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Original Article

Environmental conditions can modulate the links among oxidative stress, age, and longevity

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ABSTRACT

Understanding the links between environmental conditions and longevity remains a major focus in biological research. We examined within-individual changes between early- and mid-adulthood in the circulating levels of four oxidative stress markers linked to ageing, using zebra finches (*Taeniopygia guttata*): a DNA damage product (8-hydroxy-2′-deoxyguanosine; 8-OHdG), protein carbonyls (PC), non-enzymatic antioxidant capacity (OXY), and superoxide dismutase activity (SOD). We further examined whether such within-individual changes differed among birds living under control (*ad lib* food) or more challenging environmental conditions (unpredictable food availability), having previously found that the latter increased corticosterone levels when food was absent but improved survival over a three year period. Our key findings were: (*i*) 8-OHdG and PC increased with age in both environments, with a higher increase in 8-OHdG in the challenging environment; (*ii*) SOD increased with age in the controls but not in the challenged birds, while the opposite was true for OXY; (*iii*) control birds with high levels of 8-OHdG died at a younger age, but this was not the case in challenged birds. Our data clearly show that while exposure to the potentially damaging effects of oxidative stress increases with age, environmental conditions can modulate the pace of this age–related change.

1. Introduction

Oxidative stress is a complex, multifaceted state that arises in organisms as a consequence of an imbalance between reactive oxygen species (ROS) produced primarily during aerobic metabolism and the organisms' ROS quenching capacity (Halliwell and Gutteridge, 2015). ROS damage macromolecules, cell components and structures, which, in the absence of repair, can negatively affect performance (Martindale and Holbrook, 2002; Birben et al., 2012). The extent to which oxidative stress influences organismal health, ageing and survival will depend both on the level of damage that occurs, and the investment in repair; both of these will vary with species life histories, environmental circumstances and potentially also with age-related changes in investment priorities, and in antioxidant and repair capabilities (Finkel and Holbrook 2000; Monaghan et al., 2009; Salmon et al., 2010; Selman et al., 2012; Speakman and Garratt, 2014; Speakman et al., 2015).

Currently, we know relatively little about age-related changes in oxidative stress within individuals, how this relates to longevity, and how levels vary with environmental conditions. Population-based studies in humans have found only moderate support for a positive correlation between levels of oxidative damage and age, and even less support for age-related changes in antioxidant defences and repair efficiency (see Jacob et al., 2013 for a recent review). Robust data on these issues in other vertebrate species remain rare to date (*e.g.* Sohal et al., 1994, 1995; Hamilton et al., 2001). Most of the studies conducted so far are based on cross-sectional designs (*i.e.* comparing age classes of individuals rather than within-individual changes). Cross-sectional studies, however, can be confounded by differences in the age of death of particular phenotypes in the study population, generally termed "selective disappearance" (*e.g.* early mortality of poor quality individuals – Nussey et al., 2008; Bouwhuis et al., 2009). This can mask the true within-individual pattern of age-related variation in oxidative stress (Herborn et al., 2015). Though not always possible for a variety of reasons, repeated sampling of the same individual through time is essential to examine age-related changes in oxidative stress levels. To date only a few studies in the laboratory (Matsuo et al., 1993; Alonso-Alvarez et al., 2006) and in the wild (Bize et al., 2014; Herborn et al., 2015) have used such longitudinal sampling designs. These within-individual studies do find that selective disappearance of individuals with high oxidative stress levels does occur (Herborn et al., 2015), but

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generally also find evidence of age-related increases in oxidative stress exposure or age-related decreases in cell resistance to oxidative stress (Matsuo et al., 1993; Bize et al., 2014).

The quality of the environment might influence both oxidative stress levels at a given age and age-related change in oxidative stress levels. The undemanding conditions that animals generally experience in the laboratory might give rise to very low levels of oxidative stress, whereas these levels might be altered and more variable in more challenging environments (Salmon et al., 2010). For example, a recent study in captive black birds showed that individuals exposed to repeated immune and disturbance stressors exhibited higher levels of oxidative damage markers than control birds after one year of treatment (Hau et al., 2015). Elevated levels of stress hormones (*i.e.* glucocorticoids), generally induced by exposure to challenging and unpredictable environmental circumstances (Wingfield and Kitaysky, 2002), have been associated with elevated levels of oxidative damage in vertebrates, as shown in a comprehensive meta-analysis (Costantini et al., 2011). However, the effects of environmental conditions on oxidative stress might vary depending on the magnitude and type of environmental challenge.

The aims of this study were 1) to examine within individual changes in oxidative stress from early- to mid-adulthood; 2) to examine whether challenging environmental conditions influenced oxidative stress levels and/or age-related changes in oxidative stress levels, and 3) to examine whether oxidative stress levels were predictive of survival probability and whether such relationships were altered under more challenging environmental conditions. We used captive zebra finches (*Taeniopygia guttata*) as our study species. We manipulated the quality of the environment by exposing experimental birds to unpredictable episodes of food withdrawal throughout adulthood (see full details in Marasco et al., 2015). In this species, we have previously reported that such challenging environment moderately increased baseline levels of glucocorticoid stress hormone without overall affecting body mass (Marasco et al., 2015), leading more surprisingly to improved probability of survival to the age of at least three years (Marasco et al., 2015). In this paper, we examine four different markers of oxidative stress in a randomly chosen subset of birds from the experimental population used in Marasco et al. (2015): a DNA damage product (8-hydroxy-2′-deoxyguanosine, 8-OHdG), oxidative damage to protein (protein carbonyls), non-enzymatic antioxidant capacity (OXY), and superoxide dismutase (SOD) enzymatic antioxidant activity at two age points (early- and mid-adulthood).

We predicted: 1) that exposure to oxidative stress would increase with age (*e.g.* Finkel and Holbrook, 2000); 2) that the birds exposed to the challenging environmental protocol would show reduced oxidative damage given our previous finding of their improved survival (Marasco et al., 2015), and 3) that individuals showing higher levels of oxidative damage would have shorter lifespans irrespective of the treatment.

2. Material and methods

2.1. Experimental design

This study was performed on a subset of female zebra finches (*Taeniopygia guttata*) randomly selected from the larger study investigating the long-term effects for mothers (F0), and subsequent generations, of exposure of the F0 generation to challenging environmental conditions mentioned above. Since the main focus of this long-term transgenerational project was on maternal effects, only females were subjected to the treatments. The birds were maintained throughout the experiment at a photoperiod of 14 h:10 h light:dark cycle and the temperature was maintained between 20 and 24 °C. The environmental manipulations started when the F0 females were approximately 5 months old (mean ± SEM: 156 ± 1 day old). At this stage, they were fully grown, young adults as sexual maturation is reached by 2.5–3 months of age in the zebra finch (Zann, 1996). From the start of the experiment, females were housed in treatment-specific groups in cages (n = 7–10 per 120 × 50 × 50 cm cage), and randomly assigned to one of two experimental groups: challenging (n = 74) or control (n = 65) environments. Where possible, females that hatched in the same nest were counterbalanced between the two treatment groups and family of origin was taken into account in all analyses.

Females in the challenging environment were denied access to food for a continuous period of almost one third of the daylight hours (4.9 h a day), 4 days per week on a random schedule. For the remaining two thirds of the daylight hours, and on the non-treatment days, food was provided *ad libitum*. Access to food in the challenging environment was prevented by placing a textured paper sheet (globular embossed sheets, 180 GSM, 575MM X 485MM–DBM Scotland Ltd) at the bottom of the cages in order to assure full coverage of the food bowls and of any seed food scattered on the floor cage. The floor tray had to be briefly removed from the cage in order to place and to remove the paper sheet. Both control and experimental birds were equally spread in two experimental rooms, meaning that both groups were exposed to the same level of disturbance resulting from experimenters entering the rooms. The removal of the floor tray was a routinely conducted procedure during cage cleaning in both experimental groups.

Birds in the challenging environment were always kept on this food regime other than when breeding (three breeding events at 188 ± 1, 408 ± 1 days, and 653 ± 1 days of age, means ± SEM for all) when they received *ad libitum* food continuously for approximately 2 months. Birds in the control environment were always provided with *ad libitum* food and experienced exactly the same breeding regime as in the challenging environment. We have previously shown that the treatment had no significant overall effects on body mass, measured up to three years of age, confirming that the random withdrawal of food altered primarily the temporal predictability of resources rather than the daily overall food intake (full details in Marasco et al., 2015). Importantly, our environmental manipulation induced changes in the exposure to glucocorticoid stress hormones. We found that at the end of the episodes of food withdrawal the challenged birds showed higher baseline corticosterone (the main avian glucocorticoid) than those in the control conditions (on average 1.4 fold increase), and there was no sign of habituation of this hormonal response over time (full details in Marasco et al., 2015). Therefore, our environmental manipulation mimicked a mild/moderate environmental challenge experienced by animals living in unstable environments, such as those with unpredictable foraging conditions. The birds living in these more challenging conditions showed increased probability of survival relative to those in the control conditions based on monitoring to three years of age (treatment: exp (β) ± SE (β) = 0.53 ± 0.26, z = −2.42, $p = 0.016$ – Mixed Effects Cox Models; Marasco et al., 2015). The positive consequences of the treatment on survival emerged progressively starting from the age of about one year as mortality prior to this point (up to 379 days) was very low in the birds living in both environmental conditions (3.6% controls and 1.8% challenging – Marasco et al., 2015). Thus, the data on oxidative stress markers are not biased by mortality of individuals exhibiting a particular phenotype prior to the one year sampling. Since we had full details on the longevity and survival of individual birds living in both environments until three years of life (Marasco et al., 2015), we examined whether markers of oxidative stress (full details below) predicted longevity. In this and other studies, differences in age-specific survival patterns are detectable in the zebra finch within this time frame (Monaghan et al., 2012; Costantini et al., 2014; Marasco et al., 2015). The work was carried out under Home Office Project Licence 60/4109.

2.2. Sampling and laboratory analysis

Since we were studying the longevity of the individuals, minimally invasive techniques were used to obtain the required biological samples. In this context, blood sampling offers a good opportunity to