Assessing Stress in Arctic Lemmings: Fecal Metabolite Levels Reflect Plasma Free Corticosterone Levels

Dominique Fauteux[1,*]
Gilles Gauthier[1]
Dominique Berteaux[2]
Curtis Bosson[3]
Rupert Palme[4]
Rudy Boonstra[3]
[1]Department of Biology and Centre d'études nordiques, Université Laval, 1045 Avenue de la Médecine, Quebec, Quebec G1V 0A6, Canada; [2]Canada Research Chair on Northern Biodiversity and Centre d'études nordiques, Université du Québec à Rimouski, 300 Allée des Ursulines, Rimouski, Quebec G5L 3A1, Canada; [3]Centre for the Neurobiology of Stress, Department of Biological Sciences, University of Toronto Scarborough, 1265 Military Trail, Toronto, Ontario M1C 1A4, Canada; [4]Department of Biomedical Sciences, University of Veterinary Medicine, A-1210 Vienna, Austria

Accepted 1/12/2017; Electronically Published 3/8/2017

Online enhancements: supplemental tables, figures.

ABSTRACT

Interest in the ecology of stress in wild populations has triggered the development of noninvasive methods for quantifying stress hormones. Measurement of fecal corticosteroid metabolites (FCMs) is one such method, but it is still unclear whether FCMs can be a reliable proxy of free plasma glucocorticoids. To assess the validity of this assumption, we carried out a robust assessment on brown lemmings (*Lemmus trimucronatus*) from Bylot Island, Nunavut, Canada, that were hand captured and anesthetized and related plasma glucocorticoid levels to fecal metabolite glucocorticoid levels. We examined endogenous factors that could explain interindividual variability. Blood corticosterone was measured from samples obtained on capture and 30 min later, and FCM levels were measured from animals kept in captivity for 72 h. Plasma free corticosterone increased 135-fold over baseline values 30 min after capture, which confirmed that initial handling was perceived as a stressor. We found that FCM levels were highly related with free (marginal $R^2_m = 0.53$) but not with total ($R^2_m = 0.02$) corticosterone levels, regardless of age, sex, and reproductive condition. FCM levels started increasing 2 h after capture and reached maximum levels 4 h after capture. No circadian rhythm in FCMs was found. Plasma total corticosterone levels were much higher in adult females compared with adult males, but this difference was much smaller when measuring free corticosterone levels and FCM levels. Our results suggest that FCM levels are good measures of stress by being closely related to plasma free corticosterone levels in brown lemmings.

Keywords: small mammals, rodents, cycles, free hormone hypothesis, wildlife endocrinology.

*Corresponding author; e-mail: dominique.fauteux.1@ulaval.ca.

Physiological and Biochemical Zoology 90(3):370–382. 2017. © 2017 by The University of Chicago. All rights reserved. 1522-2152/2017/9003-6133$15.00. DOI: 10.1086/691337

Introduction

In the Arctic, the 24-h daylight that prevails during the summer in comparison to the total darkness and extreme cold in winter creates a highly contrasting environment that affects the daily activities of animals. Moreover, small rodents such as voles and lemmings are exposed to highly fluctuating predation risks and food abundance because of the strong seasonality (Gilg et al. 2009; Fauteux et al. 2015). These highly variable environmental conditions expose small rodents to multiple natural stressors, making them ideal subjects to study the endocrine responses of animals facing challenging environments.

New methods to measure endocrine responses of animals to stressful events have been increasingly developed over the past 2 decades to address both physiological and ecological questions (Boonstra 2013). The hypothalamic-pituitary-adrenocortical (HPA) axis is responsible for the secretion of glucocorticoids into the blood in response to a stressor. Glucocorticoids (cortisol or corticosterone, depending on the species) play key roles in the reproductive and immune systems of animals and influence their behavior (Sapolsky et al. 2000; Creel et al. 2013; Crespi et al. 2013). Noninvasive field methods to measure glucocorticoid metabolites in feces have proven useful to assess adrenocortical activity (Sheriff et al. 2011*a*). Enzyme immunoassays (EIAs) are commonly used to quantify those metabolites (Möstl and Palme 2002; Palme 2005) and have been successfully developed for several small rodent species (bank vole, *Myodes glareolus*: Ylönen et al. 2006; North American squirrel, *Tamiasciurus hudsonicus*: Dantzer et al. 2010; Columbian ground squirrels, *Urocitellus columbianus*: Bosson et al. 2009; chipmunks, *Tamias* spp.: Montiglio et al. 2012; Hammond et al. 2015).

The use of fecal corticosteroid metabolites (FCMs) to quantify stress levels relies on a key assumption from the free hor-

This content downloaded from 193.171.106.199 on April 08, 2017 11:36:01 AM
All use subject to University of Chicago Press Terms and Conditions (http://www.journals.uchicago.edu/t-and-c).

mone hypothesis: free glucocorticoids, which are biologically active, are mostly those metabolized in the liver, whereas glucocorticoids bound to corticosteroid-binding globulin (CBG) are biologically inactive and are not metabolized (Sheriff et al. 2010; Perogamvros et al. 2012; Breuner et al. 2013). Recognizing this distinction is crucial because glucocorticoid metabolite concentrations will be strongly affected by the metabolism. For instance, during a stressful event, glucocorticoids are produced, but some of them are quickly bound by CBG and subsequently released during the progressive return to baseline concentrations. These slowly released glucocorticoids will then be metabolized, which should maintain elevated FCM concentrations compared with the baseline for some time (Schoech et al. 2013). However, evidence of the relationship between free glucocorticoids and FCMs is still scarce (i.e., Sheriff et al. 2010), despite the importance of this assumption when assessing the physiological impacts of stress (Breuner et al. 2013). Moreover, since some evidence suggests that bound glucocorticoids may act as a reservoir for free glucocorticoids (Schoech et al. 2013), further investigations of the relationships between FCMs and total or free corticosterone are needed.

Numerous endogenous factors can affect glucocorticoid levels and hence the stress response of animals, including age, sex, reproductive state, and circadian rhythm (Boonstra and Boag 1992; Touma et al. 2003; Romero et al. 2008; Sheriff et al. 2009*a*). The inhibitive effect of testosterone on the HPA axis or the stimulating effect of estrogen can induce large differences in plasma glucocorticoid concentrations between sexes and reproductive and nonreproductive individuals (Boonstra et al. 2007). For example, Romero et al. (2008) reported that female brown lemmings (*Lemmus trimucronatus*) have substantially higher baseline levels of total corticosterone and CBG compared with males. Similar sex effects were found in other rodents (voles: Boonstra and Boag 1992; Fletcher and Boonstra 2006; squirrels: Boonstra et al. 2001*a*, 2012). Boonstra et al. (2001*b*) reported that reproductively active male ground squirrels (*Urocitellus parryii plesius*) had higher free cortisol concentrations than reproductively inactive males. Thus, even though fecal metabolites have the potential to be a good proxy of the physiological response of the HPA axis to a stressor, adequate validation must account for sex, age, and reproduction effects (Touma and Palme 2005). This is an essential prerequisite before examining the effects of external factors, such as being exposed to low-quality food or high predation risk on the stress response of individuals (McArthur et al. 2014).

We assessed the stress response of a cyclic brown lemming population in the high Arctic during the summer. Our main goal was to understand how brown lemmings respond to a standardized stressor and to determine under which conditions FCMs can be used as a good proxy for stress in this species. In order to achieve this, we obtained blood samples at capture (i.e., baseline) and 30 min later (i.e., stressed) and a complete FCM profile of the stress response over a period of 72 h. More specifically, our objectives were to determine (1) whether baseline, maximum, and relative change of plasma corticosterone and FCM levels vary as a function of age, sex, and reproductive condition; (2) the time required until maximum FCM levels are reached after capture stress; (3) whether a circadian rhythm in FCM levels occurs, even though lemmings live under 24 h of daylight during the summer; and (4) whether FCM levels are related to plasma free corticosterone and are thus a good indicator of stress. Glucose concentrations were measured in parallel with plasma corticosterone because they have been found to change under acute and chronic stress conditions and thus can provide complementary information on the state of animals (Boonstra et al. 1998; Breuner et al. 2013).

Methods

Study Area

Our study was conducted on Bylot Island in Sirmilik National Park, Nunavut, Canada (73°08′N, 80°00′W). The study site is in a large valley composed of two main habitat types, wet and mesic tundra (Duchesne et al. 2011). Both habitats are used by brown lemmings with seasonal shifts toward wetter habitat in summer and more mesic habitat when snow settles in fall. Snow typically covers the site for more than 8 mo a year from early October until mid-June, and the average annual temperature is −15°C (Cadieux et al. 2008). Animals are exposed to 24 h of daylight from early May to early August and 24 h of darkness in winter. Brown lemmings can breed almost year-round, including under the snow in winter and throughout the summer (Gruyer et al. 2010; Fauteux et al. 2015).

Capture of Animals

In June and July in 2012 and 2014, we searched for brown lemmings and captured them by hand, following the method of Romero et al. (2008). Hand capture was used to obtain baseline levels of corticosterone in the blood and in feces because the hormonal response to a stressor such as capture should appear in the blood within 3 min and FCMs should increase a few hours after capture (Palme et al. 2005; Romero and Reed 2005; Delehanty and Boonstra 2012).

Lemmings were captured between 1000 and 1600 hours by walking slowly through suitable habitat looking for movement on the ground. When a lemming was spotted, one or two observers walked slowly toward it until it ran back into a burrow. Observers waited immobile at a spot where they could monitor all potential burrow exits until the animal came out, typically a few seconds to 2–3 min later. One observer attempted to quickly grab the lemming by hand. If this failed, a second attempt was made within 2 min of the first attempt. A lemming that could not be captured within 2 min was abandoned.

Individuals were quickly sexed and their reproductive condition determined. In females, we noted whether their pubic symphysis was open and whether they were lactating or pregnant; in males, we noted whether their scrotum was well developed and testes prominent (Fauteux et al. 2015). Lemmings were immediately anesthetized by holding a delivery tube with a cotton ball

This content downloaded from 193.171.106.199 on April 08, 2017 11:36:01 AM
All use subject to University of Chicago Press Terms and Conditions (http://www.journals.uchicago.edu/t-and-c).

soaked in 20% isoflurane over the nose and mouth, following the method of Itah et al. (2004). A 75-μL blood sample was taken from the retro-orbital sinus of the anesthetized lemming with a heparinized Pasteur pipette tube. This method is a rapid and efficient way of obtaining blood samples in small mammals while minimizing harm (Bradshaw 2003). Anesthesia of lemmings was generally rapid (30–120 s), as was blood sampling (<15 s), and thus blood samples were obtained ≤3 min after capture, though we do not know the exact length of time between the lemming becoming aware of us approaching and the bleed and whether the interval was longer than 3 min. We could not record exact times because of difficulties related to hand captures in most cases, but video recordings of three captures indicated that the delay between first capture attempt and blood sampling averaged 2 min 51 s. When the lemming had regained consciousness, we carried it to the field station in a small plastic box (<20-min walk). We collected fecal samples within 15 min of capture directly from the anus of the lemmings (if available) or from the transport box. We used flat-tipped forceps disinfected with benzalkonium chloride to collect fecal samples. Field manipulations and housing procedures were approved by the Animal Welfare Committee of Université Laval (2014-061) and Parks Canada (SIR-2013-13953).

Blood and Feces Sampling after Initial Capture

A second blood and fecal sample was taken at the field station 30 min after capture. Glucose concentration (mg/dL) in blood samples (0 and 30 min after capture) was obtained with a portable electronic reader on a 0.3-μL subsample (FreeStyle, Abbott Park, IL). Blood samples were centrifuged at 13,000 *g* for 5 min in heparinized microhematocrit tubes. The plasma fraction was transferred into 0.6-mL plastic tubes and stored at −20°C before shipping to the university for hormonal analyses.

Lemmings were held at the field station for 72 h in individual 40 × 28 × 24-cm hamster cages; fed ad lib. with fresh grasses, forbs, shrubs, pieces of apple, peanut butter, and hamster chow; and supplied with water. Lemmings were held in unheated rooms with windows to ensure natural fluctuations of light and temperature. We minimized potential contamination of feces by urine by adding double bottoms made of steel wire mosquito nets.

Fecal samples of captive animals were collected at 2-h intervals for the first 8 h and at 4-h intervals afterward (i.e., 8, 12, 16 h, etc.). Lemmings were kept in captivity for 72 h to assess the temporal variations of FCMs and determine the presence or absence of a circadian rhythm. This also allowed time for lemming stress levels to return close to baseline levels after habituation to captive conditions. At each sampling occasion, we randomly collected ~1 mL of dry feces (10–15 pellets) in 1.5-mL plastic tubes and removed all other feces from the cage. Fecal samples were stored at −20°C until shipping to the university. The time associated with each fecal sample corresponds to the end of the interval during which they were produced (e.g., feces collected at 12 h were excreted between 8 and 12 h). Visibly wet feces were not collected to avoid urine-contaminated samples. At the end, all lemmings were released at their site of capture.

Plasma Corticosterone Assay

We measured total corticosterone in plasma in one run using a modified radioimmunoassay (Boonstra and Boag 1992). In order to extract steroids from plasma and saponify triglycerides, we vortexed 5 μL of plasma with 1 mL of methylene chloride and 20 μL of ammonium hydroxide for 4 min, followed by centrifugation (225 *g* for 5 min). We subsampled duplicate 200-μL aliquots of the methylene chloride suspensions, dried them to completion under filtered air, and then reconstituted them in 300 μL of phosphate buffered saline. For the assay procedure, we incubated the reconstituted aliquots overnight at 4°C with 100 μL of CJM006 corticosterone antibody (1 : 20,000; C. Munroe, University of California, Davis, CA) and 100 μL of 1,2,6,7-[^{3}H] corticosterone (2 nM). We then separated free corticosterone with 200 μL of dextran-coated charcoal (DCC) at 0°C, centrifuged them (1,750 *g* for 15 min) at 0°C, and measured the radioactivity of the supernatant. Corticosterone values for unknowns were inferred from a standard curve of known corticosterone standards.

The cross reactivity for the CJM006 antibody is reported as follows: corticosterone 100%; desoxycorticosterone 14.25%; tetrahydrocorticosterone 0.9%; 11-desoxycortisol 0.03%; prednisone <0.01%; prednisolone 0.07%; cortisol 0.23%; cortisone <0.01%; progesterone 2.65%; testosterone 0.64%; and estradiol 17b <0.01%. The average coefficient of variation for replicates was 13.5% ± 4.0% ($n = 37$). Three samples with high coefficients of variation (>50%) were included in the analyses because values obtained were in the range of values obtained for other lemmings of same age and sex.

As recommended by Delehanty et al. (2015), we measured the maximum corticosterone binding capacity (MCBC) of each plasma sample. We used a slightly modified version of the DCC separation method of Hammond and Lähteenmäki (1983). In brief, plasma was stripped of endogenous steroids at room temperature in a DCC solution for 30 min. Stripped plasma (most at a final dilution of 1 : 30,000 but a few at 1 : 3,000 because of them having relatively low MCBC) were incubated with a 3.7 nM solution of 1,2,6,7-[^{3}H] corticosterone for 1 h at 20°C. We ran two total binding replicates and one nonspecific binding tube (to which we added an additional 4,000 nM corticosterone standard) for each sample. We separated bound and free 1,2,6,7-[^{3}H] corticosterone with an ice-cold DCC solution for 10 min, followed by centrifugation (1,750 *g* for 15 min), and then measured the radioactivity (counts per minute [CPM]) of the supernatant. MCBC was estimated by subtracting each sample's nonspecific binding CPM from its total binding CPM and then calculating the ratio of 1,2,6,7-[^{3}H] corticosterone in the supernatant to the amount of 1,2,6,7-[^{3}H] corticosterone added to each tube. The average specific binding was 10.7% ± 1.9% of the total 1,2,6,7-[^{3}H] corticosterone added to each tube.

Free corticosterone concentrations were calculated using the equation of Barsano and Baumann (1989). We used the CBG affinity constant reported for brown lemmings ($K_d = 10.79$; Romero et al. 2008).

This content downloaded from 193.171.106.199 on April 08, 2017 11:36:01 AM
All use subject to University of Chicago Press Terms and Conditions (http://www.journals.uchicago.edu/t-and-c).

Extraction and Measurement of Fecal Corticosterone Metabolites (FCMs)

We extracted fecal metabolites with a protocol developed for squirrels but modified for smaller samples (Touma et al. 2003; Bosson et al. 2009; Dantzer et al. 2010). Fecal samples were freeze-dried for ≥12 h, and dried samples were refrozen with liquid nitrogen to facilitate crushing with a mortar and pestle. We subsampled 30 ± 5 mg of the resulting powder when possible and recorded the mass precisely because some samples were small ($\overline{m} = 28.4 \pm 0.3$ mg, $m_{\min} = 3.0$ mg, $m_{\max} = 33$ mg). Samples were extracted in 1 mL of an 80% methanol solution, vortexed (1,500 rpm) for 30 min, and centrifuged (2,500 *g*) for 15 min. An aliquot (0.7 mL) of the supernatant was transferred into a 1.5-mL plastic tube and stored at −20°C until analysis by the EIA.

As a starting point, we first characterized FCM metabolites by reverse-phase high-performance liquid chromatography (Touma et al. 2003) in two adult female lemmings (one pregnant, one nonreproductive) captured in 2012 and subsequently determined the immunoreactivity with two EIAs previously used in small rodents, namely, a 5α-pregnane-3β,11β,21-triol-20-one EIA (Touma et al. 2003) and an 11-oxoetiocholanolone EIA (Montiglio et al. 2012).

Statistical Analyses

Blood data were analyzed using linear models with a Gaussian distribution because we had a single measurement per individual at each time period. The only exceptions were when we used total corticosterone, free corticosterone, and MCBC to test the stress response to capture; we used linear mixed effects models with sample (i.e., baseline at $t = 0$ and stressed at $t = 30$ min) as the fixed factor and individuals as the random factor.

We developed four candidate models to test the effects of various factors at $t = 0$ and $t = 30$ min on total corticosterone concentration (total.c_0 and total.c_{30}), maximum corticosterone binding capacity (mcbc_0 and mcbc_{30}), free corticosterone (free.c_0, free.c_{30}), glucose concentration (g_0, g_{30}), and their relative change (i.e., ratio of values at $t = 30$ and $t = 0$; $r_{\mathrm{total.c}}$, r_{mcbc}, $r_{\mathrm{free.c}}$, and r_g). Model 1 tested for differences among adult females, adult males, and juveniles (groups; classified on the basis of sex and body mass; adult females: ≥28 g; adult males: ≥30 g; juveniles: all others; Fauteux et al. 2015). Model 2 examined the effect of reproductive activity only, model 3 tested for an interaction between sex and reproductive activity, and model 4 was the null model. For the second and third models, juveniles were separated according to their sex and considered nonreproductive. Females were classified as reproductively active if they had a perforate vagina, were lactating, or were pregnant. Males were classified as reproductively active if they had a well-developed scrotum. We selected the most parsimonious model on the basis of the second-order Akaike information criterion corrected for small sample sizes (AICc) and report the Akaike weight of each model. When two models were equivalent ($\Delta\mathrm{AICc} \ll 2$), we based our interpretation on the model with the smallest number of parameters (Arnold 2010). Using this model selection procedure reduced the risk of reporting spurious effects caused by small sample sizes. Because juveniles were reproductively inactive and our global model included an interaction, we could not model-average estimates (Burnham and Anderson 2002). Tukey's multiple comparisons test from the multcomp package implemented in the R software (R Development Core Team 2014) were used to compare blood characteristics among lemming groups (Hothorn et al. 2008).

We determined the delay for FCMs to appear in feces after capture with a linear model that compared FCM levels at $t = 0$ to later samples (i.e., 0.5, 2, 4, 6, and 8 h after capture). We used the same four previously described candidate models to test the effect of various factors on initial, maximal, and the relative change of FCMs between the moment of capture and the maximal level attained. We conducted autocorrelation analyses on time series to determine whether we could detect internal rhythm in ln-transformed FCM values at different time intervals: t versus $t + 4$ h, $t + 8$ h, . . . , $t + 24$ h (Venables and Ripley 2002). Analyses started 12 h after capture (i.e., $t \geq 12$ h) to exclude peak FCM concentrations induced by the capture.

Finally, we conducted an analysis to determine the relationship between total corticosterone, free corticosterone, and FCM concentrations measured by the EIA. We paired blood samples at $t = 0$ and $t = 30$ min with the initial (i.e., $t = 0$) and maximal FCM concentrations, respectively. We used mixed effects linear models with individual lemmings as the random factor.

Response variables were ln-transformed if it increased normality and homoscedasticity of residuals. Homoscedasticity was assessed visually for each model by plotting the residuals as a function of fitted values. When mixed effects models were used (i.e., when individual was included as a random effect), we estimated the coefficients of determination, following Nakagawa and Schielzeth (2013). We report the marginal (R^2_{m}, related to fixed effect) unless mentioned otherwise. All means are presented with their respective standard errors (±SE) in the text, and coefficient estimates from models are given with their respective 95% confidence intervals (CIs).

Results

Twenty lemmings were captured and 18 were held in captivity: nine adult females (six reproductive and three nonreproductive), five adult males (three reproductive and two nonreproductive), and four juveniles (two of each sex). Initial blood measurements were taken on two additional individuals (one reproductive male and one nonreproductive adult female) that had cardiorespiratory failures during anesthesia.

Plasma Levels

All lemmings ($n = 18$) strongly responded to capture and anesthesia by showing much higher levels of total corticosterone ($\beta = 0.97$, CI = 0.67–1.27, $R^2_{\mathrm{m}} = 0.09$) and free corticosterone ($\beta = 4.05$, CI = 3.28–4.81, $R^2_{\mathrm{m}} = 0.78$) 30 min

This content downloaded from 193.171.106.199 on April 08, 2017 11:36:01 AM
All use subject to University of Chicago Press Terms and Conditions (http://www.journals.uchicago.edu/t-and-c).

later, but MCBC did not change over time ($\beta = -0.05$, CI $= -0.19$ to 0.09, $R^2_m = 0.00$; fig. 1). Initial total corticosterone concentrations and MCBC were on average 10–20 times higher in adult females ($\overline{\text{total.c}_0} = 3{,}119 \pm 614$ ng/mL, $\overline{\text{mcbc}_0} = 6{,}552 \pm 1{,}421$ ng/mL, $n = 9$) compared with adult males ($\overline{\text{total.c}_0} = 228 \pm 83$ ng/mL, $\overline{\text{mcbc}_0} = 471 \pm 124$ ng/mL, $n = 5$) and juveniles ($\overline{\text{total.c}_0} = 153 \pm 17$ ng/mL, $\overline{\text{mcbc}_0} = 506 \pm 40$ ng/mL, $n = 4$; table 1). Similar results were found in samples taken 30 min after capture, with higher concentrations in adult females ($\overline{\text{total.c}_{30}} = 7{,}213 \pm 1{,}508$ ng/mL, $\overline{\text{mcbc}_{30}} = 6{,}726 \pm 1{,}333$ ng/mL) compared with adult males ($\overline{\text{total.c}_{30}} = 676 \pm 148$ ng/mL, $\overline{\text{mcbc}_{30}} = 399 \pm 95$ ng/mL) and juveniles ($\overline{\text{total.c}_{30}} = 680 \pm 59$ ng/mL, $\overline{\text{mcbc}_{30}} = 499 \pm 41$ ng/mL; table 1). The magnitude of increase in total corticosterone was higher in juveniles ($\overline{r}_{\text{total.c}} = 5.04 \pm 0.84$ times higher) than in adult females (1.99 ± 0.25 times higher) but not in adult males (3.71 ± 0.67 times higher; table 1), and there was no difference in the magnitude of increase of MCBC (r_{mcbc}) among lemming categories (fig. 1). The null models had high support when testing interindividual differences in free corticosterone concentrations for both samples taken at $t = 0$ and 30 min later (free.c_0, ΔAICc $= 0$; free.c_{30}, ΔAICc $= 0.56$), probably owing to the high interindividual variability (fig. 1; table A1; tables A1, A2, D1–D4 are available online).

Initial levels of glucose varied with reproductive condition (simplest model with ΔAICc $= 0.84$) because reproductively active lemmings ($\overline{g}_0 = 145 \pm 10$ mg/dL, $n = 9$) had higher concentrations of glucose compared with inactive ones (116 ± 5 mg/dL, $n = 9$; table 1). The relative change of glucose levels between $t = 0$ and 30 min later was also higher in juveniles compared with adults (fig. 1; tables 1, A1).

Fecal Corticosterone Metabolite Levels

Generally, both antibodies picked up several FCMs (fig. 2) and gave similar results for FCM concentrations (Pearson's $r = 0.58$; fig. B1; figs. B1, C1, D1 are available online). The 11-oxoetiocholanolone EIA detected one main metabolite eluting around high-performance liquid chromatography fraction 70. In contrast, the 5α-pregnane-3β,11β,21-triol-20-one EIA detected several metabolites that were more polar. Metabolites between fractions 32–41 were in higher concentrations in the reproductively active female compared with the inactive one, indicating slightly different patterns due to reproductive conditions. The 5α-pregnane-3β,11β,21-triol-20-one EIA reacted with more different metabolites (fractions 32–41 and 62–72) and thus may yield more stable measurements. We thus utilized the 5α-pregnane-3β,11β,21-triol-20-one EIA for all subsequent analyses. This antibody cross-reacts with metabolites having a 5α-3β,11β diol structure.

Adult males ($\overline{\text{fcm}_0} = 896 \pm 217$ ng/g) tended to have higher baseline FCM levels than juveniles (244 ± 31 ng/g), with adult females showing intermediate values (570 ± 155 ng/g), but the null model (i.e., no difference) was preferred on the basis of parsimony (ΔAICc $= 0.77$; table A2). We found no difference in FCM concentrations between $t = 0$ and 30 min ($\beta = 0.22$, CI $= -0.30$ to 0.73), but average FCM concentrations had

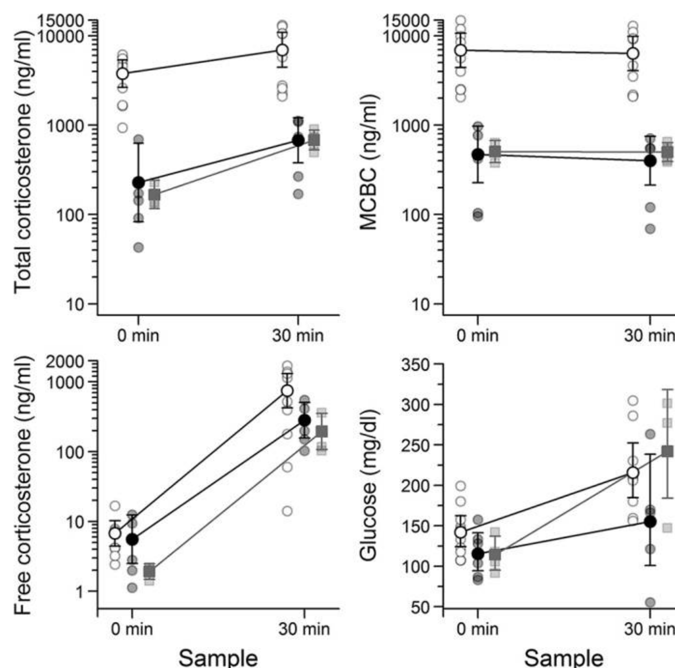

Figure 1. Average plasma concentrations of free and total corticosterone, maximum corticosterone binding capacity (MCBC), and glucose at time 0 (baseline) and 30 min after hand capture in brown lemmings with their respective 95% confidence intervals. Animals were anesthetized before bleeds at both occasions. Mean concentrations for adult females ($n_0 = 10$, $n_{30} = 9$; open circles), adult males ($n_0 = 6$, $n_{30} = 5$; filled circles), and juveniles ($n = 4$; squares). Individual values are presented for each mean values.

This content downloaded from 193.171.106.199 on April 08, 2017 11:36:01 AM
All use subject to University of Chicago Press Terms and Conditions (http://www.journals.uchicago.edu/t-and-c).

Table 1: Effects retained in the most parsimonious models testing for plasma differences in initial ($t = 0$) samples, samples taken 30 min after capture, and relative change (r; ratio between values at $t = 30$ and $t = 0$)

Response variable and comparisons	Model	R^2	β	95% CI
ln(total.c_0)	1	.82		
J-AF			−3.06[a]	−4.15 to −1.96[a]
AM-AF			−3.06[a]	−4.08 to −2.04[a]
AM-J			−.00	−1.25 to 1.24
ln(total.c_{30})	1	.73		
J-AF			−2.17[a]	−3.26 to −1.09[a]
AM-AF			−2.40[a]	−3.42 to −1.39[a]
AM-J			−.23	−1.45 to .99
$r_{\text{total.c}}$	1	.36		
J-AF			3.05[a]	.55 to 5.56[a]
AM-AF			1.72	−.78 to 4.23
AM-J			−1.33	−4.28 to 1.62
ln(mcbc_0)	1	.74		
J-AF			−2.36[a]	−3.72 to −1.01[a]
AM-AF			−2.81[a]	−3.94 to −1.69[a]
AM-J			−.45	−1.96 to 1.05
ln(mcbc_{30})	1	.78		
J-AF			−2.43[a]	−3.60 to −1.27[a]
AM-AF			−2.98[a]	−4.07 to −1.90[a]
AM-J			−.55	−1.85 to .76
g_0	2	.21		
Repro			28.98	3.66 to 54.30
r_g	1	.33		
J-AF			.67	−.07 to 1.40
AM-AF			−.35	−1.09 to .39
AM-J			−1.02[a]	−1.88 to −.15[a]

Note. Variables include total corticosterone (total.c), maximum corticosterone binding capacity (mcbc), and glucose concentrations (g). Results from model selection are presented in appendix A, available online. The coefficients (β) with their 95% confidence intervals (CIs) are presented along with the adjusted R^2 of each model. J, juvenile (females < 28 g, males < 30 g); AF, adult females; AM, adult males; Repro, reproductively active versus inactive (used as the reference group).

[a]95% CI excludes 0.

increased by 521% 2 h after capture ($\beta = 1.83$, CI = 1.32–2.34). FCM concentrations reached their highest levels 3.9 ± 0.3 h after capture across individuals (fig. 3), and samples taken 4 h after capture had on average the highest FCM concentration (1,028% increase; $\beta = 2.30$, CI = 1.791–2.81). Reproductively active lemmings ($\overline{\text{fcm}}_{\text{max}} = 8{,}117 \pm 3{,}003$ ng/g) reached higher maximum FCM concentrations compared with reproductively inactive ones (2,606 ± 390 ng/g; $\beta_{\text{fcm}} = 0.86$, CI = 0.23–1.50). Indeed, the model including only reproductive activity had similar statistical support (ΔAICc = 1.17) as the more complex model including the interaction between sex and reproductive condition. The relative change of FCMs between initial and maximal concentrations was similar among lemming groups (table A2). The 72-h FCM concentration profile of each individual lemming is presented in appendix C (apps. A–D are available online).

The autocorrelation analyses for circadian rhythm indicate that FCM levels measured at time t and $t + 4$ h were positively correlated (table 2). The autocorrelation between FCM levels measured at time t and $t + 24$ h was weak, though it approached the significance level.

When results of all lemmings were pooled, FCMs were positively related to plasma free corticosterone concentrations ($\beta_{\text{free.c}} = 0.42 \pm 0.07$, CI = 0.28–0.56, $R^2_{\text{m}} = 0.53$; fig. 4) but not to total corticosterone concentrations ($\beta_{\text{total.c}} = 0.11 \pm 0.14$, CI = −0.20 to 0.42, $R^2_{\text{m}} = 0.02$). Even after considering group, sex, and reproduction conditions in post hoc analyses, FCMs and total corticosterone were positively related ($\beta_{\text{total.c}} = 0.67 \pm 0.24$, CI = 0.19–1.15, partial $R^2 = 0.18$), but the relationship remained weaker than the one between FCMs and free corticosterone ($\beta_{\text{free.c}} = 0.43 \pm 0.06$, CI = 0.31–0.55, partial $R^2 = 0.55$; see app. D).

Discussion

We assessed the stress response of brown lemmings in a cyclic population inhabiting the high Arctic. We showed that plasma

This content downloaded from 193.171.106.199 on April 08, 2017 11:36:01 AM
All use subject to University of Chicago Press Terms and Conditions (http://www.journals.uchicago.edu/t-and-c).

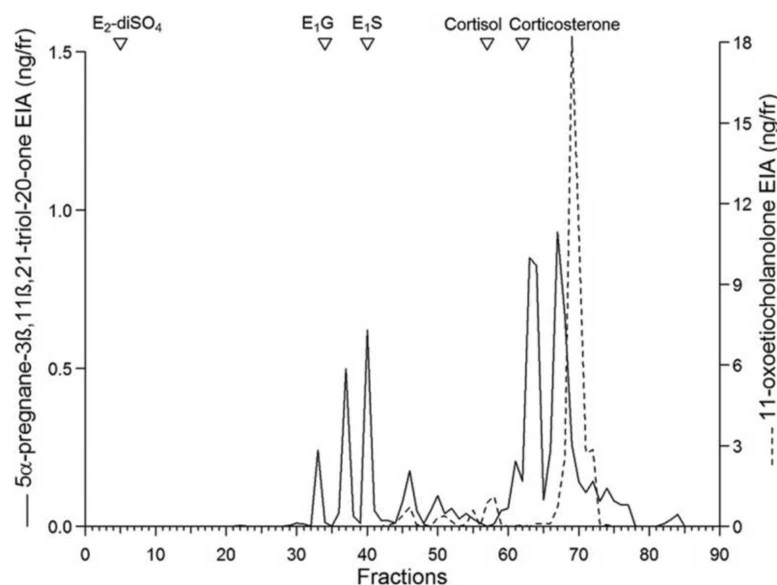

Figure 2. Reverse-phase high-performance liquid chromatography immunogram comparing cross-reactivity with lemming fecal corticosterone metabolites measured by two enzyme immunoassays (EIAs). The mass of metabolites per fraction (ng/fr) measured on one reproductively inactive adult female are presented. Peak immunoreactivity occurred at fractions 62–72 with both antibodies, indicating cross-reactivity with metabolites of similar polarity. The elution times of the estradiol disulphate (E_2-diSO$_4$), estrone glucuronide (E_1G), estrone sulfate (E_1S), cortisol, and corticosterone standards are indicated by triangles.

corticosterone and FCM increased markedly after hand capture, which confirms that both measures adequately detected the stress response of lemmings. Glucose concentrations also increased as expected under stressful conditions (Breuner et al. 2013). We found that FCM concentrations were highly related to plasma free corticosterone concentrations but not to total corticosterone concentrations, even though the latter had also increased in response to the stressor. This is only the second time that such a relationship has been found (Sheriff et al. 2010). Lemmings reached maximal FCM levels on average 3.9 h after capture, suggesting rapid metabolism of corticosterone after being stressed. Sex, reproductive status, and age had relatively weak effects on plasma free corticosterone and FCM levels but strong effects on plasma total corticosterone levels. Our findings confirm the usefulness of FCMs to measure stress levels as they reflect well the biologically active portion of corticosterone. Large interindividual variability in blood parameters could be partly explained by age, sex, or reproductive condition, though less so for FCMs, likely a consequence of our relatively small sample size. We found no evidence for a circadian rhythm in FCMs at a site where sunlight in summer is present 24 h a day.

Measurement of FCMs

We found that the 5α-pregnane-3β,11β,21-triol-20-one EIA successfully detected an endocrine response in lemming feces to hand capture and associated manipulations. Our results substantiate previous studies that show reliable detection of fecal glucocorticoid metabolites by this EIA in other rodent species (Touma et al. 2003; Bosson et al. 2009, 2013; Dantzer et al. 2010; Montiglio et al. 2012; Hammond et al. 2015). The large increase in plasma total and free corticosterone and glucose 30 min after capture coupled with the sharp increase in FCM concentrations 4 h after hand capture indicates that lemmings responded strongly to capture, anesthesia, and transport to the lab. Trapping has been shown to be stressful in several small mammals (Fletcher and Boonstra 2006; Bosson et al. 2013). The combined effect of hand capture and anesthesia clearly elicited a strong stress response and thus can be an interesting alternative to hormonal challenges (e.g., with adrenocorticotropic hormone [ACTH]) when such tests cannot be conducted because of logistical constraints (Touma and Palme 2005). The 4-h delay in the increase of FCM concentrations is similar to the one found by Rogovin and Naidenko (2010) in bank voles, which is half than in laboratory mice (*Mus musculus domesticus*; 8 h; Touma et al. 2003). This delay is also shorter than in larger rodents (7–8 h in Columbian ground squirrels, North American red squirrels, and eastern chipmunks, *Tamias striatus*; Bosson et al. 2009; Dantzer et al. 2010; Montiglio et al. 2012), which supports the hypothesis that the speed at which glucocorticoids are voided in feces may depend on gut passage time (Palme et al. 2005).

The objective in most field studies is to measure stress signatures in feces that are indicative of the true basal level and not affected by capture and handling. There is a lag between the moment of capture and the moment at which FCM concentrations start increasing substantially, and this needs to be considered when sampling wild populations. Although it took about 4 h for lemmings to reach maximum FCM concentrations, lemmings generally reached half of their maxima only 2 h after capture. This speed of excretion is surprisingly

This content downloaded from 193.171.106.199 on April 08, 2017 11:36:01 AM
All use subject to University of Chicago Press Terms and Conditions (http://www.journals.uchicago.edu/t-and-c).

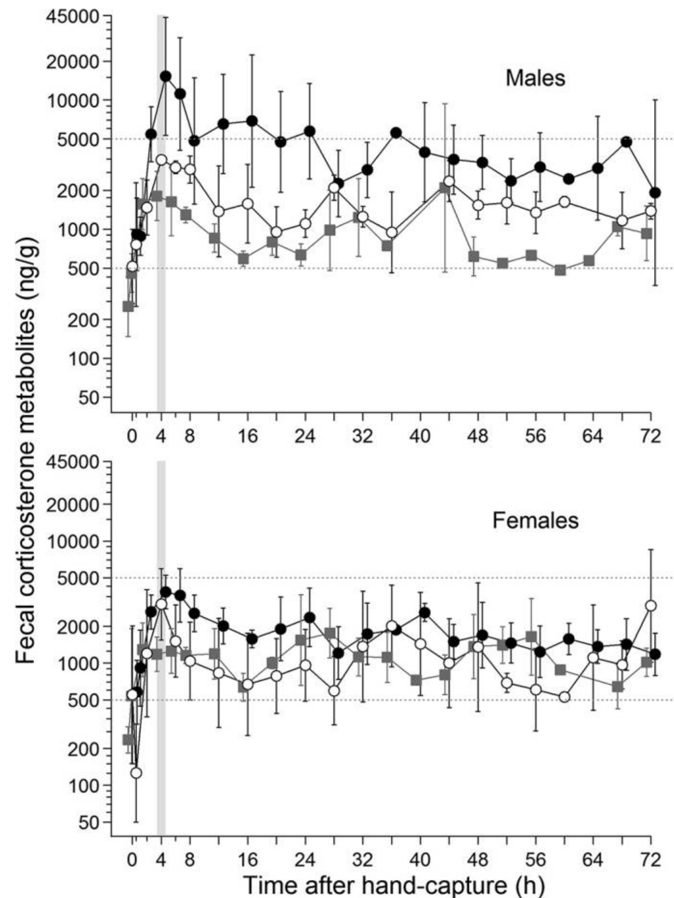

Figure 3. Time series of fecal corticosterone metabolite (FCM) concentrations (mean with 95% confidence intervals) in male and female lemmings measured by the enzyme immunoassay (see "Methods"). Filled circles, reproductive adults ($n_{\text{males}} = 3$, $n_{\text{females}} = 6$); open circles, nonreproductive adults ($n_{\text{males}} = 2$, $n_{\text{females}} = 3$); squares, juveniles ($n_{\text{males}} = 2$, $n_{\text{females}} = 2$). Lemmings were captured at time 0 and released 72 h later. Gray bar indicates the mean time when all lemmings reached their maximal FCM concentrations.

fast when comparing with other rodents, which substantiates the necessity of validating FCMs for new species under investigation. To our knowledge, this is the first time that such a rapid elevation of FCMs in feces is observed in small mammals, which could be a consequence of the intensity of the stressor. Indeed, hand capture followed by anesthetization and transportation to the field station is likely to be more stressful than simply being captured in a live trap. Nonetheless, we recommend collecting lemming fecal samples in the field <2 h after a stressful event (such as closing of a trap) to avoid artificially created high stress levels. If the traps are visited at a frequency of >0.5 visit h^{-1}, variation in FCM concentrations due to individuals entering the traps at different moments within the interval should be minimal, and FCMs should provide a reliable measure of baseline stress level.

Fecal Corticosterone Metabolites Reflect Plasma Free Corticosterone

We found that variation in FCM concentrations was highly related ($R^2_{\text{m}} = 0.53$) to that in free corticosterone recorded in the plasma following a stressful event. One potential source of variation in this relationship is that some lemmings may have already started to respond to our approach before capturing them, and thus their plasma corticosterone level may not represent true basal level. However, our results show that free corticosterone was low for all lemmings at the moment of capture ($t = 0$), suggesting that any response to our presence at that time was weak. Interestingly, FCM concentrations were not related to total corticosterone, which substantiates the hypothesis that only free corticosterone (i.e., not bound to proteins) leaves blood circulation (reviewed in Perogamvros et al. 2012; Breuner et al. 2013) when metabolized by the liver, as found in snowshoe hares (Sheriff et al. 2010). Our results do not address the possibility that bound glucocorticoids could play a role in this dynamics (Schoech et al. 2013) but are consistent with the idea that binding proteins prevent metabolism of corticosterone by the liver. Although plasma total corticosterone also increased after the stressor, the relatively small change compared to free corticosterone prevented a strong relationship with FCMs even when accounting for sex differences in total corticosterone. In contrast, differences in free corticosterone and FCMs between

This content downloaded from 193.171.106.199 on April 08, 2017 11:36:01 AM
All use subject to University of Chicago Press Terms and Conditions (http://www.journals.uchicago.edu/t-and-c).

Table 2: Results from the autocorrelation analyses for potential circadian rhythm from fecal corticosteroid metabolite (FCM) levels

Model	β	95% CI	R^2_m
t, t + 4 h	.22[a]	.07 to .37[a]	.63
t, t + 8 h	.03	−.15 to .20	.53
t, t + 12 h	.01	−.15 to .17	.57
t, t + 16 h	−.06	−.23 to .12	.55
t, t + 20 h	−.00	−.21 to .21	.47
t, t + 24 h	.16	−.06 to .38	.48

Note. Linear models with the individual as a fixed effect were used to consider high interindividual heterogeneity in FCM levels. The coefficients (β) with their 95% confidence intervals (CIs) are presented along with the marginal R^2. $t \geq 12$ h after capture to exclude peak FCM levels.

[a]95% CI excludes 0.

sexes and age were small, likely because they were buffered by the marked differences in plasma MCBC, which matched the differences observed in total corticosterone.

Some of the variation in FCM concentrations nonetheless remained unexplained. Potential reasons for this may be that we did not sample the blood when the highest free corticosterone concentrations were reached in all individuals. Indeed, we limited blood sampling to two points in time, and free corticosterone may have reached peak concentrations later than 30 min after capture (Romero et al. 2008). Another limitation is that we could not calculate the exact length of time between the moment when a lemming detected our presence and when we collected the blood sample. If it were greater than 3 min, it may have affected the baseline concentrations (Romero and Reed 2005). Moreover, measurements of plasma corticosteroids concentrations represent only a snapshot in time, whereas metabolites measured in fecal pellets represent a short window of time during which lemmings could have variable stress levels. Because FCMs measured in fecal pellets represent what occurred at least ~4 h ago, pellets collected at capture (i.e., baseline samples) may have been affected by previous but unknown stressful events. Other sources of variation may stem from the intrapopulation heterogeneity.

Intrapopulation Heterogeneity

Age, sex, and reproductive condition generally explained more variability in total (but not free) plasma corticosterone than in FCMs. Our small sample size hampered our ability to explain variations in FCMs because of the large interindividual variability recorded. For instance, two of the three reproductively active males reached very high levels (fcm_{max} = 31 and 12 μg/g), whereas the two reproductively inactive males had much lower levels (fcm_{max} = 3.6 and 3.3 μg/g; see app. C). This contrasts with plasma corticosterone levels, which was generally higher in females, probably because of the stimulatory effect of estrogen (reviewed in Boonstra et al. 2007). The large values observed in reproductively active males are interesting considering that several studies have shown that it is critical to consider reproductive condition when assessing stress levels in small mammals (Boonstra and Boag 1992; Kenagy et al. 1999; Boonstra et al. 2001*a*). High testosterone levels in reproductive males are often related to low MCBC levels, resulting in high free glucocorticoids in the blood relative to nonreproductive males (e.g., *Antechinus swainsonii*: McDonald et al. 1986; arctic ground squirrels: Boonstra et al. 2001*b*).

Adult females had much higher total corticosterone and MCBC than adult males and juveniles in both baseline and stressed samples, but these differences were not marked in free corticosterone. Although adult female lemmings have some of the highest total corticosterone concentrations of all mammals, as first observed by Romero et al. (2008), they also have very high MCBC, which explains why their free corticosterone levels were only marginally higher than those of male or juvenile brown lemmings. This means that high MCBC compensated for

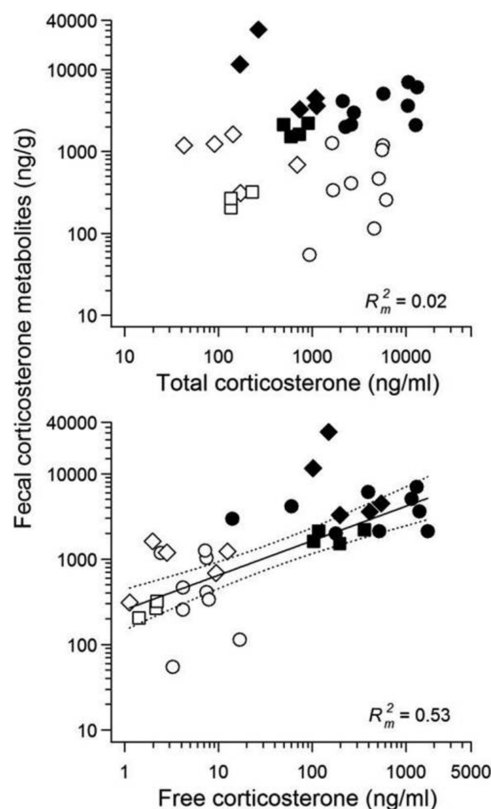

Figure 4. Relationships with their 95% confidence intervals (dotted lines) between plasma total or free corticosterone and fecal corticosterone metabolite (FCM) concentrations of lemmings. Slope and intercept were estimated using a mixed model with individual lemming as the random factor. Samples collected at $t = 0$ for plasma and FCM were paired (open symbols), whereas plasma samples collected at t = 30 min were paired with maximal FCM concentrations recorded (i.e., 2–6 h after capture, depending on each individual; filled symbols). Two observations (one for each paired samples) per lemming ($n = 18$) were used to assess the relationship. Circles, adult females; diamonds, adult males; squares, juveniles; R^2_m, marginal R^2.

This content downloaded from 193.171.106.199 on April 08, 2017 11:36:01 AM
All use subject to University of Chicago Press Terms and Conditions (http://www.journals.uchicago.edu/t-and-c).

high total corticosterone and could explain why few sex differences in FCMs were found here or in other vole species (Bian et al. 2015). In contrast to what Charbonnel et al. (2008) reported in water voles (*Arvicola scherman*), we found no evidence of variation in baseline FCM concentrations with reproductive condition in females, possibly because variability in free corticosterone concentrations was relatively small. It is still unclear why adult female lemmings maintain such high total corticosterone and MCBC levels in the plasma compared with males and other species, but this could be a useful reservoir for future highly energetic activities during more rigorous periods (i.e., reproduction in winter).

Juvenile lemmings showed a weaker stress response to capture compared with adult females but similar to adult males, as previously reported by Romero et al. (2008). This suggests that either juveniles are more resistant to stressors owing to a very efficient negative feedback or their stress response was damped by an underdeveloped HPA axis. The second hypothesis may be more plausible, as Seabloom et al. (1978) found that adrenals of juvenile meadow voles (*Microtus pennsylvanicus*) were less responsive to ACTH stimulation compared with adults. Interestingly, juveniles had the steepest increase in glucose concentrations once stressed, which indicates that they still efficiently mobilized energy in response to capture and anesthesia.

Metabolism of glucocorticoids can differ between sexes, thereby creating metabolites with different molecular structures (Touma et al. 2003; Lepschy et al. 2007). The proportion of metabolites excreted via the feces or the urine may also differ between sexes. For example, male mice excrete up to 75% of their glucocorticoid metabolites in feces, whereas females excrete 50% (Touma et al. 2003, 2004). This difference in excretion routes—in combination with sex differences in excreted FCMs and their immunoreactivity in the EIA—could have contributed to the observed patterns in plasma free corticosterone and FCM in relation to sex. Although we cannot evaluate the variation in the percentage of corticosterone metabolites detected by our EIA between sexes, it successfully detected a response to the stressor in all groups. FCM measured by the 5α-pregnane-3β,11β,21-triol-20-one EIA thus appears adequate to detect a stress response in lemmings, as revealed by free corticosterone, but comparisons of FCM levels between sexes and reproductive conditions cannot be extrapolated to plasma total corticosterone or MCBC (Touma et al. 2004).

Circadian Rhythm

Our autocorrelation analysis provided little evidence of a circadian rhythm in FCM concentrations in lemmings. This result contrasts with those of Andrews (1968), who found a circadian pattern in steroid secretion in captive brown lemmings of Alaska. A possible explanation for this difference may be the short period of habituation to captivity in our experiment coupled with the regular collection of fecal samples, which may have disrupted the normal daily activities of captive lemmings. This constant disturbance may also explain why a positive autocorrelation with a 4-h delay was present.

Diurnal rhythm in FCMs has been observed in other small mammal species, and it has been suggested that varying defecation rates according to the time of the day may play a role (Touma et al. 2003; Sheriff et al. 2009*a*). It is thus surprising that we found little evidence for such an endogenous cycle in lemmings. However, it is also possible that the 24-h daylight of the high Arctic summer inhibits such cycles, since we found that the probability of capturing lemmings during livetrapping sessions is unaffected by the time of day. It has also been suggested that reindeer (*Rangifer tarandus*) show weak or no circadian behavioral or hormonal patterns because of the shortness or the lack of photic variations during most of the year (van Oort et al. 2005; Lu et al. 2010). Thus, sampling fecal material at a different time of the day may not represent a major source of bias during summer in the high Arctic.

Conclusions

We show that a 5α-pregnane-3β,11β,21-triol-20-one EIA is well suited for evaluating adrenocortical activity via FCM in Arctic lemmings. We found that FCM is highly related with plasma free corticosterone ($R^2 = 0.53$), despite some differences related to sexes and age groups between plasma and fecal metabolites. Our study further supports the hypothesis that fecal metabolites reflect well the free, biologically active portion of plasma corticosterone. This is only the second time that such a relationship has been tested (Sheriff et al. 2010). Recent studies have found that some small mammal populations showing large-amplitude fluctuations of abundance through time have phase-dependent stress responses (Charbonnel et al. 2008; Sheriff et al. 2009*b*; Bian et al. 2015), which has the potential to explain part of their population dynamics, as shown in snowshoe hare (Sheriff et al. 2009*b*). FCM may be a useful tool to study the stress response of lemmings to external factors such as predation, food depletion, or competition (Sheriff et al. 2011*b*; Boonstra 2013). However, an important caveat when applying this technique is that fecal samples taken more than 2 h after a stressful event (e.g., livetrapping) may not be representative of baseline levels. Sex and reproductive condition of individuals should also be considered because of possible sex-specific differences in excreted glucocorticoid metabolites.

Acknowledgments

We thank Christine Lambert and David Gaspard for their help with the fieldwork. We also thank all the Bylot Island field team for their assistance in this project. The research relied on the logistic assistance of the Polar Continental Shelf Program (Natural Resources Canada) and of Sirmilik National Park. The research was funded by the Natural Sciences and Engineering Research Council of Canada (NSERC; Discovery Grants and Frontiers to Discovery programs), the Northern Student Training Program of Indian and Northern Affairs Canada, the Network of Centres of Excellence of Canada ArcticNet, the NSERC Collaborative Research and Training Experience Program in Northern

This content downloaded from 193.171.106.199 on April 08, 2017 11:36:01 AM
All use subject to University of Chicago Press Terms and Conditions (http://www.journals.uchicago.edu/t-and-c).

Environmental Sciences (EnviroNord), the W. Garfield Weston Foundation, and the Fonds de recherche du Québec–Nature et technologies. We thank Brendan Delehanty and three anonymous reviewers for their constructive comments on an early version of the manuscript.

Literature Cited

Andrews R.V. 1968. Daily and seasonal variation in adrenal metabolism of the brown lemming. Physiol Zool 41:86–94.

Arnold T.W. 2010. Uninformative parameters and model selection using Akaike's information criterion. J Wildl Manag 74:1175–1178, doi:10.1111/j.1937-2817.2010.tb01236.x.

Barsano C.P. and G. Baumann. 1989. Simple algebraic and graphic methods for the apportionment of hormone (and receptor) into bound and free fractions in binding equilibria; or how to calculate bound and free hormone? Endocrinology 124:1101–1106, doi:10.1210/endo-124-3-1101.

Bian J.-H., S.-Y. Du, Y. Wu, Y.-F. Cao, X.-H. Nie, H. He, and Z.-B. You. 2015. Maternal effects and population regulation: maternal density-induced reproduction suppression impairs offspring capacity in response to immediate environment in root voles *Microtus oeconomus*. J Anim Ecol 84: 326–336, doi:10.1111/1365-2656.12307.

Boonstra R. 2013. Reality as the leading cause of stress: rethinking the impact of chronic stress in nature. Funct Ecol 27:7–10, doi:10.1111/1365-2435.12008.

Boonstra R., J.M. Barker, J. Castillo, and Q.E. Fletcher. 2007. The role of the stress axis in life-history adaptations. Pp. 139–149 *in* J.O. Wolff and P.W. Sherman, eds. Rodent societies: an ecological and evolutionary perspective. University of Chicago Press, Chicago.

Boonstra R. and P.T. Boag. 1992. Spring declines in *Microtus pennsylvanicus* and the role of steroid hormones. J Anim Ecol 61:339–352, doi:10.2307/5326.

Boonstra R., D. Hik, G.R. Singleton, and A. Tinnikov. 1998. The impact of predator-induced stress on the snowshoe hare cycle. Ecol Monogr 68:371–394, doi:10.1890/0012-9615(1998)068[0371:tiopis]2.0.co;2.

Boonstra R., A.H. Hubbs, E.A. Lacey, and C.J. McColl. 2001*a*. Seasonal changes in glucocorticoids and testosterone in free-living arctic ground squirrels from the boreal forest of the Yukon. Can J Zool 79:49–58.

Boonstra R., C.J. McColl, and T.J. Karels. 2001*b*. Reproduction at all costs: the adaptive stress response of male arctic ground squirrels. Ecology 82:1930–1946, doi:10.1890/0012-9658(2001)082[1930:raacta]2.0.co;2.

Bosson C.O., Z. Islam, and R. Boonstra. 2012. The impact of live trapping and trap model on the stress profiles of North American red squirrels. J Zool 288:159–169, doi:10.1111/j.1469-7998.2012.00941.x.

Bosson C.O., R. Palme, and R. Boonstra. 2009. Assessment of the stress response in Columbian ground squirrels: laboratory and field validation of an enzyme immunoassay for fecal cortisol metabolites. Physiol Biochem Zool 82:291–301, doi:10.1086/597530.

———. 2013. Assessing the impact of live-capture, confinement, and translocation on stress and fate in eastern gray squirrels. J Mammal 94:1401–1411, doi:10.1644/13-MAMM-A-046.1.

Bradshaw D. 2003. Vertebrate ecophysiology: an introduction to its principles and applications. Cambridge University Press, Cambridge.

Breuner C.W., B. Delehanty, and R. Boonstra. 2013. Evaluating stress in natural populations of vertebrates: total CORT is not good enough. Funct Ecol 27:24–36, doi:10.1111/1365-2435.12016.

Burnham K.P. and D.R. Anderson. 2002. Model selection and multimodel inference: a practical information-theoretic approach. 2nd ed. Springer, New York.

Cadieux M.C., G. Gauthier, C.A. Gagnon, E. Lévesque, J. Bêty, and D. Berteaux. 2008. Monitoring the environmental and ecological impacts of climate change on Bylot Island, Sirmilik National Park: 2004–2008 final report. Centre d'étude nordique, Université Laval, Quebec.

Charbonnel N., Y. Chaval, K. Berthier, J. Deter, S. Morand, R. Palme, and J.F. Cosson. 2008. Stress and demographic decline: a potential effect mediated by impairment of reproduction and immune function in cyclic vole populations. Physiol Biochem Zool 81:63–73, doi:10.1086/523306.

Creel S., B. Dantzer, W. Goymann, and D.R. Rubenstein. 2013. The ecology of stress: effects of the social environment. Funct Ecol 27:66–80, doi:10.1111/j.1365-2435.2012.02029.x.

Crespi E.J., T.D. Williams, T.S. Jessop, and B. Delehanty. 2013. Life history and the ecology of stress: how do glucocorticoid hormones influence life-history variation in animals? Funct Ecol 27:93–106, doi:10.1111/1365-2435.12009.

Dantzer B., A.G. McAdam, R. Palme, Q.E. Fletcher, S. Boutin, M.M. Humphries, and R. Boonstra. 2010. Fecal cortisol metabolite levels in free-ranging North American red squirrels: assay validation and the effects of reproductive condition. Gen Comp Endocrinol 167:279–286, doi:10.1016/j.ygcen.2010.03.024.

Delehanty B. and R. Boonstra. 2012. The benefits of baseline glucocorticoid measurements: maximal cortisol production under baseline conditions revealed in male Richardon's ground squirrels (*Urocitellus richardsonii*). Gen Comp Endocrinol 178: 470–476, doi:10.1016/j.ygcen.2012.07.009.

Delehanty B., S. Hossain, C.C. Jen, G.J. Crawshaw, and R. Boonstra. 2015. Measurement of free glucocorticoids: quantifying corticosteroid-binding globulin binding affinity and its variation within and among mammalian species. Conserv Physiol 3:cov020, doi:10.1093/conphys/cov020.

Duchesne D., G. Gauthier, and D. Berteaux. 2011. Habitat selection, reproduction and predation of wintering lemmings in the Arctic. Oecologia 167:967–980, doi:10.1007/s00442-011-2045-6.

Fauteux D., G. Gauthier, and D. Berteaux. 2015. Seasonal demography of a cyclic lemming population in the Canadian Arctic. J Anim Ecol 84:1412–1422, doi:10.1111/1365-2656.12385.

This content downloaded from 193.171.106.199 on April 08, 2017 11:36:01 AM
All use subject to University of Chicago Press Terms and Conditions (http://www.journals.uchicago.edu/t-and-c).

Fletcher Q.E. and R. Boonstra. 2006. Impact of live trapping on the stress response of the meadow vole (*Microtus pennsylvanicus*). J Zool 270:473–478, doi:10.1111/j.1469-7998.2006.00153.x.

Gilg O., B. Sittler, and I. Hanski. 2009. Climate change and cyclic predator-prey population dynamics in the high Arctic. Global Change Biol 15:2634–2652.

Gruyer N., G. Gauthier, and D. Berteaux. 2010. Demography of two lemming species on Bylot Island, Nunavut, Canada. Polar Biol 33:725–736, doi:10.1007/s00300-009-0746-7.

Hammond G.L. and P.L.A. Lähteenmäki. 1983. A versatile method for the determination of serum cortisol binding globulin and sex hormone binding globulin binding capacities. Clin Chim Acta 132:101–110, doi:10.1016/0009-8981(83)90237-1.

Hammond T.T., R. Palme, and E.A. Lacey. 2015. Contrasting stress responses of two co-occurring chipmunk species (*Tamias alpinus* and *T. speciosus*). Gen Comp Endocrinol 211:114–122, doi:10.1016/j.ygcen.2014.11.013.

Hothorn T., F. Bretz, and P. Westfall. 2008. Simultaneous inference in general parametric models. Biometrical J 50:346–363.

Itah R., I. Gitelman, and C. Davis. 2004. A replacement for methoxyflurane (Metofane) in open-circuit anaesthesia. Lab Anim 38:280–285, doi:10.1258/002367704323133664.

Kenagy G.J., N.J. Place, and C. Veloso. 1999. Relation of glucocorticosteroids and testosterone to the annual cycle of free-living degus in semiarid central Chile. Gen Comp Endocrinol 115:236–243, doi:10.1006/gcen.1999.7307.

Lepschy M., C. Touma, R. Hruby, and R. Palme. 2007. Non-invasive measurement of adrenocortical activity in male and female rats. Lab Anim 41:372–387, doi:10.1258/002367707781282730.

Lu W., Q.-J. Meng, N.J.C. Tyler, K.-A. Stokkan, and A.S.I. Loudon. 2010. A circadian clock is not required in an Arctic mammal. Curr Biol 20:533–537, doi:dx.doi.org/10.1016/j.cub.2010.01.042.

McArthur C., P. Banks, R. Boonstra, and J. Forbey. 2014. The dilemma of foraging herbivores: dealing with food and fear. Oecologia 176:677–689, doi:10.1007/s00442-014-3076-6.

McDonald I.R., A.K. Lee, K.A. Than, and R.W. Martin. 1986. Failure of glucocorticoid feedback in males of a population of small marsupials (*Antechinus swainsonii*) during the period of mating. J Endocrinol 108:63–68, doi:10.1677/joe.0.1080063.

Montiglio P.O., F. Pelletier, R. Palme, D. Garant, D. Reale, and R. Boonstra. 2012. Noninvasive monitoring of fecal cortisol metabolites in the eastern chipmunk (*Tamias striatus*): validation and comparison of two enzyme immunoassays. Physiol Biochem Zool 85:183–193, doi:10.1086/664592.

Möstl E. and R. Palme. 2002. Hormones as indicators of stress. Domest Anim Endocrinol 23:67–74, doi:10.1016/S0739-7240(02)00146-7.

Nakagawa S. and H. Schielzeth. 2013. A general and simple method for obtaining R^2 from generalized linear mixed-effects models. Methods Ecol Evol 4:133–142, doi:10.1111/j.2041-210x.2012.00261.x.

Palme R. 2005. Measuring fecal steroids: guidelines for practical application. Ann N Y Acad Sci 1046:75–80, doi:10.1196/annals.1343.007.

Palme R., S. Rettenbacher, C. Touma, S.M. El-Bahr, and E. Möstl. 2005. Stress hormones in mammals and birds: comparative aspects regarding metabolism, excretion, and noninvasive measurement in fecal samples. Ann N Y Acad Sci 1040:162–171, doi:10.1196/annals.1327.021.

Perogamvros I., D.W. Ray, and P.J. Trainer. 2012. Regulation of cortisol bioavailability: effects on hormone measurement and action. Nat Rev Endocrinol 8:717–727, doi:10.1038/nrendo.2012.134.

R Development Core Team. 2014. R: a language an environment for statistical computing. R Foundation for Statistical Computing, Vienna. http://www.R-project.org/.

Rogovin K.A. and S.V. Naidenko. 2010. Noninvasive assessment of stress in bank voles (*Myodes glareolus*, Cricetidae, Rodentia) by means of enzyme-linked immunosorbent assay (ELISA). Biol Bull 37:959–964, doi:10.1134/s1062359010090098.

Romero L.M., C.J. Meister, N.E. Cyr, G.J. Kenagy, and J.C. Wingfield. 2008. Seasonal glucocorticoid responses to capture in wild free-living mammals. Am J Physiol 294:R614–R622, doi:10.1152/ajpregu.00752.2007.

Romero L.M. and J.M. Reed. 2005. Collecting baseline corticosterone samples in the field: is under 3 min good enough? Comp Biochem Physiol A 140:73–79, doi:10.1016/j.cbpb.2004 .11.004.

Sapolsky R.M., L.M. Romero, and A.U. Munck. 2000. How do glucocorticoids influence stress responses? integrating permissive, suppressive, stimulatory, and preparative actions. Endocrinol Rev 21:55–89, doi:10.1210/er.21.1.55.

Schoech S.J., L.M. Romero, I.T. Moore, and F. Bonier. 2013. Constraints, concerns and considerations about the necessity of estimating free glucocorticoid concentrations for field endocrine studies. Funct Ecol 27:1100–1106, doi:10.1111/1365-2435.12142.

Seabloom R.W., S.L. Iverson, and B.N. Turner. 1978. Adrenal response in a wild *Microtus* population: seasonal aspects. Can J Zool 56:1433–1440, doi:10.1139/z78-197.

Sheriff M.J., C.O. Bosson, C.J. Krebs, and R. Boonstra. 2009*a*. A non-invasive technique for analyzing fecal cortisol metabolites in snowshoe hares (*Lepus americanus*). J Comp Physiol B 179:305–313, doi:10.1007/s00360-008-0314-4.

Sheriff M.J., B. Dantzer, B. Delehanty, R. Palme, and R. Boonstra. 2011*a*. Measuring stress in wildlife: techniques for quantifying glucocorticoids. Oecologia 166:869–887, doi:10.1007/s00442-011-1943-y.

Sheriff M.J., C.J. Krebs, and R. Boonstra. 2009*b*. The sensitive hare: sublethal effects of predator stress on reproduction in snowshoe hares. J Anim Ecol 78:1249–1258, doi:10.1111/j.1365-2656.2009.01552.x.

———. 2010. Assessing stress in animal populations: do fecal and plasma glucocorticoids tell the same story? Gen Comp Endocrinol 166:614–619, doi:10.1016/j.ygcen.2009.12.017.

———. 2011*b*. From process to pattern: how fluctuating predation risk impacts the stress axis of snowshoe hares during the 10-year cycle. Oecologia 166:593–605, doi:10.1007/s00442-011-1907-2.

Touma C. and R. Palme. 2005. Measuring fecal glucocorticoid metabolites in mammals and birds: the importance of validation. Ann N Y Acad Sci 1046:54–74, doi:10.1196/annals.1343.006.

This content downloaded from 193.171.106.199 on April 08, 2017 11:36:01 AM
All use subject to University of Chicago Press Terms and Conditions (http://www.journals.uchicago.edu/t-and-c).

Touma C., R. Palme, and N. Sachser. 2004. Analyzing corticosterone metabolites in fecal samples of mice: a noninvasive technique to monitor stress hormones. Horm Behav 45:10–22, doi:10.1016/j.yhbeh.2003.07.002.

Touma C., N. Sachser, E. Möstl, and R. Palme. 2003. Effects of sex and time of day on metabolism and excretion of corticosterone in urine and feces of mice. Gen Comp Endocrinol 130:267–278, doi:10.1016/s0016-6480(02)00620-2.

van Oort B.E.H., N.J.C. Tyler, M.P. Gerkema, L. Folkow, A.S. Blix, and K.-A. Stokkan. 2005. Circadian organization in reindeer. Nature 438:1095–1096.

Venables W.N. and B.D. Ripley. 2002. Modern applied statistics with S. 4th ed. Springer, New York.

Ylönen H., J.A. Eccard, I. Jokinen, and J. Sundell. 2006. Is the antipredatory response in behaviour reflected in stress measured in faecal corticosteroids in a small rodent? Behav Ecol Sociobiol 60:350–358, doi:10.1007/s00265-006-0171-7.

This content downloaded from 193.171.106.199 on April 08, 2017 11:36:01 AM
All use subject to University of Chicago Press Terms and Conditions (http://www.journals.uchicago.edu/t-and-c).