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RESEARCH ARTICLE

Early life experience primes resistance to oxidative stress

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SUMMARY

The extent to which early stress exposure is detrimental to Darwinian fitness may depend on its severity, with mild stress exposure actually having a stimulatory and, possibly, beneficial effect through a hormetic response to the stressful stimulus. We need to understand such hormetic processes to determine how the early environment can help shape a phenotype adapted to the conditions the organism is most likely to experience in its adult environment. Using the zebra finch (*Taeniopygia guttata*), we tested the hypothesis that individuals exposed to mild heat stress earlier in life will suffer less oxidative stress when faced with high heat stress in adulthood than will individuals either not pre-exposed to heat stress or exposed to high heat stress earlier in life. Our findings demonstrate that early life exposure to mild heat stress primes the system to better withstand oxidative stress when encountering heat stress as an adult. These findings point to a potential mechanism linking early life experiences to future Darwinian fitness.

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INTRODUCTION

Pre- and post-natal exposure to stressful conditions prior to sexual maturity can have negative fitness consequences (Monaghan, 2008; Harris and Seckl, 2011; Henriksen et al., 2011). However, the extent to which early stress exposure is detrimental may depend on its severity, with mild stress exposure actually having a stimulatory and, possibly, beneficial effect (Costantini et al., 2010). There is increasing evidence that some non-essential substances or environmental stressors can have stimulatory or beneficial effects at low exposure levels while being toxic at higher levels, and that environmental priming or 'conditioning' of certain physiological processes can result in an organism then better able to deal with such adversities. These kinds of non-linear dose–response relationships are referred to as hormetic responses (Southam and Ehrlich, 1943) and have been described across a wide range of organisms (from bacteria to vertebrates), in response to exposure to at least 1000 different chemical and environmental stressors (Mattson and Calabrese, 2010). Although studies show that hormetic effects can emerge at any time throughout the individual's life (Mattson and Calabrese, 2010), they also suggest that such effects might be stronger when animals are exposed to mild stressors in early life (Bartling et al., 2003; Honma et al., 2003; Le Bourg et al., 2004). Understanding these processes may therefore be very important in determining how the early environment can help shape a phenotype adapted to the conditions the organism is most likely to experience in its adult environment.

The effect of ambient temperature on organism morphology, physiology and behaviour represents an important example of phenotypic adjustment to environmental conditions (Hill et al., 2008). Temperature has been found to induce hormetic responses in several animal species (Mattson and Calabrese, 2010). Homeothermic species are characterized by a thermoneutral zone, which is a range of ambient temperatures (T_a) within which the resting metabolic rate is independent of T_a. Above the thermoneutral zone, metabolic rate increases because the organism needs to use energy to increase the rate of water evaporation from the body surface (active evaporative cooling) and, at the same time, to counterbalance cooling in order to avoid an excessive decrease in body temperature (T_b) (Hill et al., 2008). If the T_a increases substantially, homeotherms can enter a hyperthermic state, which is characterized by a steep rise in T_b. The rise in T_b allows the animal to lose heat because T_b is then higher than T_a. However, when T_b increases to a specific temperature threshold, hyperthermia becomes detrimental and heat stress-induced mortality is observed (Calder, 1964; Hill et al., 2008).

Despite the evolution of specific adaptations to withstand increases in T_a, animals can become heat stressed when they are unable to balance production and loss of heat (Calder, 1964). The level of heat stress can be mild when T_a is close to the upper critical temperature of the thermoneutral zone, but high when T_a is above the upper critical temperature. A state of heat stress is characterized by several physiological changes beyond metabolic adjustments, such as increases in the synthesis of heat shock proteins (Feder and Hofmann, 1999), increases in the production of reactive oxygen species (Flanagan et al., 1998; Mujahid et al., 2005; Lin et al., 2008) and changes in antioxidant status (Lord-Fontaine and Averill-Bates, 2002; Lin et al., 2008). As a consequence, the animal may experience changes in oxidative balance and consequent induction of oxidative stress (Sies, 1991; Halliwell and Gutteridge, 2007; Costantini and Verhulst, 2009), disruption of redox signalling and control (Jones, 2006), and overoxidation of thiols (Sohal and Orr, 2012). It may also show reductions in body mass gain, food consumption,

oxidative metabolic efficiency, muscle integrity and/or survival (Sandercock et al., 2001; Mujahid et al., 2005; Azad et al., 2010).

While such data come from laboratory studies, heat stress is likely to be very relevant for organisms in the wild, as they are likely to experience significant variations in temperature, including on occasion conditions that will induce heat stress. Although the phenotype is programmed to deal with a wide range of temperatures, its capacity to cope with unpredictable very high temperatures is likely to have significant fitness consequences. Furthermore, the oxidative stress that may arise as a result of heat stress is likely to be a key mediator in determining the fitness of organisms in the wild, given that the resultant tissue degradation might influence reproductive performance, growth patterns, cellular senescence and survival (Alonso-Alvarez et al., 2006; Costantini and Dell'Omo, 2006; Costantini, 2008; Monaghan et al., 2009; Saino et al., 2011).

The timescale over which high temperatures are experienced might also affect their impact: for example, the levels of heat stress encountered will vary among individuals, depending on factors such as the season in which they are born and the prevailing weather conditions; those individuals that experience heat stress could actually be better placed to cope with subsequent high temperatures if the initial exposure has induced a hormetic conditioning response. There is some empirical support for such an effect. Studies on *Drosophila*, *Caenorhabditis elegans* and *Saccharomyces cerevisiae* have shown that exposure to mild heat stress early in life can increase longevity (e.g. Shama et al., 1998; Le Bourg et al., 2001; Cypser and Johnson, 2002; Olsen et al., 2006; Sørensen et al., 2007). A study on *Gallus gallus* has shown that male chicks exposed to 36–37°C when 3 days old suffered lower mortality and had lower triiodothyronine concentration (i.e. better thermotolerance) when subjected 6 weeks later to a 35°C heat shock (Yahav and McMurtry, 2001). However, we still know little about the mechanisms responsible for these apparently hormetic effects on longevity.

In this study, we tested the hypothesis that the hormetic response induced by a conditioning exposure to mild heat stress involves a greater ability to combat oxidative stress: we predicted that exposure to mild heat stress relatively early in life would lead to an improved ability to maintain oxidative balance (and reduce oxidative stress) when faced with high heat stress in adulthood. We further predicted that such a hormetic effect would not be observed when the initial exposure involved high heat stress. We also examined changes in body mass, because reducing body mass may be one strategy to assist thermoregulation and reduce energetic demands (Deerenberg et al., 1998; Speakman and Selman, 2003). We used the zebra finch, *Taeniopygia guttata* (Vieillot 1817), as our study species because much primary literature is available on its thermal and antioxidant physiology, which therefore provides a good background for designing the study and interpreting the results.

MATERIALS AND METHODS

Study birds were obtained by breeding pairs of stock zebra finches kept at Glasgow University. Cages were provided with *ad libitum* food and water at all times. As in previous studies (Blount et al., 2003), the birds were fed a diet composed of mixed seeds (foreign finch mixture, J. E. Haith, Cleethorpes, Lincs, UK, including some soaked overnight to saturation), grit and cuttlefish. The birds also received Haith's conditioning supplement, fresh spinach and Calcivet calcium supplement (Vetafarm, Wagga Wagga, NSW, Australia) once a week. The young birds were separated from their parents the day before the start of the thermal exposure (experimental day 0, see below). Males and females were housed in separate cages upon attaining adult plumage; their diet regime was the same as that of their parents. The work was conducted under licence from the UK Home Office as appropriate.

Thermal conditioning in early life

A total of 132 zebra finches (72 males and 60 females) were included in the thermal conditioning phase of the experiment when 42–45 days old. At this age, males and females are sexually immature [maturation takes between 62 and 90 days (Zann, 1996)] but can be distinguished by their plumage. Siblings were assigned randomly to three initial temperature treatment groups, which involved exposure to different temperature regimes during the conditioning phase in early life: Control early, individuals kept throughout at the standard housing temperature (24 males and 20 females); Mild early, exposure to mild heat stress (25 males and 20 females); and High early, exposure to high heat stress (23 males and 20 females). Sexes were assigned to the treatment groups to give a balanced sex ratio among groups. However, each bird was randomly assigned to a particular experimental group, and the birds from different treatments were kept together in single-sex common cages (8–10 birds per cage, following UK Home Office recommendations; balanced number among treatment groups) when not being subjected to the periods of heat treatment in order to rule out confounding cage effects. Temperatures were chosen according to Calder (Calder, 1964) (see below for more details).

The two early heat stress groups (Mild early and High early) were exposed to brief periods of higher temperatures than controls by placing the birds in small groups (3–7) in cages that in turn were put in a TLC-5 Brooder/Intensive care unit (Brinsea Products, Sandford, Avon, UK) for 3 h every second day, a total of 14 times (i.e. over a 28 day period). These care units are designed for holding small groups of birds at fixed temperatures. During these 3 h heat treatment sessions the birds were provided with water *ad libitum* (but not food). The birds in the Control early group were subjected to the same regime, being also put in a care unit with water *ad libitum* and without food for 3 h every second day, a total of 14 times, but the temperature inside the care unit for the Control early group was kept at the same room temperature at which all the birds had been reared (21–23°C).

Zebra finches occur in the arid and semi-arid regions of Australia, where they can experience high temperatures during different parts of the day/season that pose them with problems of conservation of body water and maintenance of sublethal T_b (Zann, 1996). The two heat stress regimes were chosen with reference to the thermoneutral zone and other physiological responses of zebra finches to T_a, as determined by the detailed study of thermoregulation in zebra finches by Calder (Calder, 1964). Calder recorded a minimum mean oxygen consumption at an ambient temperature of 34.9°C (Calder, 1964). Increases in oxygen consumption were observed below 29.5°C (lower critical temperature) and above 40°C (Calder, 1964). T_b was maintained relatively constant at ambient temperatures between 10 and 30°C, while above 30°C a gradual progression to mild hyperthermia was observed (Calder, 1964). Pulmocutaneous water loss (the ratio of water loss to oxygen consumption) increased gradually between 10 and 30°C, more rapidly between 30 and 40°C, and sharply above 40°C; the highest ambient temperature tested by Calder was 43.5°C and the lethal body temperature was calculated by him as 46.4°C (Calder, 1964). For the Mild early group we therefore adopted a T_a treatment of 38°C during their 3 h exposure periods, which is close to the upper critical T_a of the thermoneutral zone. The High early group was exposed to 42°C, which is above the upper limit of the zone but well below the lethal limit.

On day 0 of the experiment, a sample of blood was taken and body mass of the birds was measured using an electronic balance (0.01 g). On day 1, thermal conditioning was started. A second sample of blood was taken and body mass recorded the day after the end of the thermal conditioning, i.e. 29 days after the first blood sample.

Short-term exposure to high heat stress in adulthood

In order to test how early exposure to stress affected the later capacity to deal with a similar stressor, half of the birds from each early exposure treatment group were allocated to one of two adult treatment groups at adulthood (177–180 days): Control adult or Stress adult (42°C). Therefore, we had six experimental groups (see Fig. 1), which varied with respect to early and adult thermal experience: Control early–Control adult (12 males and 10 females), Mild early–Control adult (12 males and 10 females), High early–Control adult (11 males and 10 females), Control early–Stress adult (12 males and 9 females), Mild early–Stress adult (13 males and 9 females) and High early–Stress adult (12 males and 10 females). The plasma samples of 4 females (1 Control early–Control adult, 2 Mild early–Control adult, 1 Control early–Stress adult) were not analysed because they were turbid. However, these females were retained in the analyses of red blood cells (RBCs), giving a total sample size of 130 for assays based on RBCs and 126 for those based on plasma.

Birds were exposed to the short-term thermal adult treatment for 3 h every day for a total of 3 consecutive days, again being placed in the care units with *ad libitum* water but no food for the 3 h duration of each thermal treatment. The birds were weighed and samples of blood taken the day before the adult treatment started and again on the third treatment day just after the 3 h thermal exposure.

Bleeding procedure, storage and laboratory analyses

Blood samples were collected by venipuncture from the brachial vein using microhaematocrit heparinized capillary tubes (Vetlab Supplies, Pulborough, West Sussex, UK). Blood samples were maintained on ice and then were centrifuged to separate plasma from RBCs. Several aliquots were made for both plasma and RBC samples and were stored at –70°C. Laboratory analyses were carried out within 1 month of collection. In addition to biomarkers of blood redox state, we measured total proteins in both plasma and RBCs using the Bradford protein assay (Bio-Rad Laboratories, Hercules, CA, USA) (see Costantini et al., 2011), and used them to express values of antioxidant biomarkers per mg of proteins occurring in the matrix. Given that similar results were obtained using standardization of antioxidants for protein content, we only present outcomes of statistical models on unstandardized variables.

Quantification of blood redox status

Biomarkers of oxidative damage and non-enzymatic and enzymatic antioxidant status were measured according to established protocols (e.g. Costantini and Dell'Omo, 2006; Costantini et al., 2011). Briefly, hydroperoxides (intermediate oxidative damage products) were measured in 4 µl of plasma using the d-ROMs (reactive oxygen metabolites) assay (Diacron International, Grosseto, Italy); hydroperoxides derive from oxidation of several biomolecular substrates, such as polyunsaturated fatty acids, cholesterol, proteins and nucleic acids, and are precursors of end-products of lipid peroxidation, such as malondialdehyde, hydroxynonenal and isoprostanes (Halliwell and Gutteridge, 2007; Lajtha et al., 2009). Non-enzymatic antioxidant capacity (OXY) was measured in 2 µl diluted plasma (1:100 with distilled water) and 2 µl of erythrocyte haemolysate (RBCs diluted 1:800 with distilled water) using the OXY-adsorbent assay (Diacron International). Glutathione peroxidase (GPX) was measured in 4 µl of diluted plasma (1:40 with diluting agent) and 4 µl of diluted erythrocyte haemolysate (RBCs diluted 1:40 with diluting agent) using the Ransel assay (RANDOX Laboratories, Crumlin, UK). The concentration of thiol groups in RBCs was measured using the –SHp assay (Diacron International) using 12.5 µl of erythrocyte haemolysate (RBCs diluted 1:200 with distilled water). The absorbance was read with a Thermo Scientific Multiskan Spectrum (ThermoFisher, Vantaa, Finland) at 505 nm for ROMs, 490 nm for OXY, 340 nm for GPX and 405 nm for thiols. All analyses were run in duplicate and the intra- and inter-assay coefficients of variation were: 6.70 and 7.47% for oxidative damage; 6.84 and 7.98% for plasma OXY; 5.97 and 6.77% for RBC OXY; 6.07 and 7.30% for plasma GPX; 4.32 and 5.87% for RBC GPX; 5.48 and 7.68% for thiols.

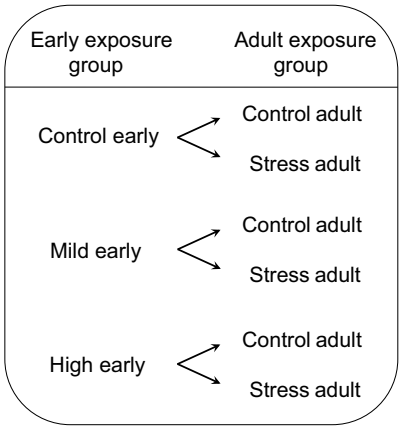

Fig. 1. Experimental design. The birds of each early conditioning treatment (Control, Mild, High) were allocated to one of two adult treatment groups: Control or high heat stress (Stress, 42°C). Therefore, we had six experimental groups, which varied with respect to early and adult thermal experience.

Statistical analyses

Data were analysed using linear mixed effect models in STATISTICA Version 10 (StatSoft, Tulsa, OK, USA). Statistical outcomes of the final models are reported in Tables 1 and 2. Assumptions of normality of residuals (Kolmogorov–Smirnov test) and homogeneity of variances (Levene's test) were respected. *Post hoc* comparisons were carried out using the Fisher LSD test. Effect size of main results is reported in the Results. Descriptive statistics of biochemical variables and body mass are reported in supplementary material Tables S1 and S2.

For the early life treatment, the model included treatment, sex and their interaction as fixed factors. Brood of origin of each juvenile was entered as a random factor to control for pseudoreplication. As a dependent variable, we used the percentage within-individual change in each variable over the conditioning period.

For the adult responses, the model included two different factors for treatment (early life treatment and adult treatment), plus sex and the following interactions: early life treatment × adult treatment; sex × early life treatment × adult treatment. As a dependent variable, we used the within-individual percentage change in each respective variable from the measurement made on the day before

the adult treatment started to the measurement made on day 3 of the adult treatment period.

Similar results for all models were obtained using the difference between the post- and pre-treatment value (data not shown). Non-significant terms were sequentially removed from the models starting from the higher order interactions and the analyses were repeated until we obtained a model with only significant terms.

RESULTS

Effect of the early thermal conditioning on blood oxidative stress

On average, all three groups showed an increase in the level of intermediate oxidative damage products recorded over the conditioning period (Fig. 2A). However, the birds exposed to high heat stress as juveniles showed a significantly greater increase in oxidative damage in comparison with controls or those experiencing mild heat stress at this time (effect size=0.78); this effect was the same for both males and females (Table 1, Fig. 2A). Plasma GPX increased marginally more in females than males, but was unaffected by treatment. While all birds gained body mass over the exposure period, there was a significant effect of treatment (effect size=0.98), with mass gain being lowest in the high stress group and highest in the controls (Table 1, Fig. 2B). However, the effect of treatment on changes in body mass was stronger in females than in males (effect size=0.59). There was no difference in the other parameters measured (Table 1). All the measured parameters, with the exception of RBC OXY, showed a significant effect of brood identity in the juvenile treatment phase (Table 1), demonstrating a familial effect.

Given that body mass increased in all birds during the trials, all the models of physiological variables were re-run including the change in body mass as a covariate. Outcomes were similar to previous results and body mass change never contributed significantly to any of the models ($F_{1,68}$=0.02 to 2.64, P=0.90 to 0.11).

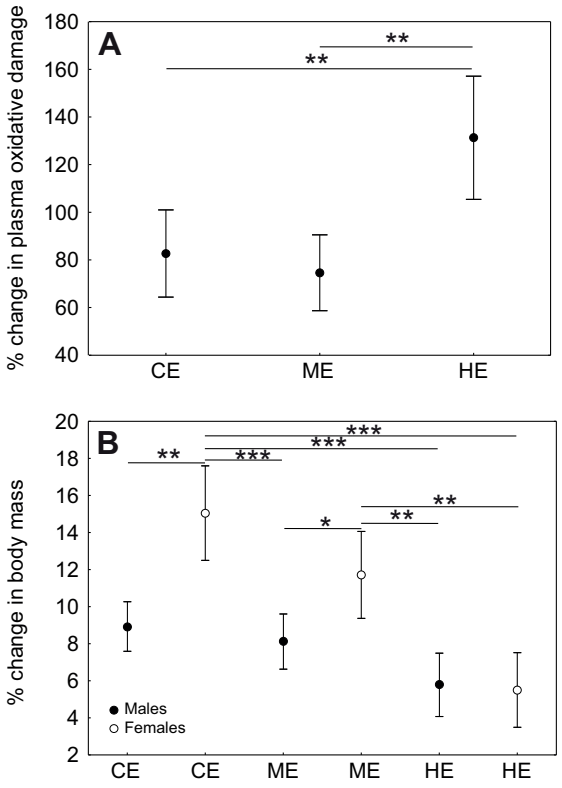

Fig. 2. Effects of thermal conditioning early in life on oxidative damage and body mass. (A) Birds of the High early group had a higher increase over the conditioning period in intermediate oxidative damage compounds than those in the Control early or Mild early groups; results from males and females were pooled because they did not differ. (B) The effect of thermal conditioning on body mass was sex dependent (see Table 1). CE, Control early; ME, Mild early; HE, High early. Data are expressed as the mean ± s.e.m. percentage change in values over the conditioning period. Significant differences among groups are highlighted as follows: *P≤0.05, **P≤0.01, ***P≤0.001.

Response to the adult short-term high heat stress protocol

There was no difference in the change of intermediate oxidative damage products recorded in the three early life treatment groups when they were exposed to the control treatment in adulthood (Table 2, Fig. 3A). This shows that the early life treatment had not caused any permanent change to levels of oxidative damage. However, the predicted hormetic conditioning effect of mild stress exposure in early life was found amongst the birds exposed to heat stress in adulthood (effect size=0.64). The change in oxidative damage over the 3 days of high heat stress exposure in adulthood was lowest in those birds that had been exposed to mild heat stress in early life, while those birds that had had no prior heat stress exposure, or had been exposed to the higher level of heat stress in early life, had higher increases in damage levels (Fig. 3A). The thiol concentration in RBCs also showed a hormetic dose–response relationship, but this was sex dependent (effect size=0.73), with a greater hormetic response in females (Table 2, Fig. 3B,C). *Post hoc* comparisons between females showed that thiols increased in those birds that had been exposed to mild heat stress in early life, but

Table 1. Results of linear mixed effect models of the early life thermal conditioning period on changes of indicators of oxidative stress and body mass

Variable	Brood	Treatment	Sex	Treatment × sex
Plasma oxidative damage	**$F_{57,72}$=4.0, P<0.001**	**$F_{2,72}$=4.8, P=0.011**	$F_{1,71}$=0.6, P=0.46	$F_{2,69}$=0.2, P=0.80
Plasma GPX	**$F_{57,73}$=2.1, P=0.001**	$F_{2,71}$=1.0, P=0.37	**$F_{1,73}$=3.8, P=0.055**	$F_{2,69}$=2.0, P=0.15
RBC GPX	**$F_{57,74}$=3.9, P<0.001**	$F_{2,71}$=0.2, P=0.82	$F_{1,73}$=0.5, P=0.47	$F_{2,69}$=0.2, P=0.86
RBC thiols	**$F_{57,74}$=3.9, P=0.005**	$F_{2,71}$=0.0, P=0.97	$F_{1,73}$=0.5, P=0.49	$F_{2,69}$=2.0, P=0.15
Plasma OXY	**$F_{57,74}$=3.9, P<0.001**	$F_{2,72}$=0.7, P=0.52	$F_{1,71}$=0.0, P=0.93	$F_{2,69}$=1.3, P=0.28
RBC OXY	**$F_{57,74}$=1.3, P=0.17**	$F_{2,72}$=1.6, P=0.21	$F_{1,71}$=0.0, P=0.90	$F_{2,69}$=0.8, P=0.46
Body mass	**$F_{57,69}$=3.3, P<0.001**	**$F_{2,69}$=9.6, P<0.001**	**$F_{1,69}$=2.8, P=0.097**	**$F_{3,69}$=3.1, P=0.048**

N=132 birds.
Outcomes in bold refer to variables included in the final model (the rest were subsequently excluded from the model because they were not significant).
GPX, glutathione peroxidase; OXY, non-enzymatic antioxidant capacity; RBC, red blood cell.

Table 2. Results of linear mixed effect models of the response to the adult heat stress regime

Variable	Brood	Early life	Adult	Sex	Early life × adult	Early life × adult × sex
Plasma oxidative damage	**$F_{57,62}$=0.9, P=0.64**	**$F_{2,62}$=2.7, P=0.08**	**$F_{1,61}$=0.8, P=0.37**	**$F_{1,62}$=7.1, P=0.01**	**$F_{2,62}$=3.6, P=0.034**	$F_{5,57}$=0.4, P=0.82
Plasma GPX	**$F_{57,68}$=1.4, P=0.11**	$F_{2,64}$=0.3, P=0.74	$F_{1,67}$=1.0, P=0.33	$F_{1,66}$=0.4, P=0.53	$F_{2,62}$=0.2, P=0.85	$F_{5,57}$=0.6, P=0.68
RBC GPX	**$F_{57,72}$=1.6, P=0.032**	$F_{2,68}$=0.2, P=0.79	$F_{1,70}$=1.5, P=0.22	$F_{1,71}$=2.6, P=0.11	$F_{2,66}$=0.4, P=0.64	$F_{5,61}$=1.1, P=0.35
RBC Thiols	**$F_{57,61}$=1.1, P=0.40**	**$F_{2,61}$=1.7, P=0.19**	**$F_{1,61}$=1.1, P=0.31**	**$F_{1,61}$=3.8, P=0.06**	**$F_{2,61}$=0.3, P=0.74**	**$F_{5,61}$=2.4, P=0.044**
Plasma OXY	**$F_{57,68}$=1.6, P=0.025**	$F_{2,64}$=0.2, P=0.81	$F_{1,67}$=3.8, P=0.06	$F_{1,66}$=0.4, P=0.53	$F_{2,62}$=1.0, P=0.38	$F_{5,57}$=0.9, P=0.51
RBC OXY	**$F_{57,72}$=1.2, P=0.22**	$F_{2,70}$=0.4, P=0.71	$F_{1,69}$=0.1, P=0.78	$F_{1,68}$=0.0, P=0.90	$F_{2,66}$=2.0, P=0.15	$F_{5,61}$=1.1, P=0.36
Body mass	**$F_{57,72}$=1.1, P=0.36**	$F_{2,70}$=1.5, P=0.23	$F_{1,69}$=1.3, P=0.26	$F_{1,68}$=0.3, P=0.59	$F_{2,66}$=0.9, P=0.41	$F_{5,61}$=0.5, P=0.80

N=126 for plasma and N=130 for RBCs and body mass.
Outcomes in bold refer to variables included in the final model (the rest were subsequently excluded from the model because they were not significant).
'Early life' refers to the early life treatment group; 'adult' refers to the adult high heat stress regime treatment group.
GPX, glutathione peroxidase; OXY, non-enzymatic antioxidant capacity; RBC, red blood cell.

decreased in those birds that had no prior heat stress exposure, or had been exposed to the higher level of heat stress in early life (Fig. 3B,C). Changes in plasma OXY and RBC GPX over the period of adult heat stress differed among broods. Changes in plasma GPX, RBC OXY or body mass were not explained by any of the variables included in the models.

DISCUSSION

In this study, we have demonstrated that mild heat stress experienced in juvenile life primes the body to better withstand oxidative stress induced by exposure to high ambient temperature in adulthood. Such early life conditioning resulted in the birds showing no increase in plasma oxidative stress on exposure to high temperatures in adulthood. In contrast, birds that had either no previous experience of heat stress or only experience of high temperatures showed a significant increase in plasma oxidative damage; they also exhibited a bigger decrease in RBC thiol antioxidants than the mild conditioning group.

Oxidative damage in Mild early birds exposed to heat stress in adulthood was around 20% lower than that observed in birds not exposed as adults to heat stress, and 35 and 55% lower than in Control early and High early birds exposed to high heat stress as adults, respectively. Such responses are consistent with the biphasic nature of a hormetic response, where stimulation and inhibition of stress response mechanisms occur at mild and high doses of a stressor, respectively. Previous studies suggest that the magnitude of stimulation is rarely higher than twice the level for the control group, and, in general, the maximum stimulation for hormesis is 30–60% higher than in control groups (Calabrese, 2010). We found no evidence of stimulation and inhibition of antioxidant mechanisms in our study. Therefore, it is plausible that the treatment differences in concentrations of intermediate oxidative damage compounds that we have measured in plasma have been primarily caused by a hormetic effect on metabolic pathways, and hence on the basal production of reactive species. For example, changes in oxygen consumption (van de Crommenacker et al., 2010) or the degree of uncoupling at the mitochondrial level (Echtay, 2007) could have modulated part of the variation in intermediate oxidative damage. Indeed, an increase in mitochondrial superoxide production in heat-stressed young cockerels has been suggested to occur because of the parallel downregulation of uncoupling proteins (Mujahid et al., 2007).

Females, but not males, showed a hormetic response in thiol concentration. Mild early females experienced an increase in thiols when exposed to the adult short-term high heat stress, and this increase was around 27% higher than Mild early females that experienced the control adult treatment, and 23 and 28% higher than Control early and High early females exposed to the high heat stress adult treatment, respectively. However, there was no difference between Mild early and Control early males in their thiol levels after the adult trials, indicating that they showed no evidence of early conditioning.

The change in thiols suggests that the effects of high temperature in juvenile life were more detrimental for females when they again experienced high environmental stress as adults. Studies on fruit flies show that hormetic effects are often larger in males than females (Salmon et al., 2001; Le Bourg et al., 2004; Le Bourg, 2005; Sørensen et al., 2007). They also show that stimulatory effects that can emerge for traits like longevity may be associated with detrimental effects for other traits, such as locomotor activity or learning (Le Bourg et al., 2004). Therefore, the depletion of thiols in females not exposed to heat stress or exposed to high stress when juvenile and then exposed to high stress at adulthood could have been traded off against other traits, whose maintenance could have been prioritized. Previous studies on birds suggested that females could for example sacrifice antioxidant protection for reproduction (Alonso-Alvarez et al., 2004; Wiersma et al., 2004), hence suffering more oxidative damage (Costantini, 2010). It is, however, unclear why females that had earlier experienced mild heat stress conditioning but no heat stress as adults experienced a decrease in thiols. It could be that these females depleted more thiols in order to control the level of oxidative damage, which was comparable to that of other Control adult groups. It is also possible that the activation of compensatory responses might have been traded off against the control of other functions later in life through, for example, a differential regulation of the expression of genes related to heat shock protein synthesis, antioxidant or DNA repair enzymes (Mattson and Calabrese, 2010). This could suggest that a physiological cost of conditioning could emerge when there is no subsequent exposure to a higher level of the stressor. We had previously suggested that the existence of such a cost would explain why natural selection has favoured a system that requires a priming effect in order to work effectively (Costantini et al., 2010). Further studies will be needed to clarify the differential responsiveness of adult males and females to a stressor in relation to early life experience.

The change in body mass in early life fitted a linear rather than a hormetic response to the thermal regime. Moreover, females were more sensitive to early life thermal conditioning than males. The mass gain in females was much less in the High early group compared with the other two groups (Control early females gained 6.1% more body mass than males; Mild early females gained 3.6% more body mass than males; High early males gained 0.3% more body mass than females). The lower increase in body mass in the

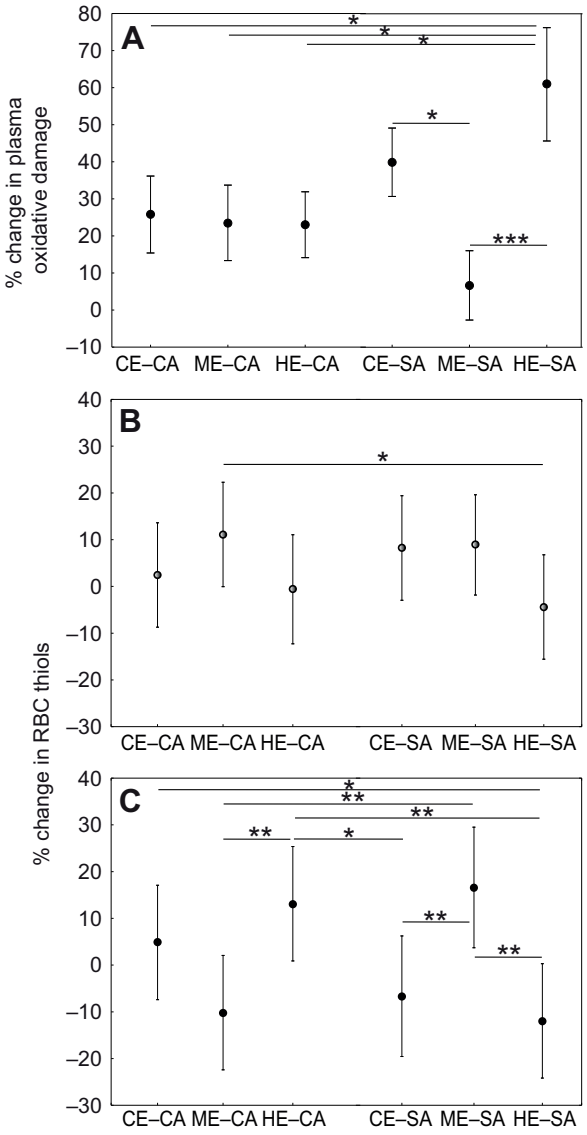

Fig. 3. Response of the birds in the three early life thermal conditioning groups to a short-term heat stress challenge when adults. Data are expressed as the mean ± s.e.m. percentage change over the period of adult heat stress. (A) Changes in plasma levels of oxidative damage. (B,C) Changes in red blood cell (RBC) thiols in males (B) and females (C). See Table 2 for statistical analysis. Treatment groups are Control early–Control adult (CE–CA), Mild early–Control adult (ME–CA), High early–Control adult (HE–CA), Control early–Stress adult (CE–SA), Mild early–Stress adult (ME–SA), High early–Stress adult (HE–SA). Significant differences among groups are highlighted as follows: $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$.

High early birds need not necessarily reflect a detrimental effect of the thermal regime. Reducing body mass may be also a strategy to reduce energetic demands (Deerenberg et al., 1998; Speakman and Selman, 2003) or to favour heat dissipation from the body at high ambient temperatures (Hill et al., 2008). When birds were exposed to high heat stress in adulthood, all birds lost body mass regardless of their early experience. In contrast, a recent experiment has shown that female zebra finches experiencing poor nutritional conditions (nutritional stress) during early development had, as adults, an increased body mass loss after food deprivation (Krause et al., 2009). Similarly, postnatal maternal separation made female but not male rats more vulnerable to the development of abnormal feeding behaviour in response to food restriction in later life (Iwasaki et al., 2000). In our case, however, there were no noticeable costs in terms of body mass loss in adults. We provide two hypothetical explanations: (1) a change in body mass may not have been detectable after only 4 days of thermal treatment; or (2) birds invested in body mass maintenance at a cost to other body functions, such as oxidative stress. Zebra finches are opportunistic breeders that inhabit the arid and semiarid regions of Australia, where breeding is triggered by aperiodic rainfall (Zann, 1996). Consequently, adult birds, and especially females, could have been programmed to prioritize investment in body stores because energy reserves are very important for successful reproduction. However, we cannot exclude the possibility that the body composition (e.g. fat *versus* lean mass) of the birds changed even if the overall body mass did not.

Our findings also show differential responsiveness of blood oxidative state components to the experimental treatments. Treatment had no effect on either the activity of the antioxidant enzyme GPX or the total non-enzymatic antioxidant capacity of plasma and RBCs. This could suggest that the oxidative stress induced by the heat stress protocol was not high enough to require upregulation of GPX or non-enzymatic antioxidants or to cause their depletion through oxidation processes. However, the lack of upregulation of GPX could also reflect a dysregulation of antioxidant mechanisms (Dröge, 2002) or that the animal de-prioritized the antioxidant response in favour of other physiological functions. With respect to the overall non-enzymatic antioxidant capacity measurements, it is also possible that any changes in circulating antioxidant levels were masked by differential deployment of antioxidants among tissues.

Finally, brood identity often explained significantly variation in some biomarkers of oxidative state, suggesting a significant genetic or maternal effect. For instance, if the effect on oxidative status primarily came through a maternal effect, this would suggest that maternal state can influence the emergence of a hormetic response in the offspring and its effects on any trade offs associated with it. Thus, a mother that experienced mild stress during the breeding phase could indirectly prime the physiological system of her offspring through deposition of hormones or antioxidants in the egg or in her interactions with the chicks after they are born. In turn, this would shape the phenotype of her offspring, possibly making them better adapted to cope with a wide range of stressor intensities compared with offspring whose mothers experienced low or high stress.

CONCLUSIONS

We have demonstrated for the first time that a thermal exposure period relatively early in life may induce short-term hormetic responses against oxidative stress and, importantly, may prime the system to withstand oxidative stress induced by heat stress in adulthood. These findings provide a plausible mechanism explaining the increase in longevity induced by hormesis. However, future studies will be needed to determine the consequences that a hormetic response may have for other physiological functions, survival and Darwinian fitness.

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Table 1. Descriptive statistics of body mass and biochemical variables collected from the early life thermal conditioning period. GPX = glutathione peroxidase; OXY = non-enzymatic antioxidant capacity; RBC = red blood cell; N = sample size. Units of measurements: body mass as grams; RBC GPX and PLA GPX as Units l^{-1} of haemolysate or plasma, respectively; PLA OXY and RBC OXY as mmol l^{-1} of plasma or haemolysate, respectively; thiols as µmol l^{-1} of –SH groups; hydroperoxides as mmol l^{-1} of H_2O_2 equivalents.

		Pre-Treatment value	Pre-Treatment value	Post-Treatment value	Post-Treatment value	
		mean	standard error	mean	standard error	N
Group	Sex	BM	BM	BM	BM	
Control Early	m	15.24	0.24	16.62	0.38	24
Control Early	f	15.21	0.30	17.47	0.47	20
Mild Early	m	15.16	0.19	16.39	0.29	25
Mild Early	f	15.29	0.31	17.09	0.55	20
High Early	m	15.20	0.25	16.03	0.26	23
High Early	f	15.81	0.37	16.64	0.43	20
		RBC GPX	RBC GPX	RBC GPX	RBC GPX	
Control Early	m	1434.33	89.89	1524.01	115.57	24
Control Early	f	1304.21	112.69	1429.69	105.54	20
Mild Early	m	1394.75	97.76	1441.39	120.00	25
Mild Early	f	1328.06	115.06	1386.19	116.52	20
High Early	m	1454.65	103.53	1541.76	110.77	23
High Early	f	1287.82	114.76	1395.53	104.93	20
		PLA GPX	PLA GPX	PLA GPX	PLA GPX	
Control Early	m	667.76	56.60	843.96	78.82	24
Control Early	f	546.37	52.97	762.38	61.71	20
Mild Early	m	646.68	51.96	744.70	53.16	25
Mild Early	f	580.01	48.34	761.70	51.76	20
High Early	m	605.33	44.79	680.41	41.64	23
High Early	f	592.98	55.20	749.75	50.92	20
		PLA OXY	PLA OXY	PLA OXY	PLA OXY	
Control Early	m	187.46	15.26	222.12	15.55	24
Control Early	f	200.54	14.29	209.77	14.92	20
Mild Early	m	186.46	13.49	209.90	9.50	25
Mild Early	f	217.36	14.37	217.95	14.24	20
High Early	m	198.29	11.71	228.12	16.91	23
High Early	f	232.24	17.17	231.94	17.73	20
		RBC OXY	RBC OXY	RBC OXY	RBC OXY	
Control Early	m	1532.39	90.03	1530.09	60.15	24
Control Early	f	1385.50	99.27	1606.39	136.46	20
Mild Early	m	1494.27	80.12	1815.33	91.14	25
Mild Early	f	1448.75	86.62	1788.86	144.70	20
High Early	m	1537.22	105.75	1651.51	99.24	23
High Early	f	1720.13	109.43	1640.62	101.41	20
		RBC THIOLS	RBC THIOLS	RBC THIOLS	RBC THIOLS	
Control Early	m	19792.31	1020.79	22533.47	1072.30	24
Control Early	f	21322.55	1218.83	22990.59	1068.47	20
Mild Early	m	21198.59	1145.94	22240.32	1178.89	25
Mild Early	f	23384.79	805.49	25596.97	1523.18	20
High Early	m	21606.48	949.72	24220.32	1162.77	23
High Early	f	22532.88	1198.14	23394.52	930.44	20
		HYDROPEROXIDES	HYDROPEROXIDES	HYDROPEROXIDES	HYDROPEROXIDES	
Control Early	m	0.40	0.04	0.63	0.06	24
Control Early	f	0.31	0.03	0.48	0.05	20
Mild Early	m	0.35	0.03	0.56	0.05	25
Mild Early	f	0.38	0.04	0.51	0.07	20
High Early	m	0.32	0.03	0.62	0.06	23
High Early	f	0.36	0.04	0.58	0.06	20

Table 2. Descriptive statistics of body mass and biochemical variables collected from the adult heat stress regime. GPX = glutathione peroxidase; OXY = non-enzymatic antioxidant capacity; RBC = red blood cell; N = sample size. Units of measurements: body mass as grams; RBC GPX and PLA GPX as Units l^{-1} of haemolysate or plasma, respectively; PLA OXY and RBC OXY as mmol l^{-1} of plasma or haemolysate, respectively; thiols as µmol l^{-1} of –SH groups; hydroperoxides as mmol l^{-1} of H_2O_2 equivalents.

			Pre-Treatment value	Pre-Treatment value	Post-Treatment value	Post-Treatment value	
			mean	standard error	mean	standard error	N
Early Group	Adult Group	Sex	BM	BM	BM	BM	
Control Early	Control Adult	m	17.85	0.57	16.07	0.49	12
Control Early	Control Adult	f	18.70	0.56	17.13	0.44	10
Mild Early	Control Adult	m	17.82	0.55	15.99	0.46	12
Mild Early	Control Adult	f	18.94	1.18	17.03	1.04	10
High Early	Control Adult	m	18.98	0.74	17.26	0.76	11
High Early	Control Adult	f	19.60	0.72	17.59	0.80	10
Control Early	Stress Adult	m	18.74	0.74	16.69	0.59	12
Control Early	Stress Adult	f	18.11	0.61	16.10	0.63	9
Mild Early	Stress Adult	m	19.33	0.75	17.38	0.61	13
Mild Early	Stress Adult	f	18.26	0.67	16.61	0.60	9
High Early	Stress Adult	m	17.05	0.57	15.10	0.43	12
High Early	Stress Adult	f	18.30	0.83	16.38	0.82	10
			RBC GPX	RBC GPX	RBC GPX	RBC GPX	
Control Early	Control Adult	m	1582.53	122.05	1560.40	109.93	12
Control Early	Control Adult	f	1358.58	159.86	1339.67	136.18	10
Mild Early	Control Adult	m	1426.23	97.64	1406.09	88.22	12
Mild Early	Control Adult	f	1725.08	162.48	1796.33	174.62	10
High Early	Control Adult	m	1407.15	125.05	1421.20	91.00	11
High Early	Control Adult	f	1820.75	116.89	1769.83	134.60	10
Control Early	Stress Adult	m	1482.90	112.62	1615.86	157.24	12
Control Early	Stress Adult	f	1627.88	158.69	1756.74	159.92	9
Mild Early	Stress Adult	m	1577.99	148.05	1617.37	143.75	13
Mild Early	Stress Adult	f	1787.90	145.60	1786.00	191.47	9
High Early	Stress Adult	m	1455.14	111.05	1553.96	107.78	12
High Early	Stress Adult	f	1572.41	135.34	1653.60	137.11	10
			PLA GPX	PLA GPX	PLA GPX	PLA GPX	
Control Early	Control Adult	m	777.32	86.03	797.07	88.67	12
Control Early	Control Adult	f	793.63	82.70	775.88	77.83	9
Mild Early	Control Adult	m	705.40	54.49	775.85	77.34	12
Mild Early	Control Adult	f	733.68	93.65	769.32	61.31	8
High Early	Control Adult	m	655.93	54.82	649.98	72.85	11
High Early	Control Adult	f	669.98	85.73	711.13	84.69	10
Control Early	Stress Adult	m	781.12	77.07	789.64	83.18	12
Control Early	Stress Adult	f	769.69	144.34	811.87	112.38	8
Mild Early	Stress Adult	m	624.30	52.17	667.93	56.71	13
Mild Early	Stress Adult	f	829.97	88.42	860.29	79.68	9
High Early	Stress Adult	m	680.65	44.99	698.92	55.55	12
High Early	Stress Adult	f	700.29	121.09	779.94	89.44	10
			PLA OXY	PLA OXY	PLA OXY	PLA OXY	
Control Early	Control Adult	m	208.87	10.86	198.57	7.39	12
Control Early	Control Adult	f	224.01	17.87	204.21	14.71	9
Mild Early	Control Adult	m	220.94	12.07	218.68	14.32	12
Mild Early	Control Adult	f	197.52	10.88	207.32	10.73	8
High Early	Control Adult	m	215.24	8.76	200.58	11.96	11
High Early	Control Adult	f	227.73	7.29	217.62	16.54	10
Control Early	Stress Adult	m	222.76	7.62	195.93	7.14	12
Control Early	Stress Adult	f	193.08	11.79	202.89	16.26	8
Mild Early	Stress Adult	m	220.17	12.98	211.36	9.71	13
Mild Early	Stress Adult	f	216.08	13.40	207.37	8.62	9
High Early	Stress Adult	m	214.70	14.23	222.85	10.46	12
High Early	Stress Adult	f	194.10	9.87	204.51	15.10	10
			RBC OXY	RBC OXY	RBC OXY	RBC OXY	
Control Early	Control Adult	m	1901.86	34.61	1930.50	72.76	12
Control Early	Control Adult	f	1795.95	65.84	1848.90	134.56	10
Mild Early	Control Adult	m	1851.98	74.82	1840.00	79.83	12
Mild Early	Control Adult	f	1825.78	73.64	1983.63	46.82	10
High Early	Control Adult	m	1661.46	50.06	1789.48	93.87	11
High Early	Control Adult	f	1936.07	59.93	1849.87	59.21	10
Control Early	Stress Adult	m	1869.10	99.40	1931.31	56.96	12
Control Early	Stress Adult	f	1744.69	83.40	1760.04	56.29	9
Mild Early	Stress Adult	m	1806.86	67.11	1924.40	56.32	13
Mild Early	Stress Adult	f	1889.25	75.54	1868.83	98.29	9
High Early	Stress Adult	m	1881.45	104.79	1911.36	63.69	12
High Early	Stress Adult	f	1927.84	84.67	1875.50	61.29	10
			RBC THIOLS	RBC THIOLS	RBC THIOLS	RBC THIOLS	
Control Early	Control Adult	m	22236.59	1124.25	22921.78	1933.49	12
Control Early	Control Adult	f	21655.65	920.01	22335.87	1396.67	10
Mild Early	Control Adult	m	20772.00	1410.55	22514.40	1247.21	12
Mild Early	Control Adult	f	23332.22	1410.76	20844.80	1338.00	10
High Early	Control Adult	m	22539.94	1465.95	22171.44	1327.96	11
High Early	Control Adult	f	22940.54	1372.03	25782.46	1550.29	10
Control Early	Stress Adult	m	21638.63	1046.93	23163.03	1266.14	12
Control Early	Stress Adult	f	24304.92	1352.06	22188.52	1281.74	9
Mild Early	Stress Adult	m	22826.42	977.93	24421.42	874.62	13
Mild Early	Stress Adult	f	20975.29	1199.10	24244.25	1216.02	9
High Early	Stress Adult	m	24497.41	1894.45	22077.52	798.22	12
High Early	Stress Adult	f	22070.91	700.92	19372.22	1059.19	10
			HYDROPEROXIDES	HYDROPEROXIDES	HYDROPEROXIDES	HYDROPEROXIDES	
Control Early	Control Adult	m	0.63	0.10	0.72	0.10	12
Control Early	Control Adult	f	0.48	0.10	0.53	0.09	9
Mild Early	Control Adult	m	0.55	0.07	0.60	0.06	12
Mild Early	Control Adult	f	0.59	0.12	0.66	0.13	8
High Early	Control Adult	m	0.39	0.05	0.50	0.04	11
High Early	Control Adult	f	0.57	0.08	0.55	0.07	10
Control Early	Stress Adult	m	0.55	0.07	0.79	0.09	12
Control Early	Stress Adult	f	0.57	0.11	0.68	0.11	8
Mild Early	Stress Adult	m	0.65	0.09	0.58	0.07	13
Mild Early	Stress Adult	f	0.60	0.10	0.62	0.08	9
High Early	Stress Adult	m	0.54	0.07	0.85	0.11	12
High Early	Stress Adult	f	0.58	0.11	0.76	0.15	10