Strong association between corticosterone and temperature dependent metabolic rate in individual zebra finches

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ABSTRACT

Glucocorticoid hormones (GCs) are often assumed to be indicators of stress. At the same time, one of their fundamental roles is to facilitate metabolic processes to accommodate changes in energetic demands. While the metabolic function of GCs is thought to be ubiquitous across vertebrates, we are not aware of experiments which tested this directly, i.e., in which metabolic rate was manipulated and measured together with GCs. We therefore tested for a relationship between plasma corticosterone (CORT, ln transformed) and metabolic rate (MR, measured using indirect calorimetry) in a between- and within-individual design in captive zebra finches (*Taeniopygia guttata*) of both sexes. In each individual, CORT and MR were measured at two different temperature levels: 'warm' (22ºC) and 'cold' (12 ºC). CORT and MR were both increased in colder compared to warmer conditions, within individuals, but also across individuals. At the between-individual level, we found a positive relationship between CORT and MR, with an accelerating slope towards higher MR and CORT values. In contrast, the within individual changes in CORT and MR in response to colder conditions were linearly correlated between individuals. The CORT-MR relationship did not differ between the sexes. Our results illustrate the importance of including variation at different levels to better understand physiological modulation. Furthermore, our findings support the interpretation of CORT variation as indicator of metabolic needs.

Keywords: corticosterone, *Taeniopygia guttata,* glucocorticoid, metabolic rate.

INTRODUCTION

Glucocorticoid (GC) hormones (e.g. cortisol, corticosterone) are often quantified to assess whether individuals or populations are 'stressed' (reviewed in Dantzer *et al*., 2014; Koolhaas *et al*., 2011). However, circulating GC concentrations can also increase during non-stressful situations, for example with the regular daily increases in energy demands that individuals routinely experience (McEwen & Wingfield, 2003; Landys *et al*., 2006; Romero *et al*., 2009; Beerling *et al*., 2011). This is in line with one of the primary functions of GCs, which is to interface with metabolism in a variety of ways. GCs have been named for their function to convert stored energy into glucose, and are therefore predicted to fluctuate in concert with metabolic demands. However, while this basic prediction underlies many concepts of GC regulation and function (McEwen & Wingfield, 2003; Romero *et al*., 2009), the existence and nature of the relationship between metabolic rate (MR) and GCs is still surprisingly unresolved (Holtmann *et al*, 2016; reviewed in Romero & Wingfield, 2015).

Multiple lines of evidence suggest that GCs and metabolism may be linked, both at the inter- and intra-specific level. Perhaps the most convincing evidence available comes from a recent comparative study on mammals, which found that both baseline and stress-induced cortisol correlated positively with mass-specific MR (Haase *et al*., 2016). At the intra-specific level, GC levels have been shown to be associated with energy expenditure (Welcker *et al*., 2015), and with factors that presumably affected energy expenditure. For example, baseline GC concentrations are generally higher with increased workload, resource limitations, reproductive investment, immune responses or thermoregulatory demands (Romero *et al*., 2009; Bonier *et al*., 2011; Miller *et al*., 2009; Bauch *et al*., 2016; Goymann *et al*. 2017; Merkling *et al*., 2017; Ouyang *et al*. 2013). Likewise, stress-induced concentrations (increases following exposure to acute stressors) can be affected by energetically-demanding processes such as molt (Cyr *et al*., 2008; Bauer *et al*., 2011; de Bruijn & Romero, 2013), climatic conditions (de Bruijn & Romero, 2011) or reproductive behaviour (Buwalda *et al*., 2012; Ouyang *et al*., 2013). In contrast, other studies have not detected a covariation between GCs and metabolism (e.g. MR, daily energy expenditure), perhaps because GCs and metabolism were not measured at the same time (e.g. Buehler *et al*., 2012, Welcker *et al*., 2009). A number of studies have employed exogenous GC administration to test for effects on metabolism (Preest & Cree, 2008; Miles *et al*., 2007; Wack *et al*., 2012; Buttemer *et al*., 1991; Wikelski *et al*., 1999; Spencer & Verhulst, 2008). However, results have been inconsistent, especially among endotherm species (Buttemer *et al*., 1991; Wikelski *et al*., 1999; Spencer & Verhulst 2008); perhaps because GC-induced increases in blood glucose levels may be required to maintain a high MR, but they may not necessarily cause a high MR.

Despite the wealth of circumstantial evidence for a GC-MR association, we are not aware of studies in which MR was simultaneously manipulated and measured in conjunction with GC measurements. The latter is an important addition, because measured effects on MR are more convincing than assumed effects. Furthermore, direct measurements are necessary for direct quantification of the GC-MR association, and individual variation in MR can otherwise not be incorporated in the analyses. We therefore tested for an association between manipulated MR and endogenous corticosterone (CORT, the main GC in birds) in captive zebra finches *Taenopygia guttata*, using both between- and within-individual approaches. For each individual, we measured CORT and MR (oxygen consumption) in 'warm' (room temperature, 22ºC) and 'cold' (12 ºC) conditions. Both temperatures are below the thermoneutral zone of zebra finches, and differ strongly in the imposed thermoregulatory demands, i.e. energy expenditure (Calder, 1964; Briga & Verhulst 2017). Based on the hypothesis that CORT variation reflects metabolic needs, we predicted that each individual will increase CORT when exposed to the cold compared to the room temperature treatment insofar as the cold treatment induced an increase in MR (within-individual approach). We tested for the same association between individuals, but have less of a prediction at this level, because there may be individual variation in the MR-CORT association leading to weak or no correlation at the between-individual level (e.g. Goymann & Dávila, 2017). Finally, we compared the MR-CORT association between the sexes, because we previously found that natural variation in ambient temperature was related to CORT in females but not in males, and this contrast can potentially be explained by sex-differences in the CORT-MR association (Jimeno *et al*., 2017).

MATERIALS & METHODS

Subjects

36 birds (18 males and 18 females) were used in this study. They were reared in our facilities at the University of Groningen, the Netherlands, in outdoor aviaries (L x H x W: 310 x 210 x 150 cm) containing 12 pairs each, with free access to food and water. After reaching independence they were moved to big single sex outdoor aviaries. One month before the experiment started they were moved to 4 separate single sex outdoor aviaries (L x H x W: 310 x 210 x 150 cm) with 10 birds each. Food and water were provided *ad libitum*. To avoid potential age effects, all birds were of similar age (8-13 months) when the experiment started, and born during the breeding season of 2014.

Blood sampling and experimental treatments

The experiment was carried out during April and May 2015. Each bird went through 4 respirometry sessions (with a minimum time of two weeks of recovery time between sessions) of 3 hours each,

and was subjected to four different treatments in random order, two of which were the warm and cold treatment mentioned above (the treatments in the other two sessions, 15 min noise stress applied either early or late during the three hour measurement session, fall outside the scope of the present paper). The identity of the bird to be sampled was pre-determined and target birds were previously marked with color-rings to facilitate their individual identification when catching. In the 'warm' treatment the ambient temperature was kept at 22ºC for the entire session (3h). In the 'cold' treatment, the ambient temperature was decreased to 12ºC after 1.5 hours, and stayed low for the remaining 1.5 hour. Average temperature in the outdoor aviaries during sampling hours was 14.38 ± 0.38 ºC (mean ± s.e). Respirometry measurements were conducted either in the morning (9:00-13:00) or in the afternoon (14:00-18:00). In each respirometry session, two birds (one male and one female) were measured simultaneously, and the sets of two birds remained the same throughout all trials.

Birds were captured from the outdoors aviaries (one bird per aviary per day) and transported indoors into the respirometer room in separate cages (L x W x H: 40 x 40 x 15 cm) with access to food. See figure 1 for a schematic overview of the measurement procedure. The birds were left undisturbed in the respirometer room for 1 h to acclimate to room temperature (22ºC), after which we took the first blood sample for CORT analysis (CORTstart). Birds were weighed to the nearest 0.1 g before going into the metabolic chambers. The door of the respirometer room was then closed, and MR measurements started. During the following 1.5h the birds remained undisturbed to further acclimatize to the metabolic chambers. After this time, for the remaining 1.5h, the temperature was either decreased to 12ºC in the cold treatment (taking 15-20 min.; temperature was changed without entering the room), or kept at 22ºC for the warm treatment. After this time the MR was at an approximately stable level (Fig. 2), birds were taken out and a second blood sample was taken (CORTend). Afterwards they were put into a cage (L x W x H: 40 x 40 x 15 cm) with food and water to recover before being returned to their aviary.
All CORT samples were taken within 3 minutes after entering the respirometry chamber to minimize disturbance effects on CORT values.

Metabolic rate

MR was measured using an open flow respirometer situated in a temperature-controlled room. Each individual was transferred to a 1.5 L metabolic chamber, without food or water. For detailed information about the technique see Bouwhuis *et al*. (2011). In brief, the air-flow through the metabolic chambers was kept at 22 l/h by mass-flow controllers (5850S; Brooks, Rijswijk, the Netherlands) calibrated with a bubble flow meter. The air was dried using a molecular sieve (3 Å; Merck, Darmstadt, Germany) and analysed using a paramagnetic oxygen analyser (Servomex Xentra 4100, Crowborough, UK). During the measurements, each metabolic chamber or reference outdoor air was sampled every 3 min for 60s. In each sampling, we measured O_2 and CO_2 concentration and oxygen consumption was calculated using Eq. of Hill (1972). An energy equivalent of 19.7 kJ /l oxygen consumed was used to calculate energy expenditure in watt (W). When analysing the data we took the average MR during the last 10 minutes of the 3h session as measure of MR (MRend).

Hormone analyses

Plasma CORT concentrations were measured using an enzyme immunoassay kit (Cat. No. ADI-900-097, ENZO Life Sciences, Lausen, Switzerland), following previously established protocols (Ouyang *et al*. 2015). Samples taken from one individual were placed in neighbouring wells, but in other respects samples were randomly distributed. Briefly, aliquots of 10 µl plasma along with a buffer blank and two positive controls (at 20ng/mL) were extracted with diethylether. After evaporation, samples were re-dissolved in 280 µl assay buffer. 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). Intra-plate coefficient of variation (CV; mean±SE) was 10.76±2.77% and inter-plate CV was 8.2% (n=11 plates; note that plate identity was included as random effect in the statistical analyses). Samples with CV's >20% were re-assayed when there was sufficient plasma. Final CORT concentrations were corrected for average loss of sample during extraction, which is 15% in our laboratory (Baugh *et al*. 2014).

Statistical analyses

We used paired t-test to test for the effect of temperature treatments on both MR and CORT. To assess the MR-CORT association we constructed general linear mixed models for both the between-individual and within-individual approaches. For the between-individual approach we used CORTend values as dependent variable and MRend, sex, body mass (as the average between the two measurements taken before and after going into the respirometer) and treatment (warm or cold) as predictors. Individual identity and assay plate (CORT analyses) were included as random factors.

Visual inspection of the data suggested the relationship between MR and CORT to be non-linear, so we tested for a quadratic effect of MR on CORT in the analysis. We also tested for potential effects of sampling variables on CORTend variation: sampling round (morning / afternoon), sampling order within the pair (1st or 2nd) and whether or not it was the first time the individual was into the respirometer. However, none of these variables had a significant effect on CORTend (Table S1), so we did not include them in further analyses.

For the within-individual approach, we used the change in CORT between the two treatments (calculated as the difference between CORTend in cold – CORTend in warm) as the dependent variable and change in MR (as the difference between MRend in cold – MRend in warm) and sex as predictors. We did not consider CORTstart a proper control for the treatment effect, because the experience of the animal prior to sampling (capture and handling) was very different from the experience prior to the sample after treatment. Assay plate was included as random factor.

While building the two models described above, we used backward elimination of least significant terms. After model selection, the Akaike Information Criterion (Akaike, 1973) was also considered to confirm that the final models had the lowest AIC values. All statistical analyses were performed using R version 3.3.2 (R Core Team, 2016) with the function "lmer" of the R package lme4 (Bates *et al.*, 2014). R^2 was calculated using the function "r.squaredGLMM" of the R package MuMIn (Barton, 2013). Logarithmic transformations were performed to normalize CORT. CORT change was calculated as ln(CORTcold) – ln(CORTwarm). Residuals of the final models showed a normal distribution. While building the models, one individual male was excluded from the between-individual analyses because it was a clear statistical outlier (this data point was 2.75 times the SD of the model residuals). That was not the case in the within-individual analysis, where its residuals were within 1 (0.25) SD of the model residuals.

Ethics

All methods and experimental procedures were carried out under the approval of the Animal Experimentation Ethical Committee of the University of Groningen, licence 5150G.

RESULTS

Treatment effects

During the cold treatment individuals maintained a significantly higher MR than during the warm treatment (t_{34} = -5.76, p<0.0001; Figure 3a). The MR response to temperature was shown by both sexes (Males: t_{17} = -3.99, p= 0.001; Females: t_{17} = -4.04, p= 0.001). Likewise individuals showed higher CORT concentrations after cold compared to the warm treatment (t_{34} = -2.70, p = 0.011; Figure 3b) and this effect was also similar in the two sexes (Males: t_{17} = -2.01, p= 0.061; Females: t_{17} = -1.80, p= 0.090).

Between individual approach

The model with CORT as the dependent variable showed a strong quadratic relationship with MR (Table 1), with the slope accelerating towards higher MR (Figure 4). The MR variation is a mixture of individual differences and a temperature effect, and these two types of variation may or may not associate with CORT in the same way. However, adding treatment to the model in Table 1 resulted in a poorer model fit (treatment effect when added to the model: P=0.4, and △AICc=3.73) indicating that the quadratic relationship between MR and CORT was independent of treatment. This implies that temperature independent individual variation in MR associated in the same way with CORT as the temperature induced variation. The association between CORT and MR was also independent of sex ($F_{1, 56.34}$=0.220, p=0.64), and did not change when adding CORTstart to the model (Table S3).

In this model we included individual identity as random effect, which increased statistical power of the model because MR and CORT were repeatable in both sexes (MR: r=0.62; CORT: r=0.37; Table S4). These estimates are within the range of estimates in our population for both MR (Briga & Verhulst 2017) and CORT (Jimeno *et al*, 2017).

Within individual approach

Within-individual changes in MR induced by cold treatment were positively correlated with the associated changes induced by ambient temperature in CORT (Table 2, Figure 5). Thus higher temperature-induced increases in MR where associated with higher increases in CORT. This association did not differ between the sexes ($F_{1, 24.34}$=0.003, p=0.96).

DISCUSSION

The generally accepted association between GCs and MR is supported by a wealth of circumstantial evidence (see introduction), but we are not aware of previous direct measurements combined with manipulations of MR in relation to CORT. We therefore manipulated MR through temperature change and we found a strong positive MR-CORT association. Speculating on the mechanism causing the observed association falls outside the scope of the present paper, but on a functional level, our interpretation of this finding is that CORT ensured increasing fuel (e.g. glucose, fatty acids) supply to match higher energetic needs, although we recognize that the evidence for such a relationship is mixed (Remage-Healey & Romero 2001; Landys *et al.*, 2004; Deviche *et al.*, 2014). Our results are in line with previous studies finding a negative association between CORT and ambient temperature (Beaulieu 2017; Jenni-Eiermann *et al*., 2008; Lendvai *et al*., 2009; de Bruijn & Romero, 2011; Jimeno *et al*., 2017), which generally has a strong effect on MR when below the thermoneutral zone (Briga & Verhulst 2017). We found this association to be consistent both within- and between- individuals and independent of temperature treatment, implying that variation in MR between individuals was associated with CORT in the same way as the temperature induced variation. Furthermore, the MR-CORT association held across a broad CORT range including baseline and stress-induced levels (as established in an earlier study on the same study population; Jimeno *et al*, 2017). The latter finding is in-agreement with the comparative study of Haase *et al*. (2016), who found the MR-GC association to be similar for baseline and stress-induced GC levels.

To our best knowledge, MR has not previously been simultaneously manipulated and measured in conjunction with CORT measurements. However, de Bruijn and Romero (2011, 2013) used heart rate as a proxy of MR in conjunction with CORT measurements to investigate effects of experimentally changed climatic conditions in captive European starlings (*Sturnus vulgaris*). To compare our findings with the results of de Bruijn and Romero (2011, 2013), we plotted average values of heart rate vs. CORT for each of their treatment groups (the authors did not report the associations between the two traits). We find that the magnitude of the treatment effects on CORT and MR were strongly correlated between the different treatments in all three groups of experiments (Fig. S1). Thus we conclude that the results of de Bruijn and Romero (2011, 2013) are in close agreement with the conclusions of the present study that there is a strong association between MR and CORT.

At the time that we took the blood sample for CORT (CORTend), the MR had been stable for some time (Fig. 2). We can therefore assume that energy turnover and fuel supply (glucose) were reasonably in balance at the time of measurement, and this may have contributed to our finding that CORT and MR were strongly correlated. Conversely, we would expect such a correlation to be

weaker or absent in the extreme case that CORT was measured immediately after an acute increase in energy expenditure (Beerling *et al*., 2011), while homeostasis is still in disequilibrium. However, such a correlation would likely become stronger with CORT levels measured after an as yet undefined time lag, when homeostasis is being restored. Thus the temporal profile of energy expenditure and the relative timing of the CORT measurement may be crucial when investigating the CORT-MR association, and this may explain the absence of such an association in studies where CORT and MR were not measured at the same time (e.g. Buehler *et al*., 2012).

MR and CORT were strongly correlated both at the between- and within- individual levels. However, the shape of the relationship differed between the two levels – being quadratic between individuals and linear within individuals (but note that CORT was logarithmically transformed prior to analysis). The explanation for this discrepancy can either be statistical or biological. A smaller range of variation within individuals when compared to the variation between individuals (see distributions along the X-axis in figures 4 and 5) may have impeded the detection of a non-linear pattern within individuals. It is possible therefore that there also exists an accelerating pattern within individuals that we cannot detect with the present data. Experiments using a wider MR range would be needed to test this explanation. Alternatively, individuals with high CORT may have a lower sensitivity to CORT (which would be the reason for having high CORT), and hence they would need to up their CORT more to achieve the same physiological response as birds with higher CORT sensitivity (and hence low CORT). Such variation in CORT sensitivity of target cells could arise at any step in the causal chain from CORT to glucose and/or other plasma metabolites in the blood stream (see Bamberger *et al.*, 1996) and could lead to associations within individuals being linear while accelerating upwards among individuals.

The present study was in part inspired by our previous finding in zebra finches of a strong relationship between ambient temperature and CORT in females housed outdoors, while this relationship was flat in males (Jimeno *et al*., 2017). A potential explanation for this finding was that the MR-CORT association is sex-dependent, being flatter or absent in males when compared to females. The present study falsifies this hypothesis, because the MR and CORT responses to a decrease in ambient temperature were indistinguishable between males and females. However, this may be different in the outdoor aviaries where the birds are housed in groups. The sexes may for example differ in their huddling behaviour when subjected to natural variation in ambient temperature.

MR variation can arise in many different ways besides the effect of temperature that we employed. It remains to be tested therefore whether other short-term MR modulating factors (e.g.

psychological stressors) will cause MR to associate with CORT in the same way. However, some considerations lead us to believe that our finding of a strong MR-CORT relationship may apply more generally, regardless of context. Firstly, our best fitting models did not include treatment, which implies that the MR-CORT relationship we observed was independent of temperature context. Secondly, we find that the results of de Bruijn & Romero (2011, 2013), where MR was manipulated using different climatic variables in addition to temperature, are in complete agreement with our finding (Fig. S1). Lastly, Buwalda *et al*. (2012) previously showed that rewarding (sex) and aversive (defeat) social stimuli and habituation to these stimuli affected both CORT and heart rate in a very similar way. Nevertheless, further research is needed to determine to what extent the MR-CORT association we observed is consistent across contexts. To this ends, studies are needed in which MR is manipulated in different ways and measured directly in conjunction with CORT measurements.

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Tables

Table 1: Between-individual analyses of CORT concentrations (ng/ml, Ln transformed) in relation to body mass and MR (watts). Main model: marginal R^2=34.6%; conditional R^2=51.9%.

	Estimate	s.e.	D.f.	F	p
Intercept	6.818	1.531	67.04		
Body mass	**-0.193**	**0.059**	**44.77**	**10.679**	**0.002**
MR	-7.668	4.221	62.86	3.300	0.074
MR^2	**7.657**	**3.127**	**62.73**	**5.995**	**0.017**
Rejected terms					
Treatment (cold)	-1.230	1.484	56.81	0.687	0.411
Sex (male)	0.338	0.827	58.01	0.167	0.684
MR x Sex	-0.591	1.260	56.34	0.220	0.640
MR x Treatment	1.806	2.281	57.77	0.627	0.432
Random factors					
		Variance			
Bird ID		0.091			
Plate		0.002			
Residual		0.259			

Table 2: Within-individual changes in CORT concentrations (lnCORTend in cold – lnCORTend in warm) in relation to within-individual changes MR (MRend in cold – MRend in warm). Main model: marginal R^2=17.7%; conditional R^2=39.9%.

	Estimate	s.e.	D.f.	F	P
Intercept	-0.163	0.271	22.20		
MR (change)	**3.880**	**1.390**	**31.15**	**7.787**	**0.009**
Rejected terms					
Sex (male)	0.024	0.506	23.03	0.003	0.962
MR x Sex	0.161	2.998	24.34	0.003	0.958
Random factors					
		Variance			
Plate		0.189			
Residual		0.513			

Figures

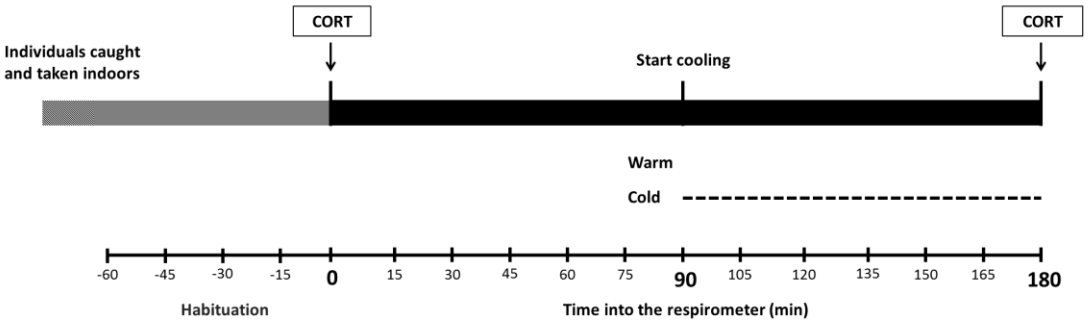

Figure 1: Timeline of the experiment

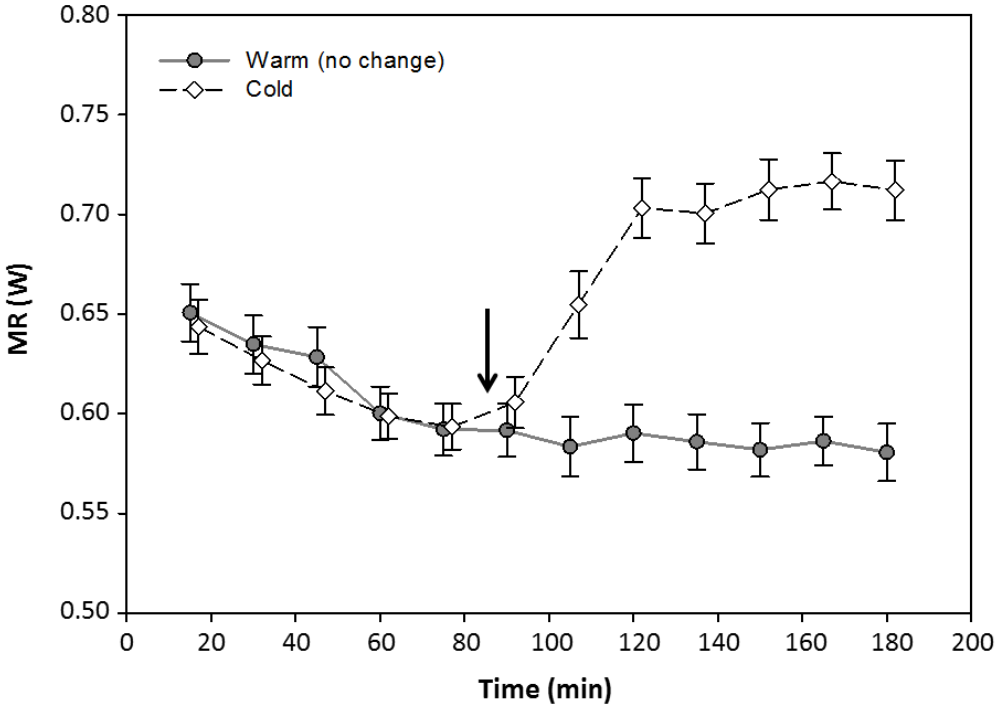

Figure 2: Metabolic rate throughout the entire trial (3 h) for the warm (filled circles, solid line) and cold treatments (open diamonds, dotted line). Bars represent mean ±s.e. for each 15 min interval. The black arrow indicates when the temperature was decreased in the cold treatment.

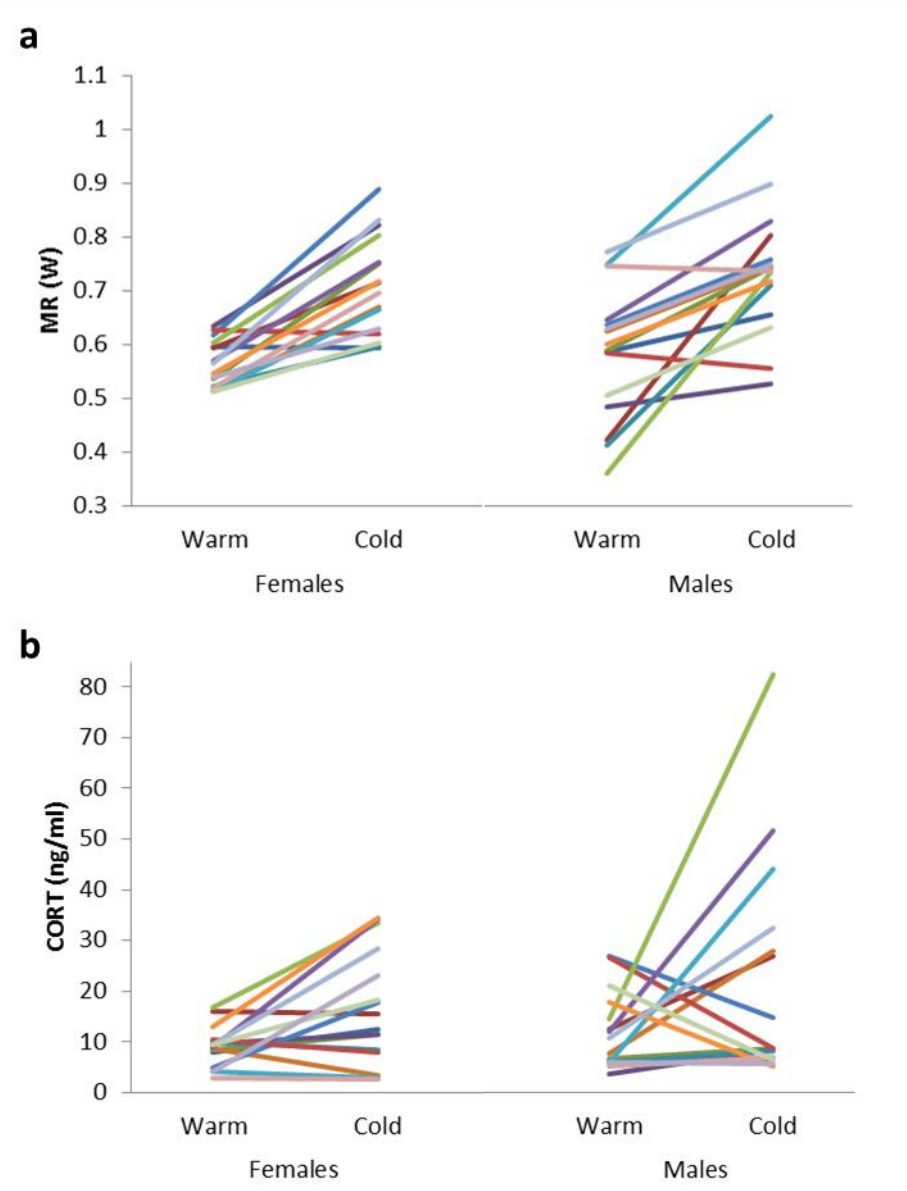

Figure 3. Effect of treatment (warm vs. cold) on metabolic rate (a) and corticosterone concentrations (b). Colors correspond to the same individuals in the two panels. CORT axis is linear, but note that the analyses were carried out using Ln transformed values.

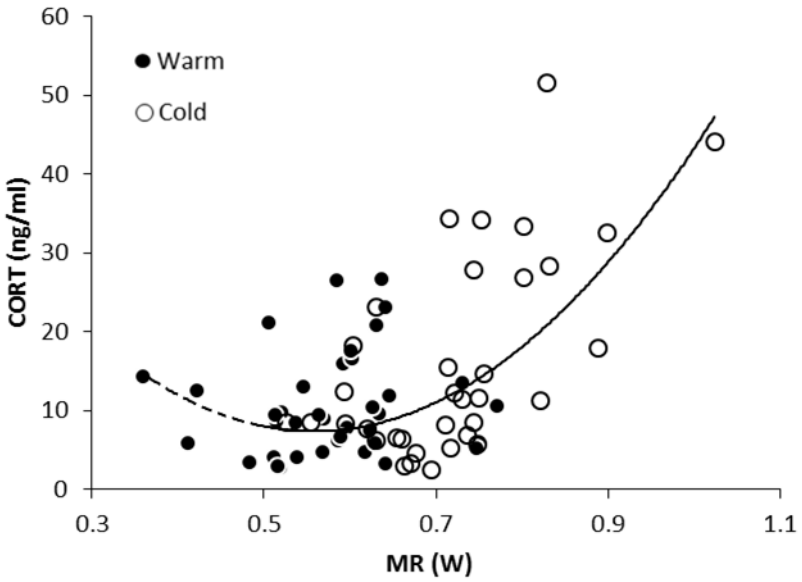

Figure 4: Corticosterone concentrations in relation to metabolic rate (between-individual approach) in warm and cold treatments. Line shows the model prediction. Note that the part of the line corresponding to lower MR values is shown dashed as an increase in CORT when decreasing MR is interpreted as not to have biological meaning. CORT axis is linear, but note that the analyses were carried out using Ln transformed values.

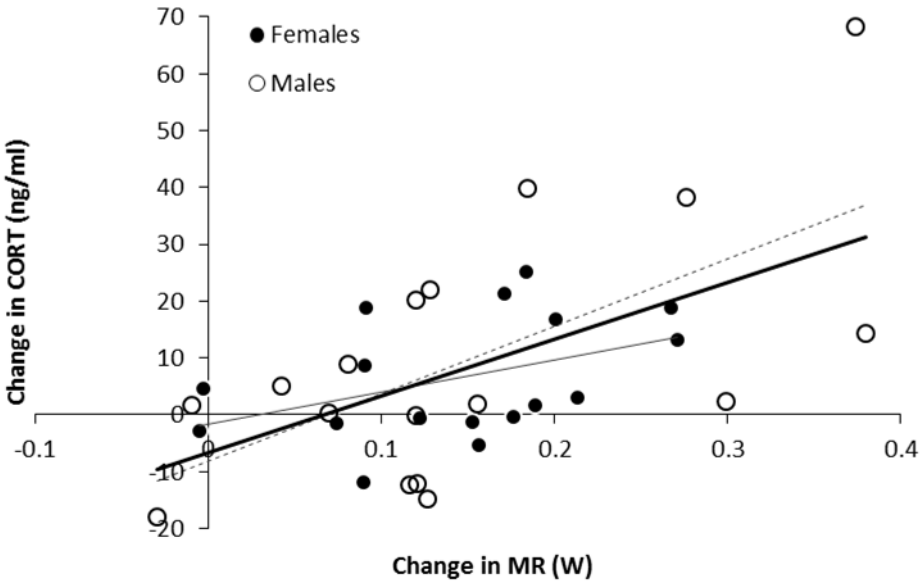

Figure 5: Within-individual changes in metabolic rate (x-axis, cold-warm) in relation to within-individual changes in corticosterone concentrations (y-axis, cold-warm) in individual males (open dots, dashed grey line) and females (filled dots, continuous grey line). Thicker black line shows the association for the sexes pooled. CORT axis is linear, but note that the analyses were carried out using Ln transformed values.

SUPPLEMENTARY MATERIAL TO: Strong association between corticosterone and temperature dependent metabolic rate in individual zebra finches

Blanca Jimeno, Michaela Hau & Simon Verhulst

Table S1. Absolute CORTend concentrations (ng/ml, log transformed) in relation to sampling variables. Sampling order (2): individual sampled second; First trial (yes): first respirometer session for that individual. Round (afternoon): individual was sampled in the afternoon.

	Estimate	Std. error	df	F	p
Intercept	2.075	0.151	41.18		
Sampling order (2)	0.305	0..180	35.76	2.892	0.098
First Trial (yes)	0.234	0.187	53.69	1.565	0.216
Round (afternoon)	0.098	0.184	42.26	0.294	0.591
Random factors					
	Variance				
Bird ID	0.089				
Plate	0.039				
Residual	0.367				

Table S2. Between-individual analyses: AIC values for models with CORTend as the dependent variable (as in Table 1). Models always include MR and body mass, but vary in their including of treatment and its interactions.

Terms	*AICc*	*⊿AICc*
MR, MR^2, Mass (main model)	***138.93***	***0.00***
MR, MR^2, Treatment, Mass	*142.66*	*3.73*
MR, Treatment, Mass, MR x Treatment	*144.25*	*5.32*
MR, Mass	*146.56*	*7.63*

Table S3. Between-individual analyses. Absolute CORT concentrations (CORTend, Ln transformed) in relation to MR (MRend). The main model differs from the model shown in the main paper in that it includes CORTstart, i.e. the CORT concentration at the beginning of the trial.

	Estimate	s.e.	D.f.	F	p
Intercept	6.187	1.501	66.76		
Body mass	**-0.192**	**0.054**	**38.45**	**12.612**	**0.001**
CORTbeg	**0.022**	**0.009**	**58.01**	**6.201**	**0.016**
MR	-6.438	4.199	65.11	2.351	0.130
MR^2	**6.655**	**3.112**	**65.47**	**4.571**	**0.036**
		Variance			
Bird ID		0.015			
Plate		0.018			
Residual		0.292			

Table S4. Repeatabilities and variance components in both the sexes for (a) MRstart (the average of MR measured during the first 10 min after going into the respirometer), (b) CORTstart and (c) Resting MR (as measured after 1.5h into the respirometer and before treatment, i.e. after the acclimation time). Shown are variances and individual repeatabilities as extracted from the null model. Note that the sample sizes correspond to number of individuals, each being measured two times.

a	Females (N=18)		Males (N=18)		Total (N=36)	
	Variance	Repeat.	Variance	Repeat.	Variance	Repeat.
Bird ID	0.0031	**47.7%**	0.0061	**70.9%**	0.0045	**61.6%**
Residual	0.0034	-	0.0025	-	0.0028	-

b	Females (N=18)		Males (N=18)		Total (N=36)	
	Variance	Repeat.	Variance	Repeat.	Variance	Repeat.
Bird ID	0.1242	**30.9%**	0.1325	**48.3%**	0.1236	**36.9%**
Residual	0.2774	-	0.1420	-	0.2111	-

c	Females (N=18)		Males (N=18)		Total (N=36)	
	Variance	Repeat.	Variance	Repeat.	Variance	Repeat.
Bird ID	0.0021	**39.6%**	0.0044	**53.01%**	0.0033	**48.5%**
Residual	0.0032	-	0.0039	-	0.0035	-

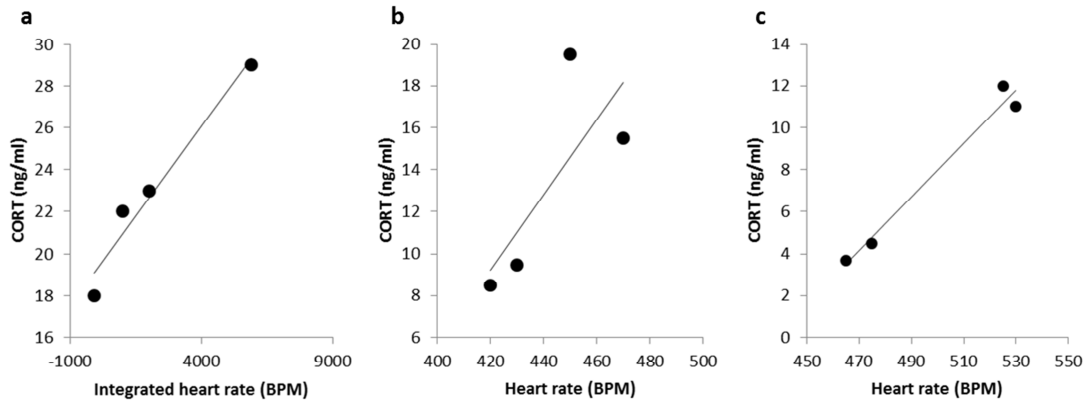

Figure S1. Corticosterone (ng/ml) in relation to heart rates (x-axis) in European starlings (data from de Bruijn & Romero 2011, 2013). Each data point represents the average of the response to experimental manipulation of climatic conditions. Heart rate is closely correlated to energy expenditure in starlings (Cyr et al., 2008) and can therefore be interpreted as proxy of MR. Because each panel shows values for the same individuals across all treatments, the linear relationship shown in these graphs corresponds to the average within-individual variation, which makes it comparable to Fig. 5 in the present paper. Treatment groups from left to right on the x-axis: (a) control, control+noise, control+air, cooling (de Bruijn & Romero, 2011); (b) rain+wind, rain, cold+wind, rain+cold+wind (non-molting birds; de Bruijn & Romero, 2013); (c) cold+wind, rain, rain+cold+wind, rain+wind (molting birds; de Bruijn & Romero, 2013). Note that graph scales are shown at different levels to make graphs comparable. For further details see de Bruijn & Romero (2011, 2013).