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Male but not female zebra finches with high plasma corticosterone have lower survival

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Abstract

1. The glucocorticoid axis is essential for coping with predictable and unpredictable environmental variation. Despite this vital function, attempts to link individual variation in the glucocorticoid axis to survival have yielded mixed results, which may be due to endocrine variation caused by uncontrolled variation in environment and life-history traits such as reproductive effort. We therefore studied the link between the glucocorticoid axis and long-term survival using captive non-breeding zebra finches.
2. We quantified the relationship between survival over a three-year period and plasma corticosterone concentrations: (1) baseline, (2) stress-induced, (3) after induction of negative feedback via dexamethasone injection and (4) after maximal adrenal stimulation via adrenocorticotropin hormone injection.
3. Only stress-induced corticosterone predicted survival, with higher concentrations being associated with lower survival. However, this effect differed significantly between the sexes, being present only in males.
4. Stress-induced corticosterone concentration is the sum of baseline corticosterone and the corticosterone increase in response to the standardized stressor, and both components were similarly associated with male survival in a model that included both variables. This implies that baseline corticosterone itself also exerts an effect on male survival, but this was only revealed when the stress-induced corticosterone increase was included in the model, presumably because this increased statistical power.
5. Given that corticosterone concentrations are highly repeatable in our study population and independent of manipulated foraging conditions, these data suggest that endocrine stress reactivity may be a major component determining male life span, presumably also in wild populations.

KEYWORDS
corticosterone, glucocorticoid, stress response, survival, *Taenopygia guttata*

1 | INTRODUCTION

The ability of individuals to cope with environmental variation can have major implications for their current and future performance. Coping strategies entail adjustments to predictable daily fluctuations in energy requirements as well as fast responses to unpredictable and challenging circumstances (Romero & Wingfield, 2015). A major physiological pathway by which vertebrates respond to both predictable and unpredictable stimuli is the hypothalamic-pituitary-adrenal (HPA) axis, which regulates the secretion of glucocorticoid hormones

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(Hau, Casagrande, Ouyang, & Baugh, 2016; Romero, 2004; Sapolsky, Romero, & Munck, 2000). Glucocorticoids (GCs) have diverse physiological functions, depending in part on their plasma concentrations. Baseline levels of GCs exhibit daily and seasonal variation (Monaghan & Spencer, 2014; Romero, 2002) in concordance with variation in metabolism and activity levels (Jimeno, Hau, & Verhulst, 2017), and as such baseline GC levels are viewed as an indicator of allostatic load (Hau et al., 2016; McEwen & Wingfield, 2003; Monaghan & Spencer, 2014; Romero, 2002). GC concentrations can also increase within minutes in response to acute challenges, inducing physiological and behavioural processes characteristic of an "emergency state" (Wingfield et al., 1998). Such increases in stress-induced GCs are thought to be adaptive as they allow the animal to allocate resources towards immediate survival functions (Sapolsky et al., 2000; Wingfield et al., 1998). However, long-lasting elevations of GC concentrations have deleterious effects on numerous neural and physiological systems (Romero, Dickens, & Cyr, 2009; Wingfield & Sapolsky, 2003). Concentrations are thus tightly regulated, and animals typically return GC to baseline levels via negative feedback within hours after a stress-induced increase (reviewed in Hau et al., 2016; McEwen & Wingfield, 2003). An optimal endocrine function therefore involves both an appropriate up- and down-regulation of glucocorticoid concentrations (Macdougall-Shackleton et al., 2009; Romero, 2004).

As GCs are instrumental when adjusting to predictable and unpredictable environmental variation, many studies have attempted to link individual variation in GC concentrations to variation in fitness. However, most studies testing for a GC-fitness relationship have focused on baseline corticosterone concentrations and reproduction (Angelier, Weimerskirch, Dano, & Chastel, 2007; Bauch, Riechert, Verhulst, & Becker, 2016; Bonier, Moore, Martin, & Robertson, 2009; Bonier, Moore, & Robertson, 2011; Love, Breuner, Vézina, & Williams, 2004; Moore & Jessop, 2003; Ouyang, Sharp, Dawson, Quetting, & Hau, 2011; Williams, Kitaysky, Kettle, & Buck, 2008). Other aspects of the stress response such as the regulatory components of GC secretion (MacDougall-Shackleton, Schmidt, Furlonger, & MacDougall-Shackleton, 2013) and their associations with survival have been less well studied, and the link between natural GC variation and fitness remains largely unresolved (reviewed in Bonier, Martin, Moore, & Wingfield, 2009; Breuner, Patterson, & Hahn, 2008; Crespi, Williams, Jessop, & Delehanty, 2013). Baseline GC concentrations often show no relationship with survival (Angelier, Holberton, & Marra, 2009; Blas, Bortolotti, Tella, Baos, & Marchant, 2007; Macdougall-Shackleton et al., 2009; Ouyang, Sharp, Quetting, & Hau, 2013), whereas stress-induced glucocorticoid concentrations have been reported to show a positive (Angelier et al., 2009; Patterson, Hahn, Cornelius, & Breuner, 2014), negative (Blas et al., 2007; Cavigelli & McClintock, 2003; Macdougall-Shackleton et al., 2009) or no relationship (Angelier et al., 2009; Ouyang et al., 2013) with survival rates. While the strength of these studies is that most of them were conducted on natural populations, the downside of studying a natural population is that it is usually difficult to account for variables that are known to influence GC levels, like reproductive effort or age (Bonier, Martin, et al., 2009; Breuner et al., 2008; Crespi et al., 2013; MacDougall-Shackleton et al., 2013). This issue is illustrated by the finding that the repeatability of GC traits is often rather low (Hau et al., 2016). This implies that reliable characterization of individual variation in GCs usually requires repeated sampling, which can be difficult in practice. We propose that testing for the GC-survival relationship in more controlled environments and for multiple GC traits may help resolving the contradictions between studies.

We here test for the relationships between corticosterone (CORT, the main avian glucocorticoid) and survival in male and female zebra finches *Taenopygia guttata* in outdoor aviaries. The captive setting allowed us to repeatedly sample known-age individuals, control for life-history variation by keeping individuals in same-sex groups (preventing breeding), and minimize individual differences in diet, social setting and other factors that may affect GC levels. At the same time, we recognize that providing a more controlled environment is also a limitation of our study, because the effects of ecologically relevant variables such as predation and reproduction, and their potential interactions with sex, are excluded from our experimental design. For each individual, we assessed four aspects of the GC axis: plasma concentrations of (1) baseline CORT (Bas-CORT) and (2) stress-induced CORT (SI-CORT) after a capture-restraint stressor (Wingfield, Smith, & Farner, 1982). We further assessed (3) the strength of negative feedback by determining CORT levels following dexamethasone injection (DEX-CORT, see Hau et al., 2015; Kriengwatana et al., 2014; MacDougall-Shackleton et al., 2013; Romero & Wikelski, 2010) and (4) the maximal adrenal capacity to secrete CORT after adrenocorticotropin-injection (ACTH-CORT, see Dickens, Earle, & Romero, 2009; Hau et al., 2015; Kriengwatana et al., 2014; MacDougall-Shackleton et al., 2013; Rich & Romero, 2005; Romero & Wikelski, 2010). Given the mixed results in the literature, we did not have strong predictions. However, in light of conceptual considerations we expected that low baseline (indicating low allostatic load; McEwen & Wingfield, 2003), and high responsiveness to the restraint protocol, and to both dexamethasone and ACTH injections, would be positively associated with survival in both sexes. We previously found all of these CORT traits to be repeatable in our population, with repeatabilities of measurements taken 1 year apart of $r = .45$ for Bas-CORT, $r = .55$ for SI-CORT, $r = .60$ for DEX-CORT and $r = .70$ for ACTH-CORT (Jimeno, Briga, Verhulst & Hau, 2017; unpubl. data). Thus, we can characterize individuals in our population based on their CORT traits.

2 | MATERIALS AND METHODS

2.1 | Animals and housing

This study was carried out at the University of Groningen, the Netherlands, as part of an ongoing full-factorial long-term experiment described in detail in Briga, Koetsier, Jimeno, Boonekamp, and Verhulst (2017) with the general aim to study the effects of developmental and adult environmental conditions on ageing, life span and the underlying physiological mechanisms. In brief, all birds were reared in either a small or large brood, and moved to aviaries when 3 months old in which foraging conditions were either easy or hard. Specifically,

when the chicks were a maximum of 5 days old, all chicks in the nest were weighed and cross-fostered randomly to create experimentally small (two, sometimes three chicks) and large (six, sometimes five chicks) broods. These brood sizes are within the range observed in the wild (Zann, 1996) and in captivity (Griffith et al., 2017). From 35 until *c*. 120 days of age, young birds were separated by sex and housed in indoor aviaries (153 × 76 × 110 cm) with about 40 other young and two male/female adults (tutors) to allow for sexual imprinting. Once they reached sexual maturity individuals were housed in eight outdoor aviaries (310 × 210 × 150 cm) for a long-term adult treatment in which they spent the rest of their lives. Four aviaries provided an easy foraging environment with low foraging costs and four provided a hard foraging environment with high foraging costs. In brief, food was offered in boxes that were suspended from the ceiling, and there were holes in the boxes through which the birds can reach the food. Underneath the holes, there was either a perch (easy foraging), or not (hard foraging), in which case the birds had to fly to the food box and back for every seed (see Koetsier & Verhulst, 2011 for details of the foraging manipulation). In each aviary, there was only one sex present, while both brood size treatments were balanced. This is a long-term experiment and every year some young birds were added to the treatments to maintain *c*. 20 birds per aviary, as well as keep the sex and brood size treatment balance.

2.2 | Blood sampling

We sampled one bird per aviary per day (n = 8/day), in order to minimize disturbance and possible changes in CORT (see Jimeno, Briga, et al., 2017 for details). We chose to follow this procedure because the size of our aviaries made it difficult for more than one person to catch and bleed a bird within 2 min (note that longer times would have prevented us from getting baseline concentrations). Sampling was balanced with respect to sex, age group and experimental treatments. Blood samples (N = 211) were taken from the brachial vein of 162 individuals in the same month in two consecutive years: 91 individuals in May 2014 (0.88–8.29 years old, M = 3.82) and 120 individuals in May 2015 (0.93–8.81 years old, M = 3.33). Of those birds, 49 were sampled in both years. We determined Bas-CORT concentrations by sampling within 2 min after the door of the aviary was opened. After the first sample, the birds were placed in an opaque cloth bag where they waited for 20 min (restraint), after which the stress-induced sample was collected (SI-CORT). Immediately after the second sample, we tested the adrenal′s maximal ability to down-regulate the CORT response via negative feedback by administrating a dexamethasone (DEX, CORT analogue) injection into the pectoral muscle (1,000 μg/kg; MacDougall-Shackleton et al., 2013; Romero & Wikelski, 2010) and taking a third blood sample at minute 80 (DEX-CORT). Finally, we administered an adrenocorticotropic hormone (ACTH, CORT precursor) injection (100 IU/kg; Hau et al., 2015) to determine the maximum CORT release capacity and took the final blood sample at minute 100 after first disturbance (ACTH-CORT). Thus for each bird, four blood samples (max total 150 μl) for CORT analyses were taken (Figure S1). Plasma was separated through centrifugation and stored at −20°C until analysed. A few individuals (n = 18) had lost the maximum amount of blood already after the third sample, in which case the fourth sample was not taken. After blood sampling, birds were allowed to recover with food and a heat lamp before being returned to their aviary.

Birds were weighed 1 week before blood sampling to calculate the mass-adjusted doses of DEX and ACTH (birds were not weighed at sampling to minimize handling). Body mass is highly repeatable in our population (r = .72; Briga & Verhulst, 2017).

2.3 | Hormone analysis

We determined plasma CORT concentrations 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 the same individual were placed in adjacent wells, but in other respects samples were randomly distributed. Aliquots of either 10 μl (for Bas-CORT and DEX-CORT) or 7 μl plasma (SI-CORT and ACTH-CORT) along with a buffer blank and two positive controls (at 20 ng/ml) were extracted twice 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). In 2014, intra-plate coefficient of variation (CV; $M \pm SE$) was 9.63 ± 5.1% and inter-plate CV was 15.23 ± 3.2% (n = 10 plates). In 2015, the intra-plate CV was 11.43 ± 7.05% and inter-plate CV was 9.99 ± 2.67% (n = 16 plates). Note that inter-plate variation was accounted for in the statistical analyses by including plate identity as a random effect in the models. Final CORT concentrations were corrected for average loss of sample during extraction (15% in our laboratory; see Baugh, van Oers, Dingemanse, & Hau, 2014).

2.4 | Statistical analyses

Associations between CORT and survival were analysed using Cox proportional hazards models (CPH, coxme package; Therneau, 2012) with number of days from sampling till death as the dependent variable. Birds still alive on 1 March 2017 (31 males and 24 females) and birds that died from various causes unrelated to the present dataset (12 males and four females; mainly accidents where the birds got themselves trapped inside the aviary) were censored. CPH analyses require predictors to be proportional, i.e. parameter coefficients and mortality rates need to be constant with time (Therneau, 1997, p. 127). We checked for the proportionality assumption using Schoenfeld residuals and with the "cox.zph" function, and this was the case for all variables except for age. However, the effect of sampling age on mortality accelerated with time (adding 1 year of sampling age has a stronger effect on the mortality of old than of young birds) and hence was not proportional. We solved this issue by categorizing age at sampling into three groups of equal range and included this as "strata" in the models (Therneau, 1997, p. 45 & 145), which fulfilled the proportionality assumption (p > .1). Linearity and influential

TABLE 1 Correlation matrix (*r*) for the four CORT variables measured (log-transformed) in males (below diagonal) and females (above diagonal). All correlations were positive. *N* = 192 complete sets (i.e. four samples). Bas-CORT: baseline CORT, SI-CORT: stress-induced CORT, DEX: CORT after DEX injection, ACTH: CORT after ACTH injection. Significance levels: **p* < .05; ***p* < .01; ****p* < .001

	Bas-CORT	SI-CORT	DEX	ACTH
Bas-CORT		0.34***	0.13	0.21*
SI-CORT	0.28**		0.62***	0.64***
DEX	0.21*	0.45***		0.65***
ACTH	0.25*	0.47***	0.38***	

data points were checked with Martingale and deviance residuals respectively. In all CPH models, sampling year (2014 or 2015), assay plate and individual identity nested within aviary were included as random factors. CORT variables were log-transformed and standard-normalized in all analyses to allow for a comparison of the model coefficients. We also tested for a quadratic effect of every CORT variable on survival but these were never statistically significant (not shown). To compare model fits on data, we used the Akaike information criterion (AIC_c; Burnham & Anderson, 2003). Because the study population was subjected to two experimental manipulations, i.e. of developmental conditions (natal brood size) and foraging conditions in adulthood, the survival analyses were carried out with and without the experimental treatment factors and their interaction in the model. This comparison is in itself of interest, because it sheds light on the extent to which the experimental effects on survival are mediated by experimentally induced adjustments in the GC-phenotype.

The four CORT variables were not independent, with sex-specific correlations between the variables ranging from 0.13 to 0.65 (Table 1), being significant in most cases. We therefore first analysed their associations with survival separately, and followed this up with further tests to address specific questions arising from the results.

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

3 | RESULTS

By 1 March 2017, 61 of the 91 birds (67%) sampled in 2014 and 59 of the 120 birds (49%) sampled in 2015 had died a natural death, well in agreement with the age-dependent survival rates we observed in this population (Briga et al., 2017). Some birds (*N* = 49) were sampled twice, and of the 162 birds sampled in total, 107 (66%) had died (Table S1). Of the four CORT variables, SI-CORT showed the strongest and the only significant association with survival (Figure 1; Table 2). However, a significant interaction between sex and SI-CORT on survival indicated this association to be sex-specific (*p* = .007; Table 2b). We thus ran separate models for each sex for SI-CORT.

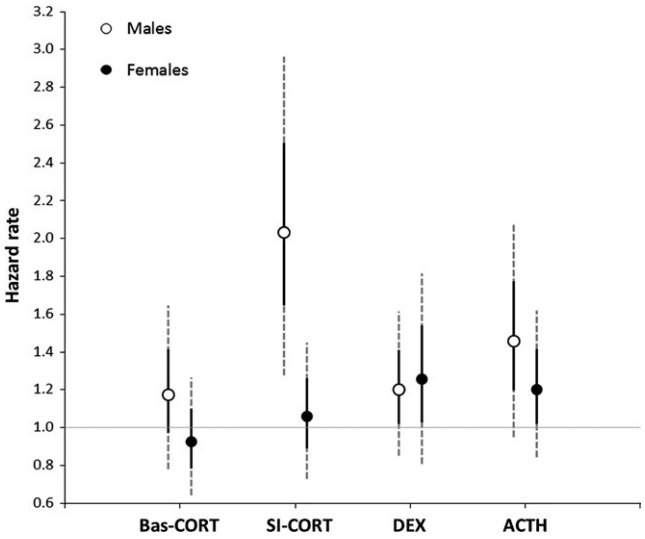

FIGURE 1 Relationship between CORT and mortality for each of the CORT variables. Shown are hazard rates from models as in Table 2, run separately for each sex. A hazard ratio of one implies no effect, and values >1 indicate increasing mortality with an increase in the independent variable. Females: filled symbols, males: open symbols. Error bars show *SE*. (black, continuous) and 95% confidence interval (grey, dashed). Bas-CORT: baseline CORT, SI-CORT: stress-induced CORT, DEX: CORT after DEX injection, ACTH: CORT after ACTH injection

Males with higher SI-CORT concentrations had higher mortality (Figure 2; $z = 3.37$, $p = .0007$), while there was no discernible association between SI-CORT and survival in females (Figure 2; $z = 0.33$, $p = .74$). Additionally, as previously observed in this population (Briga et al., 2017), there was an effect of sex on survival, independently of CORT variables, with females having lower survival than males (Figure 3; Table 2). These results remained unchanged when controlling for early and late environmental conditions: rearing brood size, foraging treatment and their interaction (Table S2). Thus, only SI-CORT predicted survival and in males only. SI-CORT concentrations did not differ between years (2014 vs. 2015). This was found both on a between-individual level (Jimeno, Briga, et al., 2017; Jimeno, Hau, et al., 2017) as well as within-individuals, and irrespective of sex (Figure S2). Furthermore, the difference in SI-CORT between the first and the second year (SI-CORT in 2015—SI-CORT in 2014) was not related to age at first sampling, with no differences between sexes (Figure S3). Note however, that this finding does not provide information on age effects on SI-CORT, as we cannot distinguish these from year effects given that we sampled 2 years only.

There was a tendency for higher ACTH-CORT levels to be related to lower survival (Table 2d; Table S2d). However, this relationship disappeared when controlling for SI-CORT levels in the analysis ($z = 1.31$, $p = .19$), with only SI-CORT remaining as the variable with a significant effect on survival. This finding was confirmed by a model selection procedure, comparing AIC_c values of the male survival model (as in Table S3b). A model with SI-CORT fitted the data substantially better than a model with only ACTH-CORT ($\Delta AIC = -3.41$) and was indistinguishable from a model with both parameters ($\Delta AIC_c = -0.42$). Thus

TABLE 2 Cox proportional hazards models of the relationship between each of the CORT variables and survival. (a) Baseline CORT (Bas-CORT), (b) Stress-Induced CORT, (c) CORT after DEX and (d) CORT after ACTH. To make coefficients comparable, the CORT variables were log-transformed and standardized (mean zero, *SD* = 1). Each table shows two models, without (left) and with (right) the interaction between sex and CORT. Note that Exp(coef) estimates are hazard ratios. A hazard ratio of one implies no effect, and values >1 indicate increasing mortality with an increase in the independent variable

Fixed effects	**Coef ± *SE***	**Exp(coef)**	***z***	***p***	**Coef ± *SE***	**Exp(coef)**	***z***	***p***
(a) Bas-CORT								
Bas-CORT	0.027 ± 0.111	1.027	0.24	.810	−0.070 ± 0.138	0.932	−0.51	.610
Sex (male)	−0.627 ± 0.246	0.534	−2.54	.011	−0.643 ± 0.243	0.526	−2.64	.008
Sex × Bas-CORT	—	—	—	—	0.268 ± 0.232	1.307	1.16	.250
Random effects	**Variance**				**Variance**			
Bird ID/aviary	0.000				0.000			
Bird ID	0.481				0.448			
Plate	0.116				0.108			
Year	0.027				0.037			
Fixed effects	**Coef ± *SE***	**Exp(coef)**	***z***	***p***	**Coef ± *SE***	**Exp(coef)**	***z***	***p***
(b) SI-CORT								
SI-CORT	0.311 ± 0.120	1.364	2.58	.010	0.067 ± 0.155	1.069	0.43	.670
Sex (male)	−0.605 ± 0.251	0.546	−2.41	.016	−0.672 ± 0.266	0.511	−2.52	.012
Sex × SI-CORT	—	—	—	—	0.694 ± 0.256	2.001	2.71	.007
Random effects	**Variance**				**Variance**			
Bird ID/aviary	0.103				0.168			
Bird ID	0.400				0.543			
Plate	0.132				0.150			
Year	0.061				0.030			
Fixed effects	**Coef ± *SE***	**Exp(coef)**	***z***	***p***	**Coef ± *SE***	**Exp(coef)**	***z***	***p***
(c) CORT after DEX								
CORT DEX	0.178 ± 0.116	1.195	1.54	.120	0.148 ± 0.159	1.159	0.93	.350
Sex (male)	−0.616 ± 0.240	0.540	−2.56	.010	−0.614 ± 0.241	0.541	−2.54	.011
Sex × CORT DEX	—	—	—	—	0.063 ± 0.217	1.065	0.29	.770
Random effects	**Variance**				**Variance**			
Bird ID/aviary	0.092				0.077			
Bird ID	0.257				0.295			
Plate	0.157				0.157			
Year	0.064				0.060			
Fixed effects	**Coef ± *SE***	**Exp(coef)**	***z***	***p***	**Coef ± *SE***	**Exp(coef)**	***z***	***p***
(d) CORT after ACTH								
CORT ACTH	0.250 ± 0.113	1.284	2.22	.027	0.138 ± 0.134	1.148	1.03	.300
Sex (male)	−0.588 ± 0.217	0.555	−2.72	.007	−0.594 ± 0.213	0.551	−2.79	.005
Sex × CORT ACTH	—	—	—	—	0.293 ± 0.214	1.341	1.37	.170
Random effects	**Variance**				**Variance**			
Bird ID/aviary	0.010				0.000			
Bird ID	0.010				0.000			
Plate	0.150				0.153			
Year	0.149				0.157			

AIC_c values for only SI-CORT and for SI-CORT together with ACTH-CORT were indistinguishable, but lower than for only ACTH-CORT, suggesting that the association between ACTH-CORT and survival is mainly caused by the positive correlation between SI-CORT and ACTH-CORT (r = .47, Table 1).

SI-CORT concentrations are the sum of Bas-CORT and the increase induced by a stressor. We therefore compared the relative contributions of these two components to the association between SI-CORT and survival in males. To this end, we ran a model including both Bas-CORT and CORT "increase", to compare the slopes of their associations with survival (Table 3). The coefficients of the two variables were indistinguishable, and we therefore conclude that variation in Bas-CORT and the CORT increase after stress contributed similarly to the association between SI-CORT and male survival.

4 | DISCUSSION

Male zebra finches with higher stress-induced CORT concentrations survived less well than conspecific males with lower SI-CORT concentrations. This relationship was independent of early and late life manipulations of environmental conditions (which did not affect SI-CORT; Jimeno, Briga, et al., 2017), and was absent in females. Furthermore, SI-CORT concentrations did not change with age in this population (Jimeno, Briga, et al., 2017), so we can dismiss age driving the observed effect. Of the different aspects of the glucocorticoid axis we tested, only SI-CORT was related to survival. Given the consistency of individual CORT phenotypes (see Section 1), and the independence from foraging conditions, our findings suggest that SI-CORT concentration is potentially a major determinant of variation in male life span in natural populations.

The relationship between SI-CORT and survival rate could either be due to the absolute SI-CORT level, to the CORT increase following a stressor, or to Bas-CORT insofar this was correlated with SI-CORT. We investigated these options by comparing the hazard rates of Bas-CORT and CORT increase after the stressor when fitted together in one model (Table 3). Both traits associated

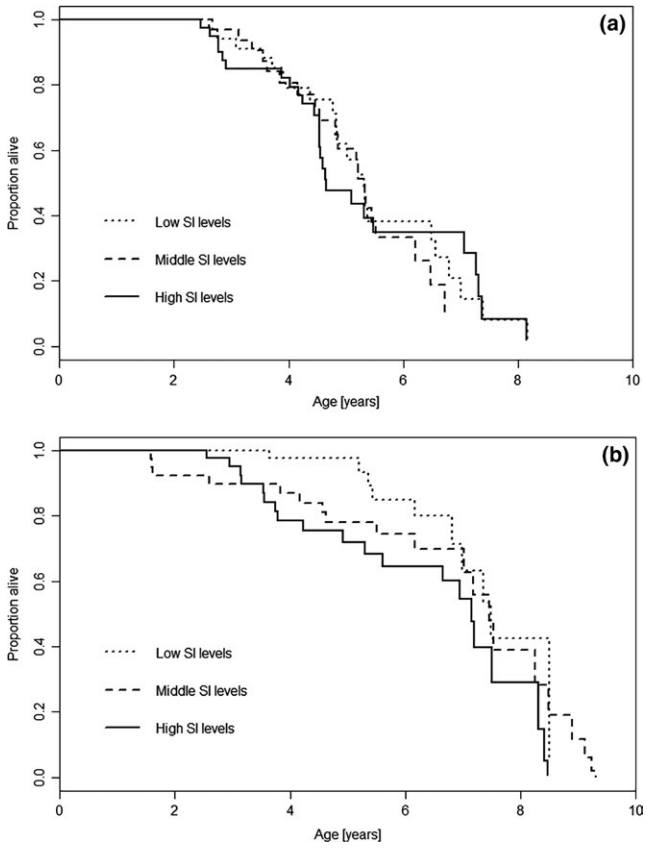

FIGURE 2 Survival curves (proportion alive) vs. age for (a) females and (b) males, grouped by SI-CORT level (tertiles). Solid lines correspond to high SI-CORT concentrations (ng/ml, upper tertile), dashed lines to intermediate SI-CORT concentrations (middle tertile), and dotted lines to low SI-CORT concentrations (lower tertile). Note that this grouping is for illustrative purposes only; CORT concentrations were entered as a continuous variable in the analyses. Also, note that the minimum age at sampling was *c.* 1 year (see Materials and methods), and hence there can be no mortality prior to this age

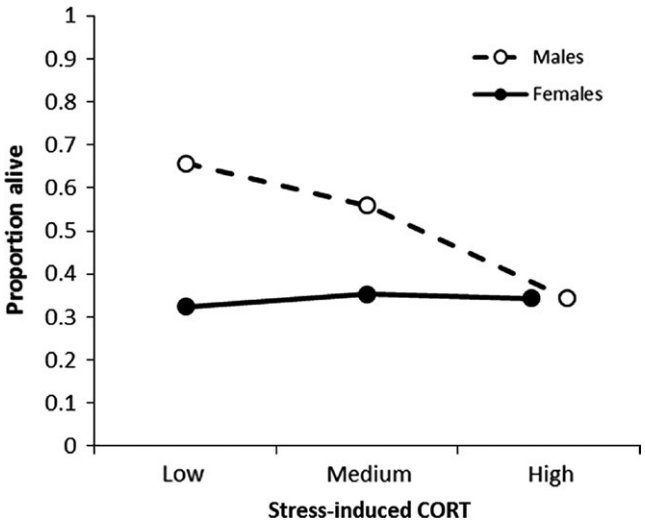

FIGURE 3 Percentage of individuals alive by 1 March 2017, grouped by relative SI-CORT concentrations. Low, medium and high concentrations correspond to the lower, middle and upper tertiles of SI-CORT distributions. Note that this categorization is for illustrative purposes only, as CORT concentrations and time until death were used as continuous variable in the analyses. Females: filled symbols and solid lines, males: open symbols and broken lines

TABLE 3 Cox proportional hazards models showing the relationship between baseline CORT and CORT increase and male survival. Baseline CORT (Bas-CORT) and stress-induced CORT (SI-CORT) were both log-transformed (but not standardized as in Table 2), and CORT increase was taken to be the difference between the log-transformed values. Note that Exp(coef) estimates are hazard ratios. A hazard ratio of one implies no effect, and values >1 indicate increasing mortality with an increase in the independent variable

Fixed effects	Coef ± *SE*	Exp(coef)	*z*	*p*
Bas-CORT	1.327 ± 0.568	3.771	2.34	.019
CORT increase	1.433 ± 0.522	4.192	2.75	.006
Random effects	**Variance**			
Bird ID/aviary	0.404			
Bird ID	0.846			
Plate	0.095			
Year	0.012			

similarly with survival, in the sense that the hazard rates were indistinguishable. This implies that we can attribute the association between SI-CORT and survival to the absolute SI-CORT levels rather than the CORT increase. It also implies that Bas-CORT by itself was associated with survival, which contrasts with the results of the initial analysis where the association between Bas-CORT and survival did not reach significance (p = .4; Figure 1). Inclusion of CORT increase in the model, which reduces residual variation and thereby increases statistical power, may explain this apparent contrast. Bas-CORT and SI-CORT concentrations exert most of their actions by binding to different receptors (Romero, 2004; Romero & Wingfield, 2015), suggesting that binding to both the mineralocorticoid and the glucocorticoid receptors contribute to the effects of SI-CORT on survival.

The negative association between SI-CORT and survival appears contradictory to the supposed benefits of a CORT stress response, which is thought to accommodate coping with acute challenging conditions such as predator attacks, thus increasing short-term survival. However, these short-term benefits may be achieved at the expense of long-term costs, for example because persistent high CORT levels increase disease susceptibility and decrease capacity to cope with additional stressors (e.g. McEwen & Wingfield, 2003; Romero et al., 2009). In our experimental set-up, there were presumably few benefits of a high CORT because life-threatening challenges (e.g. predators and large energetic challenges) were absent, which presumably differs from the environment in which the HPA axis of zebra finches evolved, leaving long-term costs to dominate the survival results. Short-term benefits of high SI-CORT are likely to be of key importance in natural populations, and, together with the selection shadow diminishing long-term costs (due to declining survival), outweigh any long-term effects. A more detailed study in which short- and long-term survival effects of high SI-CORT can be distinguished is required to test this explanation.

The association between SI-CORT and survival rate was sex-dependent, being strong in males, but absent in females (Figure 1). We are not aware of similar sex differences emerging from other studies, but note that the sex dependence of the association between CORT and survival is not always explicitly tested (e.g. Angelier et al., 2009; Blas et al., 2007; Patterson et al., 2014), and that the demonstration of such interactions requires a fairly large sample size. We can only speculate with respect to the possible cause of the large sex difference, but note that trade-offs between the CORT stress response and other functions may differ between sexes (e.g. physiological interactions with processes mediated by androgens). Alternatively, males may suffer the same damage from CORT as females but may invest differently in repair, e.g. by prioritizing investment into sexual traits over investment into antioxidant or immune defences (Schmidt, Kubli, MacDougall-Shackleton, & MacDougall-Shackleton, 2015). Finally, high stress reactivity can also be related to the social status of the individual (Creel, Dantzer, Goymann, & Rubenstein, 2013; Rubenstein, 2007), or to differences in behavioural responses or personality traits (e.g. dominance interactions; Baugh et al., 2012; Baugh, van Oers, Naguib, & Hau, 2013; Hau & Goymann, 2015), and the intensity or relevance of such associations could then differ between sexes. Thus, more information on the sex-specific associations between SI-CORT and other behavioural and physiological traits, such as immune function and oxidative stress, may explain why SI-CORT predicts survival in males, and not in females.

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CONFLICT OF INTEREST

The authors declare they have no conflict of interest.

AUTHORS' CONTRIBUTIONS

B.J., M.H. and S.V. designed the study. S.V. conceived and designed the long-term experiment in captivity, which was run by B.J. and M.B. B.J. collected the samples. B.J. analysed the data with the help of M.B. and S.V. B.J., M.H. and S.V. wrote the paper, and all authors edited later stages of the manuscript.

DATA ACCESSIBILITY

Data are archived in the Dryad Digital Repository https://doi.org/10.5061/dryad.c0g0b (Jimeno, Briga, Hau, & Verhulst, 2017).

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