Glucocorticoid-temperature association is shaped by foraging costs in individual zebra finches

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ABSTRACT

Glucocorticoid (GC) levels vary with environmental conditions, but the functional interpretation of GC variation remains contentious. A primary function is thought to be metabolic, mobilizing body reserves to match energetic demands. This view is supported by temperature-dependent GC levels, although reports of this effect show unexplained heterogeneity. We hypothesised that the temperature effect on GC concentrations will depend on food availability through its effect on the energy spent to gather the food needed for thermoregulation. We tested this hypothesis in zebra finches living in outdoor aviaries with manipulated foraging conditions (i.e. easy vs. hard), by relating within-individual differences in baseline GCs between consecutive years to differences in ambient temperature. In agreement with our hypothesis, we found the GC-temperature association to be significantly steeper in the hard foraging environment. This supports the metabolic explanation of GC variation, underlining the importance of accounting for variation in energy expenditure when interpreting GC variation.

KEYWORDS: Corticosterone, metabolic rate, energy expenditure, foraging environment, glucose

INTRODUCTION

Increased concentrations of glucocorticoid hormones (GCs) are often assumed to be an indication of "stress" (reviewed in Dantzer et al. 2015; Koolhaas et al. 2011). The term 'stress', however, has been under scientific debate since its first use in physiological and biomedical research by Hans Selye (1950), and in the field of ecology has been used to refer to different concepts including noxious stimuli, coping responses by organisms, and the overstimulation of such responses that results in disease (reviewed in Koolhaas et al. 2011). Through the synthesis and release of GCs, organisms mobilize body reserves (i.e. glucose, fatty acids and proteins; Remage-Healey et al. 2001; Sapolsky, Romero & Munck 2000) to provide the resources needed to cope with a current or anticipated increase in energy expenditure (Herman et al. 2016; McEwen & Wingfield 2003; Romero et al. 2009). In this context, GCs are considered as mediators of allostasis (i.e. achieving stability through change, McEwen & Wingfield 2003), integrating physiology and associated behaviours in response or anticipation to changing internal and external conditions (McEwen & Wingfield 2003; Romero et al. 2009). GC levels fluctuate in daily and seasonal patterns (i.e. not only in response to perturbations), and also rise rapidly when a perturbation occurs. For example, a GC increase is often observed in response to colder weather, which induces a higher metabolic rate (Jenni-Eiermann et al. 2008; Lendvai et al. 2009; Thiel et al. 2011; Jimeno et al. 2017a reviewed in Jessop et al. 2016). Despite this fact, much research on GCs in the last decades has focused on establishing associations between variation in GC concentrations and animal welfare or fitness prospects under the assumption that GCs are indicators of stress, usually without explicitly considering the role of variation in energy metabolism. This tendency contrasts with the numerous studies published during the early stages of GC research that provided insights on their metabolic role (e.g. Snedecor et al. 1963; Edens & Siegel 1975; Harlow et al. 1987; reviewed in Siegel 1980, Munck et al. 1984).

While the negative association between ambient temperature and GCs is often found, it is not ubiquitous (e.g. differing between sexes or among taxa; Lendvai et al. 2009; Jessop et al. 2016;

Jimeno et al. 2017b), for reasons that are not well understood. A potential explanation for the heterogeneous findings is that there is environmental or individual variation in the extent to which ambient temperature affects metabolic rate and hence GCs. Negative results when testing for a GC-metabolism relationship may also arise from a reliance on cross-sectional data, in which variation between individuals can partially mask existing patterns within individuals (Briga & Verhulst 2017). In the present study we therefore concentrated on within-individual variation.

Lower ambient temperature requires higher heat production, leading individuals to increase their energy requirements (Jimeno et al. 2017a, Cohen et al. 2008). When food acquisition costs energy, which will usually be the case in the wild, but rarely so in captivity, a lower ambient temperature further increases foraging effort, because the foraging costs themselves need to be covered with more foraging. Building on the hypothesis that GCs are primarily regulated with respect to energetic demands, we predicted the GC-temperature associations to be steeper in environments with higher foraging costs. We tested this prediction in captive zebra finches living permanently in outdoor aviaries with either low or high foraging costs, by comparing baseline corticosterone (CORT, the main bird glucocorticoid) measurements taken on the same individuals in two consecutive years at different ambient temperatures.

MATERIALS AND METHODS

Housing and rearing conditions of the birds used in this study are described in Briga et al. (2017). In brief, individuals were bred indoors and when the oldest chick was maximally 5 days old, chicks were randomly cross-fostered to create small and large broods, always within the range observed in the wild. After reaching 100 days of age, individuals were assigned randomly to one of eight outdoor aviaries (310×210×150 cm), evenly distributed between easy and hard foraging environments. Each aviary contained 20-25 individuals of one sex, and an approximately equal number of birds reared in small and large broods.

The foraging manipulation is described in detail in Koetsier & Verhulst (2011). In brief, in each aviary a food container with 5 holes on each side was suspended from the ceiling. In the easy foraging environment food-boxes had perches just below the holes, allowing birds to perch while eating (low foraging costs). In the hard foraging environment the perches were absent, forcing birds to stay on the wing when obtaining food (high foraging costs). Birds could sustain themselves well in these conditions (Koetsier & Verhulst 2011), but in the long run the manipulation shortened lifespan of birds reared in large broods, but not of birds reared in small broods (Briga et al. 2017). The foraging manipulation did not on average over all groups affect baseline corticosterone levels, but more complex patterns emerged in more detailed analyses (Jimeno et al. 2017b).

Ambient temperature at the aviaries was recorded each hour (HOBO, Onset computer corporation). Following Jimeno et al. (2017b), for temperature we used the average ambient temperature during the hour prior to sampling.

Blood samples were collected in May 2014 and May 2015, always within 2 min of entering the aviary, in the context of the study described in Jimeno et al. (2017b). Samples were taken from the brachial vein, collected in heparinized microcapillary tubes and stored on ice until centrifugation. Plasma was separated from all samples and stored at −20 °C until analysed.

Plasma CORT concentrations were determined using an enzyme immunoassay kit (Cat. No. ADI-900-097, ENZO Life Sciences, Lausen, Switzerland), following previously established protocols (Jimeno et al. 2017b). In brief, aliquots of 10 µl along with a buffer blank and two positive controls (at 20 ng/ml) were extracted twice with diethylether and redissolved in 280 µl assay buffer after evaporation. On the next day, two 100 µl duplicates of each sample were added to an assay plate and taken through the assay. Buffer blanks were at or below the assay's lower detection limit (27 pg/ml). Samples with CV's higher than 20% were re-assayed. Final hormone concentrations were corrected for average loss of sample during extraction in our laboratory (i.e. 15%).

To test our hypothesis we applied model selection using the Akaike Information Criterion (AICc, Burnham & Anderson 2002). Difference in corticosterone (2015 – 2014) was the dependent variable, and the model representing our hypothesis contained temperature difference (2015-2014), foraging treatment, and their interaction. The alternative models we considered are listed in Table 1. Statistical analyses were performed using R version 3.2.2 (R Core Team, 2015) with the function "lm" of the R package nlme (Pinheiro et al. 2014), and the functions "dredge" and "r.squaredGLMM" of the R pckage MuMIn (Barton 2013). Logarithmic transformations were performed to normalize CORT; corticosterone change was calculated as the difference between lnCORT2015 – lnCORT2014.

We previously showed that being reared in a small or large brood had no effect on the association between ambient temperature and corticosterone (Jimeno et al 2017b). Analyses on the present dataset confirmed this result and therefore we do not report the effects of the brood size manipulation in the present analyses.

RESULTS AND DISCUSSION

CORT difference in response to a temperature difference was known for 49 individuals that were sampled in both years (Fig. S1; 28 in easy and 21 in hard foraging environment; 27 females and 22 males). In agreement with our prediction, the model that best explained the within-individual change in CORT concentrations (i.e. lowest AICc) included temperature difference, foraging treatment, and their interaction (Table 1), while the next best model ($\triangle$AICc = +1.25) was similar except for the addition of sex as main effect. Thus between-year differences in ambient temperature were associated with differences in CORT, and this association was affected by foraging treatment, with birds living in the energetically more demanding environment showing a steeper slope (Fig. 1). The effect of temperature differences on CORT differences did not differ between the sexes: i.e. including the interaction between sex and foraging treatment or temperature resulted in poorer model fits ($\triangle$AICc > 5.9). The lack of an interaction with sex is in agreement with our earlier results collected under temperature controlled conditions (Jimeno et al. 2017a). Removing the interaction between foraging treatment and temperature difference from any of the models always increased AICc values (△AICc > 4). Thus, when experiencing natural variation in ambient temperature, individuals that had to expend more energy to forage (i.e. fly more to obtain food) showed stronger CORT responses to variation in ambient temperature, compared to individuals in the less demanding foraging environment.

The increase in metabolic demands induced by lower temperatures will require an increase in fuel supply (i.e. glucose, the main fuel molecule in birds) to match those needs. Glucose can be absorbed in the intestine during digestion, or synthetized in the liver from glycogen or fat reserves (Braun & Sweazea 2008). As GCs are required for the latter process, a correlation between energetic (i.e. glucose) needs and GCs may only be detected when individuals do not have access to food, or when access to food is energetically costly, as in our hard foraging treatment. Glucose synthesis and mobilization may also be needed when food is easily available but the short-term energetic demands are too high to be fulfilled by current absorption from food; however further research is needed to test this idea and the involvement of the glucose mobilization processes. Nevertheless, this strong effect of the foraging costs, together with reliance on cross-sectional measurements, could partly explain the inconsistency between studies testing for correlations between metabolism and GCs (e.g. Wikelski et al. 1999; Buehler et al. 2012).

Our results can be integrated into the Allostasis Model (McEwen & Wingfield 2003), which provides a framework for understanding GC secretion through modelling the energetic requirements of an individual in relation to the energy available in its environment. This model incorporates concepts that allow replacing the controversial word “stress” (Blas 2015): *Allostatic load* is the cumulative energetic requirement of an organism (the “workload”) at a particular moment, including predictable and unpredictable demands. Meanwhile, *allostatic overload* is defined as the state in which energy requirements exceed the capacity of the animal to replace that energy from environmental resources. According to this theoretical framework, birds in a hard foraging environment would have higher allostatic load compared to birds in an easy foraging environment, because the energy required to obtain food will be higher in the hard foraging environment. This difference will be larger at colder temperatures, because birds need to allocate more energy on thermoregulation, and at very low temperatures the individuals in the hard foraging treatment will have higher risk of experiencing allostatic overload unless energy saving mechanisms such as hypothermia are triggered (Briga & Verhulst 2017).

In conclusion, our findings underline the importance of accounting for variation in energy metabolism when interpreting variation in glucocorticoid concentrations. More generally, this study illustrates the importance of investigating variation in (physiological) traits in multiple environments that differ in ecologically relevant variables, in particularly in laboratory conditions that usually differ strongly from the environment in which the trait evolved.

ETHICS

All methods and experimental procedures were carried out under the approval of the Animal Experimentation Ethical Committee of the University of Groningen, licence 5150E, and in accordance with the approved guidelines.

ACKNOWLEDGEMENTS

We thank Sabine Jörg for expertly running all assays and students who helped in the sampling sessions.

COMPETING INTERESTS

The authors declare no conflict of interest.

AUTHORS´ CONTRIBUTIONS

BJ, MH and SV designed the study. BJ collected the samples. BJ analysed the data with the help of SV. BJ wrote the first version of the paper, and MH and SV contributed to finalizing the manuscript.

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DATA AVAILABILITY

The data generated during the current study are available from the corresponding author on reasonable request.

FUNDING

B.J. was funded by the University of Groningen and the Max Planck Institute for Ornithology. Hormone assays were funded by the Max Planck Society to M.H.

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Table 1: Within-individual differences in plasma corticosterone concentrations in relation to temperature differences and foraging treatment. Best fitting model (R^2=0.34) and alternative models. Note that differences in both temperature and corticosterone represent the difference between the second (2015) and the first (2014) sample (for corticosterone, change = lnCort2015-lnCort2014).

	Estimate	**s.e.**	**d.f.**	**F**	**p**
Intercept	-0.023	0.136			
Temperature (difference)	0.031	0.017	1,45	11.42	0.002
Foraging	-0.092	0.096	1,45	0.32	0.583
Temperature x Foraging	-0.037	0.012	1,45	9.44	0.004
Alternative models					
			AICc	**△AICc**	**weight**
Temperature, Foraging, Temperature x Foraging			153.98	0.00	0.40
Temperature, Foraging, Sex, Temperature x Foraging			155.24	1.25	0.22
Temperature, Foraging, Sex, Temperature x Foraging, Foraging x Sex			156.47	2.48	0.12
Temperature, Foraging, Sex, Temperature x Foraging, Temperature x Sex			157.37	3.39	0.07
Temperature			158.07	4.08	0.05
Temperature, Sex			158.77	4.79	0.04
Temperature, Foraging, Sex, Temperature x Foraging, Foraging x Sex, Temperature x Sex			159.12	5.13	0.03
Temperature, Sex, Sex x Temperature			159.96	5.98	0.02
Temperature, Foraging			160.34	6.36	0.02
Temperature, Foraging, Sex			161.01	7.02	0.01
Temperature, Foraging, Sex, Sex x Foraging x Temperature			161.77	7.79	0.01
Temperature, Foraging, Sex, Sex x Temperature			162.21	8.23	0.01
Temperature, Foraging, Sex, Foraging x Sex			163.14	9.16	0.00

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Figures

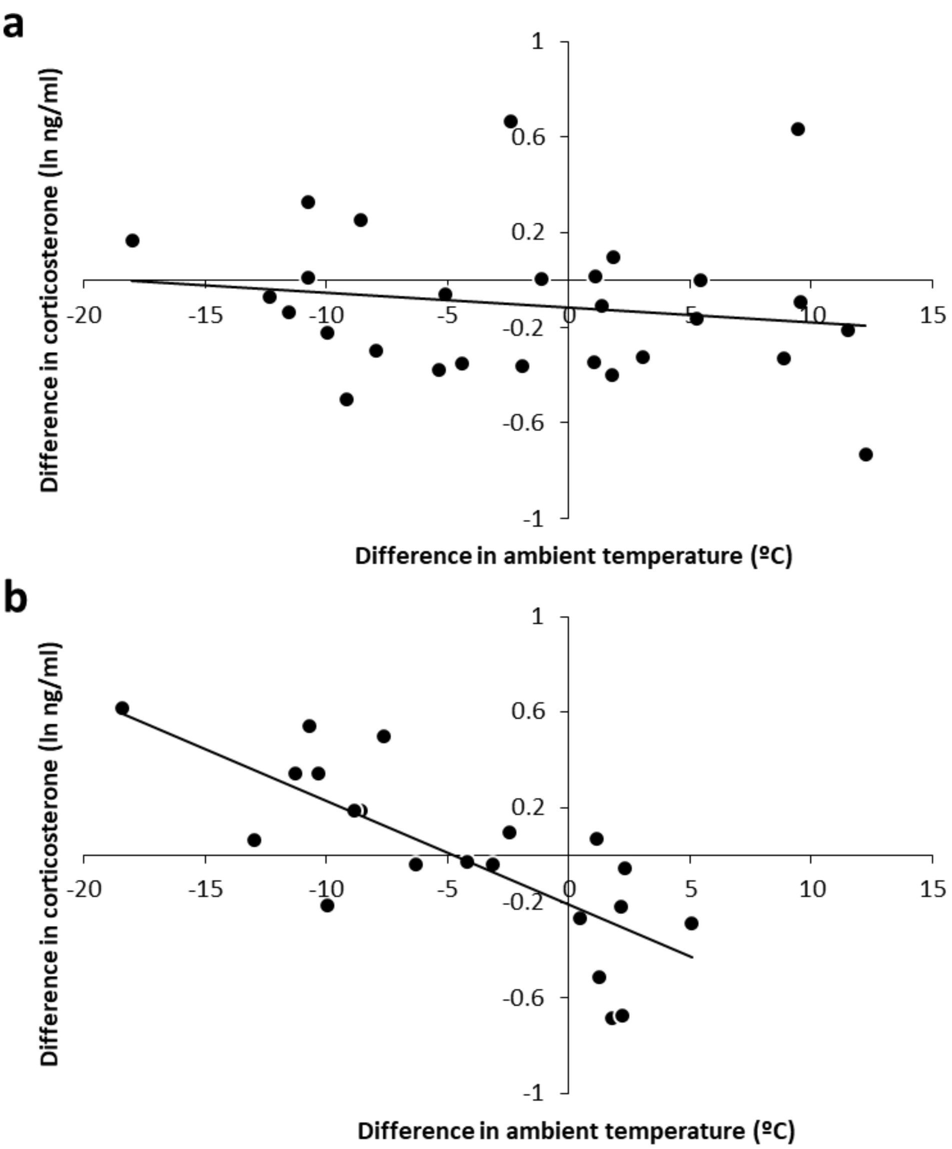

Fig. 1: Relationship between the difference in plasma corticosterone (ng/ml) and the difference in ambient temperature at sampling (within-individual approach) in (a) easy (N=28) and (b) hard (N=21) foraging environments. Changes in both temperature and CORT represent the differences between the second (2015) and the first (2014) sample (corticosterone values were ln transformed, change = lnCort2015-lnCort2014).

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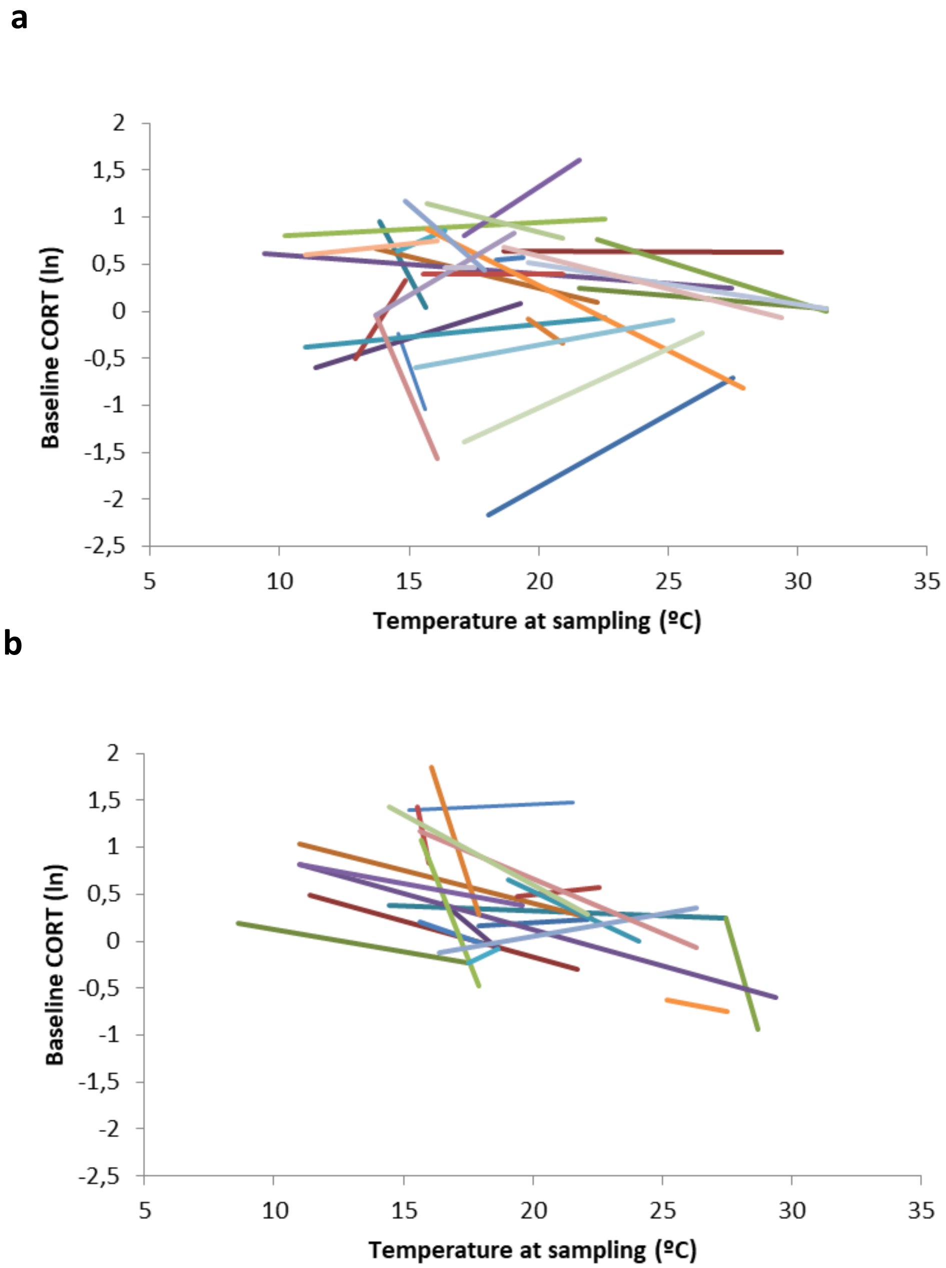

Fig. S1: Baseline CORT and ambient temperature at sampling of individual zebra finches in (a) easy (N=28) and (b) hard (N=21) foraging environment.