3A). It is unlikely that this result is due to a low-temperature-related decrease of gut passage time, the more as increases in corticosterone are related with faster, not slower gut passage in geese (Kotrschal et al., 2000). As this response is a few hours delayed, it may reflect physiological adjustment to temperature rather than an immediate stress response. Additionally, experiments in geese have shown that social and handling stress produce values of fecal corticosterone metabolites which may be approximately 10 times higher than the present response to temperature (Frigerio and Kotrschal, 1999).

The results are consistent with the main metabolic function of glucocorticosteroids, i.e., promoting survival

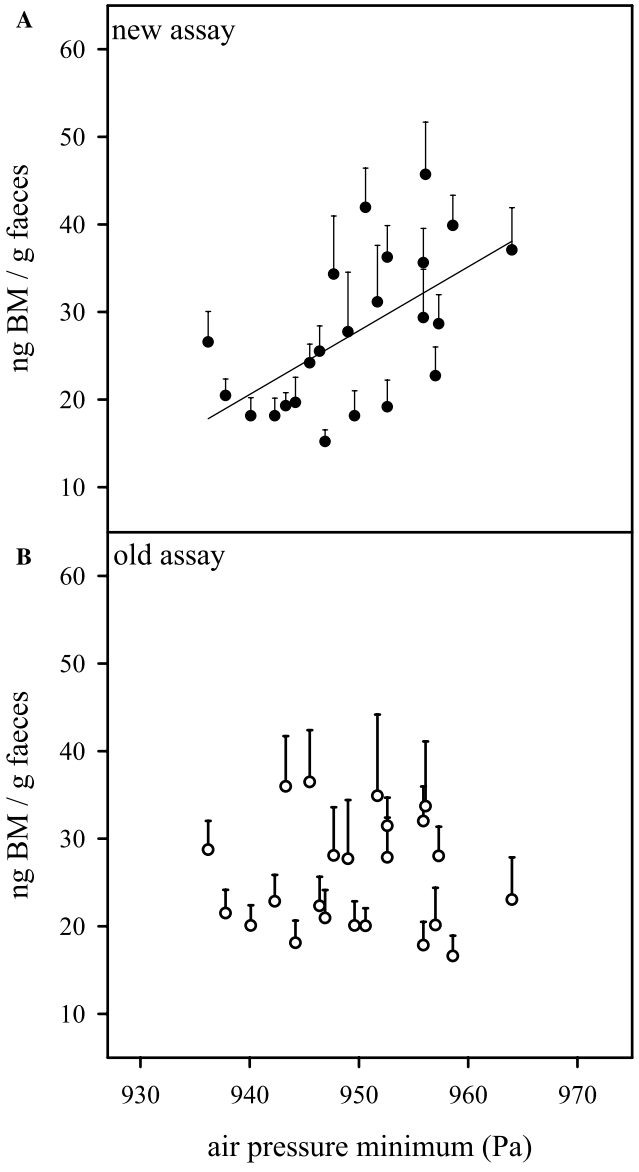

Fig. 4. Minimum values of daily air pressure of the foregoing day and mean levels of excreted corticosterone immuno-reactive metabolites as determined by (A) the "new assay" (+SE; full circles; Spearman, $r_s = 0.62$, $P < 0.008$; $r^2 = 0.35$) and (B) the "old assay" (+SE; open circles; $r_s = -0.049$, $P > 0.05$).

via energy mobilization and energy allocation, at the same time maintaining body's physical condition (Siegel, 1980; Wingfield et al., 1997a). In the present data set, BM increased with decreasing ambient temperature. This could suggest a role of increased adrenal activity in thermoregulation. In fact, even though thyroid hormones play the major role with respect to thermogenesis and basal metabolic rate, corticosteroids are also involved. They are at least modulators and mediators, due to their profound effects on glucose transport and intermediary metabolism (see Bentley, 1998). It has also been shown that adrenalectomized rats, for example, have difficulties in thermoregulation when trying to adapt to a cold environment (Deavers and Musacchia, 1979).

Similarly, the lower the air pressure measured on the previous day, the lower the levels of BM on the next morning (Figs. 2B and 4A). Such a long lag between stimulus and response is consistent with the idea that air pressure is, indeed, used a predictor. This may reflect a function in down-regulation of activity patterns with low air pressure. Several studies have been describing the effects of extreme weather on bird behavior: individuals may leave the breeding territory or the winter home range (irruptive migrations; Elkins, 1988; Newton, 1998; see also Silverin, 1998) alternatively, in other cases, they may become inactive, reducing energy expenditure in an attempt to "ride out" the period of inclement weather (Elkins, 1988). The novel aspect of this study is that reactions to climatic changes did not involve extreme weather conditions. The temperature and air pressure changes were well within the range of seasonally occurring fluctuations. Behaviorally, corticosterone may increase or decrease activity patterns depending on severity and duration of adverse weather (see also Wingfield et al., 1997a) and elevated corticosterone may affect feeding, which is adaptive to balance energetic costs caused by environmental unpredictability (Silverin, 1986). Significant effects of air pressure and ambient temperature on activity levels and behavior of the geese were found in a follow-up study (Dorn, unpublished data). Therefore, the fluctuations in weather and physiology found in this study may represent the lower end of a grated adrenal and behavioral response to adverse weather.

The elapsed time between the recorded value of ambient temperature and air pressure and the correlation with excreted BM might reflect time needed by the organism to adapt to changes in the physical environment, the more as systemic hormone surplus is excreted quickly in geese (maximum excretion at about 90 min; Kotrschal et al., 2000; Krawany, 1996). For example, Elkins (1965) reported that spring movements of wintering ducks from the sea to the coasts always occurred three days after an incursion of mild air. In turn, Astheimer et al. (1995) and Romero et al. (2000) observed changes in behavior of Arctic-breeding passerines within 24–72 h after the beginning of a storm. As in the present study samples were frozen within less than an hour after collection, we can exclude any potential direct effect of air temperature on the procedures of sample collection and storage before freezing. This was additionally examined by comparing changes in the concentrations of excreted metabolites from feces collected, processed, and stored at different temperature and with different time lags (own unpublished data).

Unexpected severe weather conditions have been shown to delay the onset of breeding despite functional

gonads, by depressing circulating levels of gonadal steroids (e.g., Wingfield et al., 1983). In the present study, no significant effect of ambient temperature and air pressure were observed on levels of excreted AM. However, this was not to be expected, because of the generally low levels of excreted androgens outside the reproductive season (Hirschenhauser et al., 1999).

One of the major methodological messages from our work is that, despite the significant correlation between the results of the two assays (Fig. 1), and their relationship with ACTH application (own unpublished data), only the "new assay" revealed positive relationships with weather conditions. Such differences could be explained by the different corticosterone metabolites measured by the two assays. In ruminants, for example, fecal cortisol metabolites are representative for the stress of transport and novel environment but not for that caused by invasive diagnostic procedure (Hopster et al., 1999; Möstl et al., 2002). From the analytical point of view, our results suggests that the assay selection for non-invasive monitoring procedures should also include a test for resolving power, as not all assays may show the same biological sensitivity.

Another, more general methodological conclusion from our present results is the need to consider ambient temperature and air pressure as factors to correct for when using corticosterone or its excreted metabolites in behavioral endocrinology.

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