

SHORT COMMUNICATION

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 hypothesized 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 versus 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.

KEY WORDS: 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., 2014; 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; Ramage-Healey and Romero, 2001; Sapolsky et al., 2000) to provide the resources needed to cope with a current or anticipated increase in energy expenditure (Herman et al., 2016; McEwen and Wingfield, 2003; Romero et al., 2009). In this context, GCs are considered as mediators of allostasis (i.e. achieving stability through change; McEwen and Wingfield, 2003), integrating physiology and associated behaviours in response or anticipation to changing internal and external conditions (McEwen and 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 into their metabolic role (e.g. Snedecor et al., 1963; Edens and Siegel, 1975; Harlow et al., 1987; reviewed in Siegel, 1980; Munck et al., 1984).

Although 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 and 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 [*Taeniopygia guttata* (Vieillot 1817)] 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, zebra finches 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

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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 and Verhulst (2011). In brief, in each aviary, a food container with five holes on each side was suspended from the ceiling. In the easy foraging environment, food boxes had perches just below the holes, allowing the birds to perch while eating (low foraging costs). In the hard foraging environment, the perches were absent, forcing birds to remain in flight when obtaining food (high foraging costs). Birds could sustain themselves well in these conditions (Koetsier and 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, affect baseline corticosterone levels over all groups, 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 (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^{-1}) 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^{-1}). Samples with coefficients of variation 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 corrected for small sample size (AICc; Burnham and Anderson, 2004). 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 (<https://www.r-project.org/>) with the function 'lm' in the R package nlme (<http://cran.r-project.org/package=nlme>), and the functions 'dredge' and 'r.squaredGLMM' in the R package MuMIn (<https://cran.r-project.org/package=MumIn>). Logarithmic transformations were performed to normalize CORT; corticosterone change was calculated as the difference between $\ln\text{CORT}_{2015}$ and $\ln\text{CORT}_{2014}$.

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.

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.

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 the easy and 21 in the 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 ($\Delta\text{AICc}=+1.25$) was similar except for the addition of sex as a 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

Table 1. Within-individual differences in zebra finch plasma corticosterone concentrations in relation to temperature differences and foraging treatment

	Estimate	s.e.	d.f.	<i>F</i>	<i>P</i>
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×Foraging	-0.037	0.012	1,45	9.44	0.004
Alternative models			AICc	ΔAICc	Weight
Temperature, Foraging, Temperature×Foraging			153.98	0.00	0.40
Temperature, Foraging, Sex, Temperature×Foraging			155.24	1.25	0.22
Temperature, Foraging, Sex, Temperature×Foraging, Foraging×Sex			156.47	2.48	0.12
Temperature, Foraging, Sex, Temperature×Foraging, Temperature×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×Foraging, Foraging×Sex, Temperature×Sex			159.12	5.13	0.03
Temperature, Sex, Sex×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×Foraging×Temperature			161.77	7.79	0.01
Temperature, Foraging, Sex, Sex×Temperature			162.21	8.23	0.01
Temperature, Foraging, Sex, Foraging×Sex			163.14	9.16	0.00

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= $\ln\text{CORT}_{2015}-\ln\text{CORT}_{2014}$).

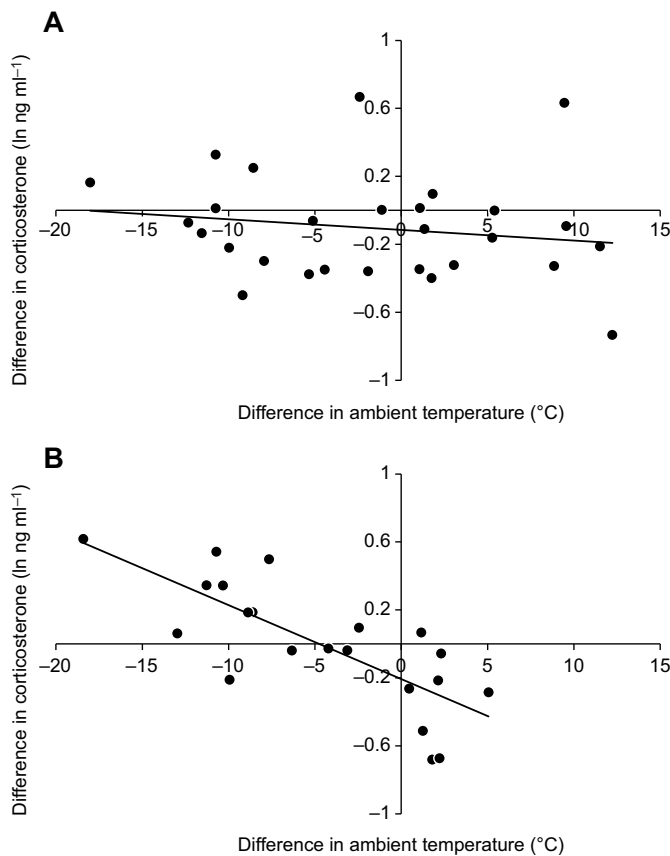


Fig. 1. Relationship between the difference in plasma corticosterone (CORT; ng ml⁻¹) and the difference in ambient temperature at sampling (within-individual approach) in zebra finches under different foraging conditions. (A) Easy ($N=28$) and (B) hard ($N=21$). Changes in both temperature and CORT represent the differences between the second (2015) and the first (2014) sample (CORT values were ln transformed, change= $\ln\text{CORT}_{2015}-\ln\text{CORT}_{2014}$).

interaction between sex and foraging treatment or temperature resulted in poorer model fits ($\Delta\text{AICc}>5.9$). 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 ($\Delta\text{AICc}>4$). 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 with 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 synthesized in the liver from glycogen or fat reserves (Braun and 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 and 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 a higher allostatic load compared with 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 to thermoregulation, and at very low temperatures the individuals in the hard foraging treatment will have a higher risk of experiencing allostatic overload unless energy-saving mechanisms such as hypothermia are triggered (Briga and Verhulst, 2017).

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

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: B.J., M.H., S.V.; Methodology: B.J., M.H., S.V.; Software: B.J.; Validation: B.J., S.V.; Formal analysis: B.J., S.V.; Investigation: B.J.; Resources: M.H., S.V.; Data curation: B.J., S.V.; Writing - original draft: B.J.; Writing - review & editing: B.J., M.H., S.V.; Visualization: B.J., M.H., S.V.; Supervision: M.H., S.V.; Project administration: M.H., S.V.; Funding acquisition: M.H., S.V.

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Supplementary information

Supplementary information available online at <http://jeb.biologists.org/lookup/doi/10.1242/jeb.187880.supplemental>

References

- Blas, J. (2015). Stress in birds. In *Sturkie's Avian Physiology* (ed. C. G. Scanes), 6th edn, pp. 769-810. Elsevier.
- Braun, E. J. and Sweazea, K. L. (2008). Glucose regulation in birds. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **151**, 1-9.
- Briga, M. and Verhulst, S. (2017). Individual variation in metabolic reaction norms over ambient temperature causes low correlation between basal and standard metabolic rate. *J. Exp. Biol.* **220**, 3280-3289.
- Briga, M., Koetsier, E., Boonekamp, J. J., Jimeno, B. and Verhulst, S. (2017). Food availability affects adult survival trajectories depending on early developmental conditions. *Proc. R. Soc. B* **284**, 20162287.

- Buehler, D. M., Vézina, F., Goymann, W., Schwabl, I., Versteegh, M., Tieleman, B. I. and Piersma, T. (2012). Independence among physiological traits suggests flexibility in the face of ecological demands on phenotypes. *J. Evol. Biol.* **25**, 1600-1613.
- Burnham, K. P. and Anderson, D. R. (2004). Multimodel inference: understanding AIC and BIC in model selection. *Sociol. Method. Res.* **33**, 261-304.
- Cohen, A., Hau, M. and Wikelski, M. (2008). Stress, metabolism, and antioxidants in two wild passerine bird species. *Physiol. Biochem. Zool.* **81**, 463-472.
- Dantzer, B., Fletcher, Q. E., Boonstra, R. and Sheriff, M. J. (2014). Measures of physiological stress: a transparent or opaque window into the status, management and conservation of species? *Conserv. Physiol.* **2**, cou023.
- Edens, F. W. and Siegel, H. T. (1975). Adrenal responses in high and low ACTH response lines of chickens during acute heat stress. *Gen. Comp. Endocrinol.* **25**, 64-73.
- Harlow, H. J., Thorne, E. T., Williams, E. S., Belden, E. L. and Gern, W. A. (1987). Adrenal responsiveness in domestic sheep (*Ovis aries*) to acute and chronic stressors as predicted by remote monitoring of cardiac frequency. *Can. J. Zool.* **65**, 2021-2027.
- Herman, J. P., McKlveen, J. M., Ghosal, S., Kopp, B., Wulsin, A., Makinson, R., Scheimann, J. and Myers, B. (2016). Regulation of the hypothalamic-pituitary-adrenocortical stress response. *Compr. Physiol.* **6**, 603-621.
- Jenni-Eiermann, S., Glaus, E., Gruebler, M., Schwabl, H. and Jenni, L. (2008). Glucocorticoid response to food availability in breeding barn swallows (*Hirundo rustica*). *Gen. Comp. Endocrinol.* **155**, 558-565.
- Jessop, T. S., Lane, M. L., Teasdale, L., Stuart-Fox, D., Wilson, R. S., Careau, V. and Moore, I. T. (2016). Multiscale evaluation of thermal dependence in the glucocorticoid response of vertebrates. *Am. Nat.* **188**, 342-356.
- Jimeno, B., Hau, M. and Verhulst, S. (2017a). Strong association between corticosterone levels and temperature-dependent metabolic rate in individual zebra finches. *J. Exp. Biol.* **220**, 4426-4431.
- Jimeno, B., Briga, M., Verhulst, S. and Hau, M. (2017b). Effects of developmental conditions on glucocorticoid concentrations in adulthood depend on sex and foraging conditions. *Horm. Behav.* **93**, 175-183.
- Koetsier, E. and Verhulst, S. (2011). A simple technique to manipulate foraging costs in seed-eating birds. *J. Exp. Biol.* **214**, 1225-1229.
- Koolhaas, J. M., Bartolomucci, A., Buwalda, B. D., de Boer, S. F., Flügge, G., Korte, S. M., Meerlo, P., Murison, R., Olivier, B. and Palanza, P. (2011). Stress revisited: a critical evaluation of the stress concept. *Neurosci. Biobehav. Rev.* **35**, 1291-1301.
- Lendvai, A. Z., Loiseau, C., Sorci, G. and Chastel, O. (2009). Early developmental conditions affect stress response in juvenile but not in adult house sparrows (*Passer domesticus*). *Gen. Comp. Endocrinol.* **160**, 30-35.
- McEwen, B. S. and Wingfield, J. C. (2003). The concept of allostasis in biology and biomedicine. *Horm. Behav.* **43**, 2-15.
- Munck, A., Guyre, P. M. and Holbrook, N. J. (1984). Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. *Endocr. Rev.* **5**, 25-44.
- Remage-Healey, L. and Romero, L. M. (2001). Corticosterone and insulin interact to regulate glucose and triglyceride levels during stress in a bird. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **281**, R994-R1003.
- Romero, L. M., Dickens, M. J. and Cyr, N. E. (2009). The reactive scope model - A new model integrating homeostasis, allostasis, and stress. *Horm. Behav.* **55**, 375-389.
- Sapolsky, R. M., Romero, L. M. and Munck, A. U. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocr. Rev.* **21**, 55-89.
- Selye, H. (1950). Stress and the general adaptation syndrome. *Br. Med. J.* **1**, 1383.
- Siegel, H. S. (1980). Physiological stress in birds. *Bioscience* **30**, 529-534.
- Snedecor, J. G., King, D. B. and Henrikson, R. C. (1963). Studies on the chick glycogen body: effects of hormones and normal glycogen turnover. *Gen. Comp. Endocrinol.* **3**, 176-183.
- Thiel, D., Jenni-Eiermann, S., Palme, R. and Jenni, L. (2011). Winter tourism increases stress hormone levels in the capercaillie *Tetrao urogallus*. *Ibis* **153**, 122-133.
- Wikelski, M., Lynn, S., Breuner, C. and Wingfield, J. C. (1999). Energy metabolism, testosterone and corticosterone in white-crowned sparrows. *J. Comp. Physiol. A* **185**, 463-470.