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Causes and consequences of glucocorticoid variation in zebra finches

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Chapter 7

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

Blanca Jimeno, Michaela Hau & Simon Verhulst

Submitted

ABSTRACT

Glucocorticoid hormones (GC) mediate adjustments to environmental conditions in vertebrates, but the functional interpretation of GC variation is still contentious. One of their main functions is to elevate glucose release to the blood stream. In agreement with this metabolic function we recently showed a strong association between temperature-induced changes in metabolic rate and plasma corticosterone concentrations in zebra finches in controlled conditions. Building on this finding, we hypothesised that the temperature effect on metabolic rate will depend on food availability, because, in addition to the increased thermoregulatory costs, individuals will have to spend energy to gather the extra food needed for thermoregulation. We tested this hypothesis in zebra finches using natural temperature variation in outdoor aviaries with either an easy or a hard foraging environment. We measured baseline GCs in two years, together with variation in ambient temperature. Our within-individual analyses confirmed a negative association between ambient temperature and GCs that was significantly steeper in the hard foraging environment. This finding supports the hypothesis that GC concentrations are regulated to maintain homeostasis in the face of fluctuations in energy metabolism, and underlines the importance of variation in metabolic rate as explanatory factor of variation in glucocorticoid concentrations.

Background

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). However, through the synthesis and release of GCs, organisms mobilize body reserves (i.e. glucose, fatty acids and proteins; Remage *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). 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; reviewed in Jessop *et al.* 2016). Hence GCs primary function may be metabolic, but to what extent GC’s are primarily regulated with respect to energetic demands remains a contentious issue.

While the association between ambient temperature and GCs is often found, it is not ubiquitous (e.g. Lendvai *et al.* 2009; Jessop *et al.* 2016), for reasons that are not well understood. For example, in zebra finches *(Taeniopygia guttata)* living in outdoor aviaries, we found a temperature-CORT association in females but not in males (Jimeno *et al.* 2017a), and such findings are reason to question whether GCs are primarily regulated with respect to energetic demands. A potential explanation for the heterogeneous findings is that there is 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 reliance on cross-sectional data, in which variation between individuals could partially mask existing patterns within individuals. In our study we therefore concentrated on within-individual variation.

Lower ambient temperature requires higher heat production, leading individuals to increase their energy expenditure (Jimeno *et al.* 2017b) and food intake. When food acquisition costs energy, which will usually be the case in the wild, a lower ambient temperature further increases foraging effort because the foraging costs themselves need to be covered with more foraging. Thus, building on the hypothesis that GCs are primarily regulated with respect to energetic demands, we predict 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 (Briga *et al.* 2017), by comparing baseline corticosterone 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 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 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). Ambient temperature at the aviaries was recorded each hour (HOBO, Onset computer corporation). Following Jimeno et al 2017a, 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 as described in Jimeno *et al.* (2017a). 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. For the present study we used all 49 individuals (27 females and 22 males) that were sampled in both years.

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.* 2017a). 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%).

We applied model selection using the Akaike Information Criterion AICc (Burnham & Anderson 2002) to test our main hypothesis. 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 3.2.1 (R Core Team, 2015) with the function “lm” of the R package nlme (Pinheiro *et al.* 2014). Logarithmic transformations were performed to

normalize CORT, thus corticosterone change was calculated as the difference between lnCORT2015 – lnCORT2014.

Results and discussion

In agreement with our predictions, 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). Thus larger between-year differences in ambient temperature at time of blood sampling were associated with greater 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). This relationship did not differ between sexes (i.e. including sex and its interactions always resulted in a poorer model fit, and including sex and rearing brood size never improved the model). Furthermore, removing the interaction between foraging treatment and temperature difference from the models, our main test, always increased AICc values. Thus when experiencing naturalistic variations in ambient temperature, individuals that had to expend more energy to forage (i.e. fly more to obtain food) also increased their CORT concentrations more strongly when temperatures decreased, compared to the less demanding foraging environment. Our results corroborate the existence of a relationship between energy expenditure and GC concentrations observed previously in climate-controlled rooms (Jimeno *et al.* 2017b), but highlight the relevance of the food availability and the ensuing foraging costs on determining the strength (and hence detectability) of such association.

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 reserves (Braun & Sweazea 2008). As GC release is required for the latter process (Remage-Healey *et al.* 2001), 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 (which is often the case in the wild, but rarely so in captivity), as in our high foraging costs treatment. Glucose synthesis in the liver may also be needed when food is easily available but energetic demands are too high to be fulfilled by food intake; 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 the lack of within-individual 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 findings suggest that at the within-individual level, GCs fluctuate proportionally to energy expenditure, presumably through their effect on glucose supply. More generally, this study illustrates the importance of investigating (physiological) traits in multiple environments that differ in ecologically relevant variables such as for example food availability and ambient temperature.

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.

Table 1: Within-individual differences in corticosterone concentrations in relation to temperature differences and foraging treatment. Best fitting model, R^2=0.34. 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		
Foraging, Temperature, Sex, Temperature x Foraging (b)			1.25		
Temperature (c)			4.08		
Temperature, Foraging (a)			6.36		
Sex, Temperature, Sex x Temperature (d)			7.45		
Sex, Foraging, Temperature, Sex x Foraging x Temperature (e)			7.79		

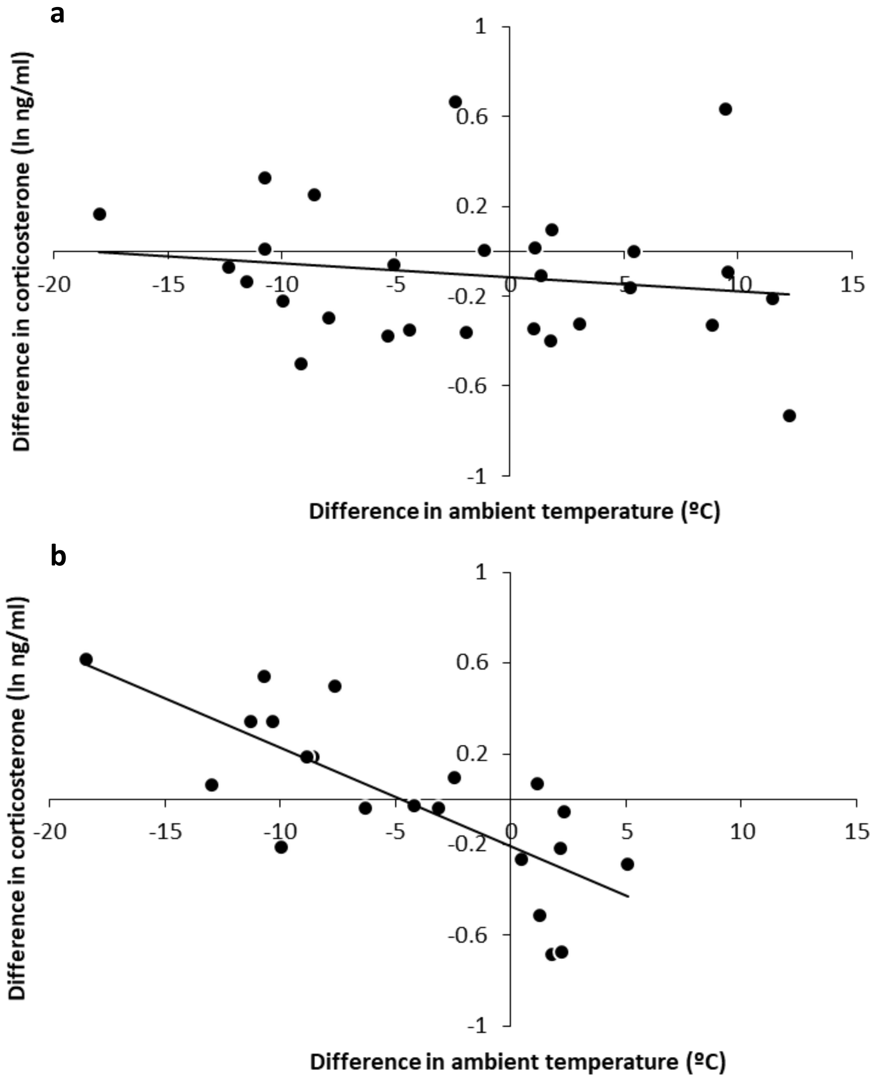

Figure 1: Relationship between the difference in corticosterone (ng/ml) and the difference in ambient temperature at sampling (within-individual approach) in (a) easy and (b) hard foraging environment. Changes in both temperature and CORT represent the differences between the second (2015) and the first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

SUPPLEMENTARY INFORMATION to:

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

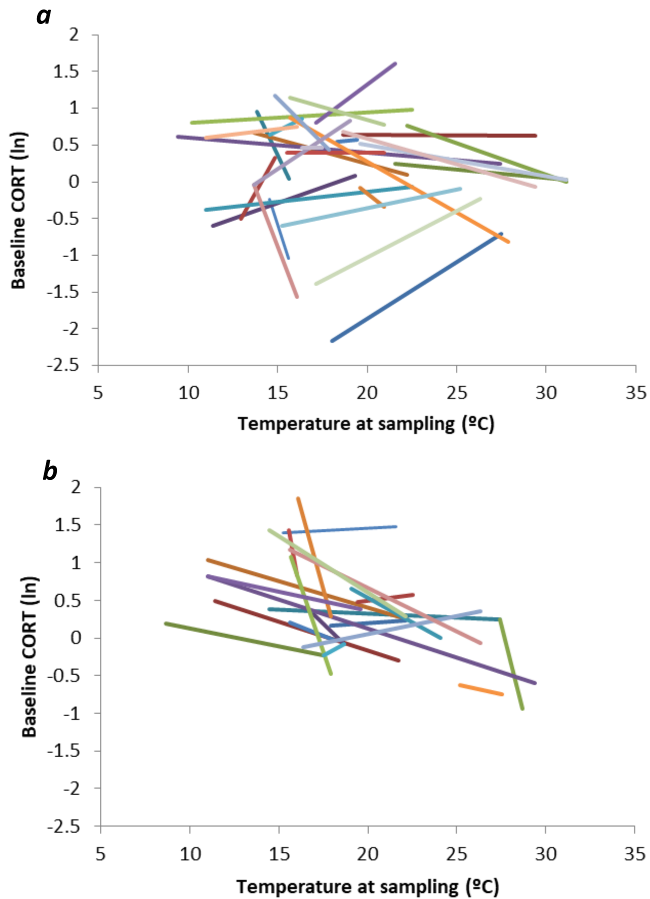

Figure S1: Baseline CORT and ambient temperature at sampling of individual zebra finches in easy (a) and hard (b) foraging environment.

BOX D

Effects of developmental and adult environments on metabolism: Daily energy expenditure

Blanca Jimeno

Previous results in our zebra finch population (see Fig. 2 in the introduction for details on the experimental design) have reported lower basal metabolic rate (BMR) and standard metabolic rate (SMR) in individuals living in hard foraging conditions, with no effect of brood size manipulation (Briga & Verhulst 2017). However, this leaves open the questions whether this holds for more integrative measures of metabolism, and whether brood size manipulation has an effect on other metabolic traits. Furthermore, when the foraging costs are increased (as in our hard foraging environment), everything else remaining constant, we would expect the total energy expenditure to increase accordingly (Wiersma, Salomons & Verhulst 2005). Hence we would predict birds living in easy vs. hard foraging conditions to differ in energy expenditure.

We therefore tested for the effects of our experimental treatments (brood size manipulation and adult foraging environment) on daily energy expenditure (DEE). We estimated DEE by the doubly labelled water method (Lifson & McClintock 1966; Butler *et al.* 2004; Welcker *et al.* 2015) for 128 individuals sampled between October and December of 2008.

DEE was 9.8% lower in birds reared in large broods (F_{112}=4.51, p=0.03, Fig. 1), with no effect of foraging environment (F_{112}=0.33, p=0.59) or their interaction (F_{112}=0.14, p=0.71). Including structural size, body mass or ambient temperature as predictor variables did not qualitatively change the results, although higher ambient temperatures were associated with lower DEE (slope: -1.37 ± 0.44; F_{112}=9.83, p<0.01). Differences in metabolism between treatments may indicate absolute differences in metabolism, or else a change in energy allocation, by favouring other processes that maximise fitness in that specific environment (e.g. foraging efficiency in the hard foraging environment). The fact that total DEE was affected by developmental conditions, whereas BMR and SMR were only affected by foraging environment, point at different energy balance strategies (or divergent energy allocation priorities) between treatment groups, determined by both developmental and adult conditions. Individuals may down-regulate their metabolic rate under certain circumstances (e.g. during the night) to be able to increase their energetic scope without the need to acquire more energy. This might also be facilitated by a reduction in

potentially costly processes (e.g. immune responses or decrease in the metabolism of vital organs; Deerenberg *et al.* 1997). This "compensation" (Wiersma, Salomons & Verhulst 2005) is more expected to occur in animals operating close to an energetic ceiling (Welcker *et al.* 2009, 2015).

These analyses were carried out on the basis of an experiment and data collection by Gertjan van Dijk, Femke Tamminga, Egbert Koetsier and Simon Verhulst.

METHODS

Daily energy expenditure: An hour after the injection an initial blood sample was collected, and twenty-four hours after the initial blood sample the final blood samples were taken. Body mass was measured at the time of injection and when taking the final blood sample. Analysis of isotopic enrichment of blood was performed by isotope ratio mass spectrometry as described in Speakman & Krol (2005). The rate of CO_2 production (rCO_2) was calculated using Speakman's single-pool model equation 7.17 (Speakman 1997). Initial isotope dilution spaces were calculated by the intercept method (Coward and Prentice 1985). Total body water was converted to grams using a molecular mass of 18.020 for body water, and expressed as a percentage of body mass. Finally, rCO_2 was converted to energy expenditure assuming an energetic equivalent of 22.0 kJ/L CO_2^{-1} based on the Weir equation (Weir 1949), and average respiratory quotient from our respirometry measurements (0.95). Final body water was inferred from final body mass assuming a constant fraction of body water throughout the experiment.

Statistical analyses: We ran general linear models (GLMM) with DEE as dependent variable and experimental treatments and sex as predictors. We also tested for the effects of mean daily temperature, structural size and individual body mass (corrected by size) by including them as covariates. Model residuals were normally distributed.

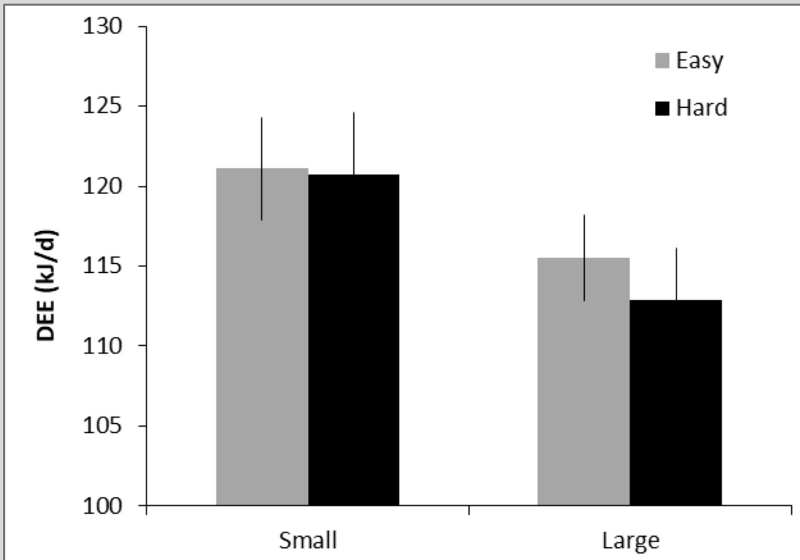

Fig. 1: Daily energy expenditure (DEE, mean ± s.e.) by in relation to manipulated brood size (small vs. large broods) and foraging environment (easy vs. hard) in zebra finches.