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Short Note

Seasonal glucocorticoid secretion in mountain hares (*Lepus timidus*)

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Abstract

In temperate climates mammals might show elevated glucocorticoid secretion in winter to facilitate catabolism. We investigated the influence of season on the concentration of faecal glucocorticoid metabolites (GCMs) in mountain hares throughout the course of 1 year. The results show that mountain hares did not have higher GCM values in winter. Our results suggested that this lagomorph copes with harsh environmental conditions by reducing metabolic rate instead of depleting fat reserves.

Keywords: faeces; glucocorticoids; mountain hare; non-invasive; seasonal changes.

It has been shown in several mammalian species dwelling in temperate climate zones that the concentration of glucocorticoids is higher in winter than in summer. Therefore, glucocorticoids are not only an indication of acute or chronic stress but also of catabolism (Huber et al. 2003, Touma and Palme 2005, Dalmau et al. 2007), i.e., metabolising fat or proteins to keep body temperature stable. One could assume that these changes in glucocorticoid production should be more pronounced during the Alpine winter, but only in species which build up fat reserves to survive this season. Therefore, we investigated glucocorticoid secretion in mountain hares (*Lepus timidus*), a glacial relict species inhabiting elevations from 1500 to 3500 m a.s.l. in the Alps (Gamboni 1997, Slotta-Bachmayr 1998, Nodari 2006, Rehnus 2009), which does not store large fat reserves in autumn to survive winter (Pyörnilä et al. 1992, Thulin and Flux 2003). Consequently, we hypothesised that we should not find pronounced changes in glucocorticoid production in this species across seasons. To test this, we measured glucocorticoid metabolites (GCMs) in faecal pellets of mountain hares, a non-invasive method which has been developed and successfully applied in field studies (Touma and Palme 2005, Arlettaz et al. 2007, Thiel et al. 2008, Bosson et al. 2009, Monclús et al. 2009).

The Swiss National Park (46°39′ N, 10°11′ E) has a continental climate. A meteorological station at Buffalora, which is located near the Swiss National Park, at 1970 m a.s.l. recorded a mean precipitation of 54 mm in January and of 104 mm in July and a mean temperature of -9.2°C in January and of 10.3°C in July (Aschwanden et al. 1996). In our study, the average hourly mean of ambient temperature (°C) and precipitation (mm) were measured at two meteorological stations (open land and forest values) which are located in the study area and calculated as mean values over a 3-day sampling period.

In the study area we assumed a high density of mountain hare based on the high number of hunted mountain hares in the area around the Swiss National Park (canton Grisons: annual mean 1121±39 individuals in 1955–2007; Federal Office for the Environment 2010) and on the related small mean 95% MCP home range size of 57.5 ha (mean ± 28.3 ha), which was found in a neighbouring national park (Nodari 2006). Collection of faecal pellets was conducted in two steps between July 2007 and May 2008 within a network of 31 trial plots, which we preselected by using GIS in a grid of approximately 200 m in a selected study area of 56.7 km^2. In the first step we removed all hare faeces from the trial plots. Three nights later, we collected at least seven fresh faecal pellets from each plot, which were termed as faecal samples. The selected interval was based on a previously performed storage experiment, which is necessary if samples cannot be frozen immediately in the field (Palme 2005). Measured GCM concentrations did not change significantly during that time interval at 10°C and 25°C ambient temperatures (Rehnus et al. 2009). After field collection, all samples were frozen immediately at -20°C until analysis. We discarded 47 out of 101 samples from analyses, because precipitation occurred within the 3-day period, as Rehnus et al. (2009) showed a ‘‘washing-out effect’’ of precipitation on faecal GCM concentrations.

To determine faecal cortisol metabolites an 11-oxoaetiocholanolone enzyme-immunoassay (EIA) and a 5α-pregnane-3β,11β,21-triol-20-one EIA were found to be suited for measuring faecal GCM to evaluate adrenocortical activity in mountain hares (Rehnus et al. 2009). In the present study, only the 11-oxoaetiocholanolone EIA, which measures GCM with a 5β-3α-ol-11-one structure, was used to reduce costs and time of laboratory analysis. After field collection, samples were homogenised and 0.15 g of dry weight was extracted with 5.0 ml of methanol (80%). After shaking on a hand vortex (1 min) and centrifugation (2500×*g*, 15 min) the amounts of GCMs were determined in the supernatant (after a 1:10 dilution with assay buffer) by the 11-oxoaetiocholanolone EIA (Möstl et al. 2002).

All statistical tests were conducted using R 2.8.1 (R Development Core Team, Vienna, Austria). Faecal samples were grouped to the following seasons: summer (July/August), autumn (October), winter (February) and spring (May). Influences on the GCM concentration were tested by analysis of variance with the GCM concentration as a dependent variable and season as a predictor variable (statistical significance: $p<0.05$). Data were log-transformed because they were not normally distributed. The Tukey test for post-hoc testing was conducted to analyse differences between seasons. Data were plotted as mean and interquartile ranges of GCM concentration and average mean of temperature in each season.

Concentrations of GCMs in faecal samples of 1 year (n=54) of mountain hares varied significantly across seasons ($F_{3,51}=2.85$, $p=0.047$). Faecal GCM values were lower in winter than in other seasons, but these differences were not significant.

Moreover, the post-hoc tests showed no significant differences in GCM concentration between individual seasons (Figure 1). Even the direct comparison between winter and summer values revealed no significant difference (t-test: $t=-2.635$, $p=0.051$).

Our results are in contrast to other studies which showed that peak GCM levels were reached in winter and these effects were mainly explained by the process of catabolism (red deer: Huber et al. 2003; Chamois: Dalmau et al. 2007). One could assume that elevated GCM levels in winter could be restricted to larger mammals which are able to store large energy reserves, but other studies in ungulates are in contrast to this assumption and in line with our finding (reindeer: Lund-Larsen et al. 1978, Bubenik et al. 1998; axis deer: Bubenik and Brown 1989; Eld's deer: Monfort et al. 1993). Millspaugh et al. (2001) even found reduced GCM values in red deer (*Cervus elaphus*) from North America during winter which could be an indication for reduced winter metabolism in this species as an adaptation to harsh winter seasons (Arnold et al. 2004). For mountain hares it has been shown that this lagomorph can downregulate its basal metabolic rate by 18% in winter (Pyörnilä et al. 1992) and decrease the body core temperature by 0.4°C (Nieminen and Mustonen 2008) while significant body fat reserves are absent (Pyörnilä et al. 1992, Thulin and Flux 2003). These abilities in line with results from a study on fatty acid composition in heart muscles of mountain hares suggest a winter adaptation with decreased basal metabolic rate and heart rate at lower temperatures in mountain hares (Kaiser 2009). Increased GCM concentrations in spring and summer might be a consequence of reproductive activity (Touma et al. 2003, Franceschini et al. 2007) which occurs in April–July (Baumann 1949, Couturier 1964), or heat stress (Silanikove 2000). Higher glucocorticoid secretion due to the influence of human disturbance (Dehnhard et al. 2001, Arlettaz et al. 2007, Thiel et al. 2008) can be excluded for spring, because the park area was closed to the public during winter and spring time. GCM concentrations can also be influenced by predator stress (Monclús et al. 2009, Sheriff et al. 2009). However, as the occurrence of hare predators in our study area does not change over the year (H. Haller personal communication) this factor can be excluded.

In our study, we investigated adrenocortical activity in free-living mountain hares in the Alps for the first time.

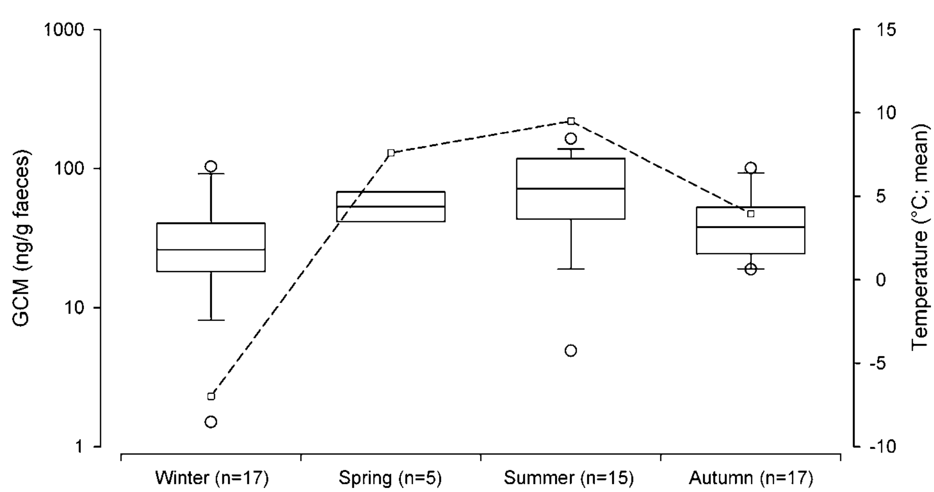

Figure 1 Boxplot of concentrations of GCM (note the logarithmic scale) in different seasons and mean values of measured temperature (- □-- ; °C) during 1 year.

Mountain hares did not have higher GCM values in winter, suggesting that this lagomorph copes with harsh environmental conditions by reducing metabolic rate instead of depleting body reserves. However, further studies are necessary to prove this hypothesis.

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