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RESEARCH ARTICLE

Consequences of being phenotypically mismatched with the environment: no evidence of oxidative stress in cold- and warm-acclimated birds facing a cold spell

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ABSTRACT

Seasonal changes in maximal thermogenic capacity (M_{sum}) in wild black-capped chickadees suggests that adjustments in metabolic performance are slow and begin to take place before winter peaks. However, when mean minimal ambient temperature (T_a) reaches −10°C, the chickadee phenotype appears to provide enough spare capacity to endure days with colder T_a, down to −20°C or below. This suggests that birds could also maintain a higher antioxidant capacity as part of their cold-acclimated phenotype to deal with sudden decreases in temperature. Here, we tested how environmental mismatch affected oxidative stress by comparing cold-acclimated (−5°C) and transition (20°C) phenotypes in chickadees exposed to an acute 15°C drop in temperature with that of control individuals. We measured superoxide dismutase, catalase and glutathione peroxidase activities, as well as lipid peroxidation damage and antioxidant scavenging capacity in pectoralis muscle, brain, intestine and liver. We generally found differences between seasonal phenotypes and across tissues, but no differences with respect to an acute cold drop treatment. Our data suggest oxidative stress is closely matched to whole-animal physiology in cold-acclimated birds compared with transition birds, implying that changes to the oxidative stress system happen slowly.

KEY WORDS: Thermoregulation, Mitotic tissue, Post-mitotic tissue, Rate of change, Cold drop, Seasonality

INTRODUCTION

Phenotypic flexibility allows animals to adjust to fluctuating abiotic conditions by reversibly altering their phenotype with respect to the prevailing demands (Piersma and Drent, 2003; Swanson, 2010; McWilliams and Karasov, 2014). In small-bodied temperate bird species, seasonally changing thermoregulatory demands are often dealt with by altering metabolic performance (McKechnie, 2008; McKechnie and Swanson, 2010; Swanson, 2010; Swanson and Vézina, 2015). For example, in winter some birds increase their summit metabolic rate (M_{sum}) (Swanson, 2010) and citrate synthase (CS) activity in pectoralis muscle (Zheng et al., 2008; Liknes and Swanson, 2011), as a means to maintain homeostatic set points in the face of falling temperatures. This allows birds to match their physiological capacity with the requirements of their environment and survive the winter (Piersma and Drent, 2003; Petit et al., 2017; Latimer et al., 2018). However, with climate change posing increases in the frequency and duration of cold snaps (Jentsch et al., 2007), birds may also face prolonged and unexpected thermal challenges that may cause a mismatch between physiological capacity and demands (i.e. phenotypic mismatches). This can be buffered to a point as cold-acclimated phenotypes also seem to include spare capacity for sudden, unpredictable environmental changes (McWilliams and Karasov, 2014; Petit and Vézina, 2014b). However, the rate at which physiological changes occur during sudden environmental mismatch is only beginning to be elucidated.

There are physiological trade-offs of altering metabolic rate as a result of changing environmental conditions at the cell level, such as potentially altering the rate of reactive oxygen species (ROS) production, thus creating changes to the oxidative stress system. Mitochondrial function is phenotypically flexible in birds across seasons (Liknes and Swanson, 2011) and ROS possess various metabolic functions playing crucial signaling roles (Sena and Chandel, 2012; Scialò et al., 2017). However, uncontrolled ROS production may lead to increases in superoxide anions, and other toxic molecules, which can cause tissue damage, if not balanced by enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), or low molecular weight antioxidants such as vitamin E (Skrip and McWilliams, 2016). SOD, as a first line of defense, reacts with superoxide anions, often forming the relatively unreactive molecule H_2O_2 (Skrip and McWilliams, 2016). H_2O_2 can be further reduced to H_2O by CAT and GPx; however, when not reduced, it can react with ferrous ions and form a highly reactive hydroxyl radical that plays a major role in the continuation of lipid peroxidation (LPO) (Cooper-Mullin and McWilliams, 2016). LPO is one of the most insidious types of oxidative damage. ROS molecules can damage lipids, especially polyunsaturated fatty acids (PUFA), leading to the release of toxic derivatives such as malondialdehyde (MDA) (Hulbert et al., 2007). Although the relationship between whole-animal metabolism and ROS production is not linear (Stier et al., 2014), it is often thought that increasing metabolic rates during thermal challenges could also increase ROS production. At the whole-animal level, oxidative stress may also vary in diurnal cycles and increase during times of energetic stress, such as migration (Franson et al., 2002; Norte et al., 2009; Costantini et al., 2010; Costantini and Bonadonna, 2010). However, how oxidative stress and subsequent ROS production are altered across seasons and during thermal environmental mismatch remains to be tested in wild populations (Costantini, 2014).

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Seasonal variation in maximal thermogenic capacity in wild black-capped chickadees (*Poecile atricapillus*) suggests that M_{sum} adjustments are slow and begin to take place well before winter peaks (Petit and Vézina, 2014a,b). However, when mean minimal ambient temperature (T_a) reaches −10°C, their phenotype appears to provide enough spare capacity in cold endurance to buffer days with T_a of −20°C or below. As cold acclimation or acclimatization involve more than just heat production (Swanson, 2010), it is likely that whole-animal spare capacity should also be detectable in other systems. For example, wintering birds could maintain a higher antioxidant capacity as part of their cold-acclimated phenotype to buffer periods of cold spells where sudden elevation in metabolic rate should be required to compensate for heat loss (Petit and Vézina, 2014a,b). In contrast, birds facing temperature drops during the transition season (autumn) should not be as well prepared and may accrue more oxidative damage as a cost. However, the rate of change in these cell-level processes after a sudden environmental change has not been well documented. The black-capped chickadee is one of the smallest birds capable of overwintering at high latitudes, illustrating its ability to effectively thermoregulate (Hill et al., 1980; Cooper and Swanson, 1994; Sharbaugh, 2001). Here, black-capped chickadees were measured under two different phenotypes, the first in birds acclimated to a winter-like, cold temperature and the second in birds acclimated to a temperature typical of the transitional autumn period, when birds are adjusting their phenotype to the cold (Petit and Vézina, 2014b). We examined oxidative stress in black-capped chickadees by assessing the activity of CAT, SOD and GPx, total antioxidant capacity with respect to peroxyl and hydroxyl scavenging capacity, and LPO damage in muscle, brain, intestine and liver tissues. Because we chose four metabolically active organs, we were able to differentially test oxidative stress responses across different tissues, so as to gain a 'global' perspective on oxidative stress in the organism. We expected that only transition birds would increase the activity/concentration of their antioxidant system and/or show increases in oxidative damage during temperature drops.

MATERIALS AND METHODS

Capture, maintenance and temperature protocol

Black-capped chickadees, *Poecile atricapillus* (Linnaeus 1766), were captured near Rimouski, QC, Canada, in the Forêt d'Enseignment et de Recherche Macpès, using mist nets. All captured birds (N=38; N=20 transition group and N=18 cold; described below) were transported back to the Avian Research facility at the Université du Québec à Rimouski (UQAR). All birds were fitted with a unique color band, housed individually (41×42×32 cm cages) and randomly assigned to an experimental group (described below). Procedures were approved by the UQAR Animal Care Committee (CPA-69-17-191) and were conducted under scientific and banding permits from Environment and Climate Change Canada – Canadian Wildlife Service (10889).

We used a factorial design combining two temperature treatments and two phenotypes derived from the reaction norm for M_{sum} reported by Petit and Vézina (2014a,b). Birds in the cold phenotype were held at −5°C for 21 days, and individuals in the transition phenotype were held at 20°C also for 21 days. Each of the two phenotypes was divided into two treatments: a control group (no temperature change) and a cold drop group, which were acutely exposed to a 15°C decrease over 3 h at a rate of −5°C h^{-1}. Birds in the cold were under a 10 h 15 min light:13 h 45 min dark photoperiod (i.e. roughly mimicking an average February for this location) and transition birds were under a 13 h 23 min light:10 h 37 min dark cycle (i.e. roughly mimicking an average September for this location). All birds remained at their final room temperature for 3 h prior to sampling. We then alternated removing one bird from each room until each bird had been sampled, euthanized and dissected. Immediately after removal from their cage, we measured total body mass (±0.01 g). Birds were euthanized using CO_2 asphyxiation and quickly (under 5 min) dissected for their brain, liver, intestine and pectoralis muscle. All samples were flash frozen in liquid nitrogen and stored at −80°C until further analysis. For the transition birds, final sample sizes were based on N=20 individuals: N=10 control and N=10 cold drop. In the cold group, sample sizes varied between N=10 (N=5 control, N=5 cold drop) and N=17 (N=8 control, N=9 cold drop).

Laboratory analyses

Homogenates for CAT, SOD and GPx activity and oxygen radical absorbance capacity (ORAC) assay

To estimate CAT, SOD, GPx activity and total antioxidant capacity, we homogenized (Fisher Scientific Homogenizer Model 125) approximately 0.1 g of each tissue (1:9 mass:volume) in buffer solution containing 20 mmol l^{-1} Hepes, 1 mmol l^{-1} EGTA and 90 mmol l^{-1} mannitol. We then centrifuged homogenates for 20 min at 32,000 ***g*** using an Eppendorf centrifuge (5417C) at 4°C. The supernatant was extracted and stored at −80°C until further testing. All samples were run within 1 week of homogenizing. We used commercial kits (Cayman Chemical Company, Ann Arbor, MI, USA) to measure the activity of CAT (cat. no. 707002), SOD (cat. no. 706002) and GPx (cat. no. 703102), according to the manufacturer's instructions.

We estimated antioxidant capacity against peroxyl and hydroxyl radicals, two of the more damaging forms of ROS, by using a microplate-based version of the competitive ORAC assay (Cao and Prior, 1999; Prior and Cao, 1999). In this assay, when *in vitro* production of radicals exceeds the antioxidant capacity of the tissue, these ROS modify the algal pigment phycoerythrin (545 nm/575 nm) and decrease its fluorescence. The decrease in phycoerythrin was monitored using a temperature-controlled (30°C) microplate reader (Infinite M200 Pro, Tecan, Grödig, Austria). Peroxyl radicals were generated by 320 mmol l^{-1} 2,2′-azobis (2-amidinopropane) dihydrochloride, and hydroxyl radicals were generated in separate plates by adding 0.25 µl per well of 10 mmol l^{-1} $CuSO_4$ and 0.667 mol l^{-1} ascorbate mixture. ORAC values for peroxyl and hydroxyl radicals were determined by integrating the area under the fluorescence decay curve. Tissue from each individual was measured in duplicate for both peroxyl and hydroxyl radical absorbance capacity; replicates were averaged prior to statistical analysis. This method has previously been used and validated for tissue homogenates (Cao and Prior, 1999; Prior and Cao, 1999).

LPO damage assay

We estimated LPO damage by using a microplate-based version of the ferrous oxidation of Xylenol Orange (FOX) assay (Gay and Gebicki, 2003; Hermes-Lima et al., 1995; Wolff, 1994). Tissues were weighed, and approximately 0.1 g of tissue was placed (1:9 mass:volume) in a solution of methanol containing 4 mmol l^{-1} 2,6-di-tert-butyl-4-methylphenol (BHT). We homogenized each tissue (Fisher Scientific Homogenizer Model 125) and centrifuged homogenates at 2000 ***g*** in an Eppendorf centrifuge (5417C) at 4°C for 5 min. Supernatants were immediately incubated in a 90% methanol solution containing 36 mmol l^{-1} sulfuric acid, 0.25 mmol l^{-1} ammonium iron sulfate and 0.1 mmol l^{-1} Xylenol Orange for 30 min before plating in duplicate wells in a 96-well plate. Lipid hydroperoxide concentration

was monitored using a microplate reader (Infinite M200 Pro, Tecan) at an absorbance of 595 nm. Standard curves were generated using cumene hydroperoxide as a positive control.

Statistical analysis

All statistical analyses were run using JMP (version 13.1.0). We used a 2-way ANOVA, with phenotype/treatment (a 4-level variable: cold/control, cold/cold drop, transition/control and transition/cold drop) and tissues as fixed factors and bird ID as a random variable. Non-significant interactions were removed from models. Tukey's HSD was used for *post hoc* analysis. LPO damage was not normally distributed even after log transformation. We thus used the Sheirer–Ray–Hare extension of the Kruskal–Wallis test for LPO damage data.

RESULTS

We found significant differences among phenotypes and tissues in the activity of all enzymes as well as in the tissue capacity for scavenging peroxyl and hydroxyl. We also found an effect of phenotype on lipid peroxidation damage. However, no significant difference in enzymatic activity or scavenging capacity was found within the cold and transition phenotypes when comparing control birds with those that had been exposed to the temperature drop. In other words, the sudden increase in thermoregulatory demand did not trigger a detectable response in the variables we measured, whether the birds were acclimated to cold or not. However, LPO damage was affected by phenotype (see below).

CAT activity did not differ among phenotype/treatments (P=0.43), but showed significant differences among tissues (F_3=275.6, $P\leq$0.0001; Fig. 1G; Fig. S1). Indeed, although CAT activity differed significantly in all tissues, it was much lower in the brain than in all other tissues, being 2.8 times lower than in muscles and 4 times lower than in the liver (Fig. 1G). We also found a significant tissue×phenotype/treatment interaction (F_9=9.4, $P\leq$0.0001). However, for each tissue, birds did not differ between treatments within phenotypes (Fig. S1). GPx activity differed between phenotypes (F_3=4.1, P=0.013) and tissues (F_3=19.4, $P\leq$0.0001) with 43.1% higher activity in the transition phenotype than in the cold phenotype (Fig. 1A). Post-mitotic tissue (brain and muscle) did not differ significantly and had the highest GPx activity; on average 3 times higher than that in mitotic tissue (liver and intestine), which also did not differ (Fig. 1E). SOD activity was also significantly different across phenotypes (F_3=8.1, P<0.0003) and tissues (F_3=241.9, $P\leq$0.0001). Its activity was 15% higher in the cold compared with the transition phenotype (Fig. 1B). The liver showed the highest SOD activity of all organs, followed by the intestine and muscle, and the lowest SOD activity was measured in the brain, being only 20.8% of that measured in the liver (Fig. 1F).

Hydroxyl scavenging capacity was significantly different across phenotypes (F_3=7.7, P=0.0009) but not across tissues (P=0.082), with scavenging capacity being 19.7% higher in the transition phenotype compared with that in cold-acclimated birds (Fig. 1C). Peroxyl scavenging capacity differed across phenotypes (F_3=4.7, P=0.012) but also tissues (F_3=125.7, $P\leq$0.0001). Cold-acclimated birds had a 15.8% higher scavenging capacity compared with birds in the transition phenotype (Fig. 1D), and scavenging capacity was higher in the intestine than in all other organs. The liver also showed better peroxyl scavenging capacity than the brain and muscle, which did not differ (Fig. 1H). LPO damage was significantly different across phenotypes (H_3=10.8, P=0.013) but not across tissues (P=0.171; Fig. 2). LPO damage was 3.3 times higher in the transition phenotype compared with that in cold-acclimated birds.

DISCUSSION

Animals can maintain spare capacity as part of physiological acclimation/acclimatization. This spare capacity allows for rapid upregulation of physiological performance, up to a certain level, above routine when confronted with sudden changes in demand (McWilliams and Karasov, 2014). At the whole-organism level, forest-inhabiting birds such as dark-eyed juncos (*Junco hyemalis*) and black-capped chickadees have been reported to adjust their basal metabolic rate (BMR) and M_{sum} within 14–30 days following changes in T_a, whereas other species normally found in open, more thermally variable areas showed metabolic changes within 8 days (Dubois et al., 2016). Whole-animal changes, such as metabolic rate, also seem to happen more rapidly in cold than in warm temperatures (Barceló et al., 2009), and BMR was shown to respond to temperature at a faster rate than M_{sum} or maximal metabolic rate (MMR) in experimental conditions (Dubois et al., 2016), although McKechnie (2008) pointed out that the time course of BMR adjustments in birds remains unknown. However, the rate at which physiological changes can happen at the cell level has been understudied. We hypothesized that birds in winter phenotype would have a better reserve capacity against cold spells than birds in a transition phenotype. By inference, then, we expected that only transition birds would increase the activity/concentration of their antioxidant system and/or show increases in oxidative damage during temperature drops but our data did not support this hypothesis. Instead our data only showed significant differences between tissues and phenotypes.

Because the antioxidant system responds to ROS production, and ROS production is not proportional to whole-animal metabolism (Stier et al., 2014), various aspects of the system may react differently across tissues (Cooper-Mullin and McWilliams, 2016). Tissues within animals are said to have differences with respect to their ability to accumulate oxidative damage based on whether they are mitotic or post-mitotic (Selman et al., 2000). Skeletal muscles may thus exhibit relatively low antioxidant enzyme activities, although they contain post-mitotic cells that accumulate ROS damage over time (Selman et al., 2000). In contrast, in Sprague–Dawley rats, oxidative damage to DNA in the form of 8-hydroxy-2′-deoxyguanosine (8-OH-dG) increases with age in mitotic tissues such as liver, kidney and intestine, but not in the post-mitotic brain (Fraga et al., 1990), potentially indicating that post-mitotic tissue may accrue less DNA oxidative damage compared with mitotic tissues. Here, we sampled muscle and brain tissues as representatives of post-mitotic tissue, and liver and intestine as representatives of mitotic tissue. We did not find consistent patterns across mitotic and post-mitotic tissue; rather, each tissue seemed to follow a functional phenotype with regards to oxidative stress. For example, liver SOD activity was highest out of the tissues measured, potentially due to the liver's detoxifying functions for the organism. Brain tissue demonstrated the lowest levels of CAT and SOD activity and peroxyl scavenging capacity, and did not accumulate higher amounts of LPO damage. Although it may seem surprising that one of the body's most metabolically active tissues has low antioxidant capacity, high oxygen consumption is a general characteristic of this organ and not an acute response to increase temporary performance of the mitochondria, and thus it is not expected to have high rates of ROS production (Munro and Treberg, 2017). In contrast, CAT activity was highest in the liver to potentially deal with a higher concentration of H_2O_2 produced by SOD. Indeed, as the affinity of CAT for H_2O_2 is low, H_2O_2 must be present at high concentrations for this enzyme to be efficient (Hulbert et al., 2007). We also found that GPx activity across tissues

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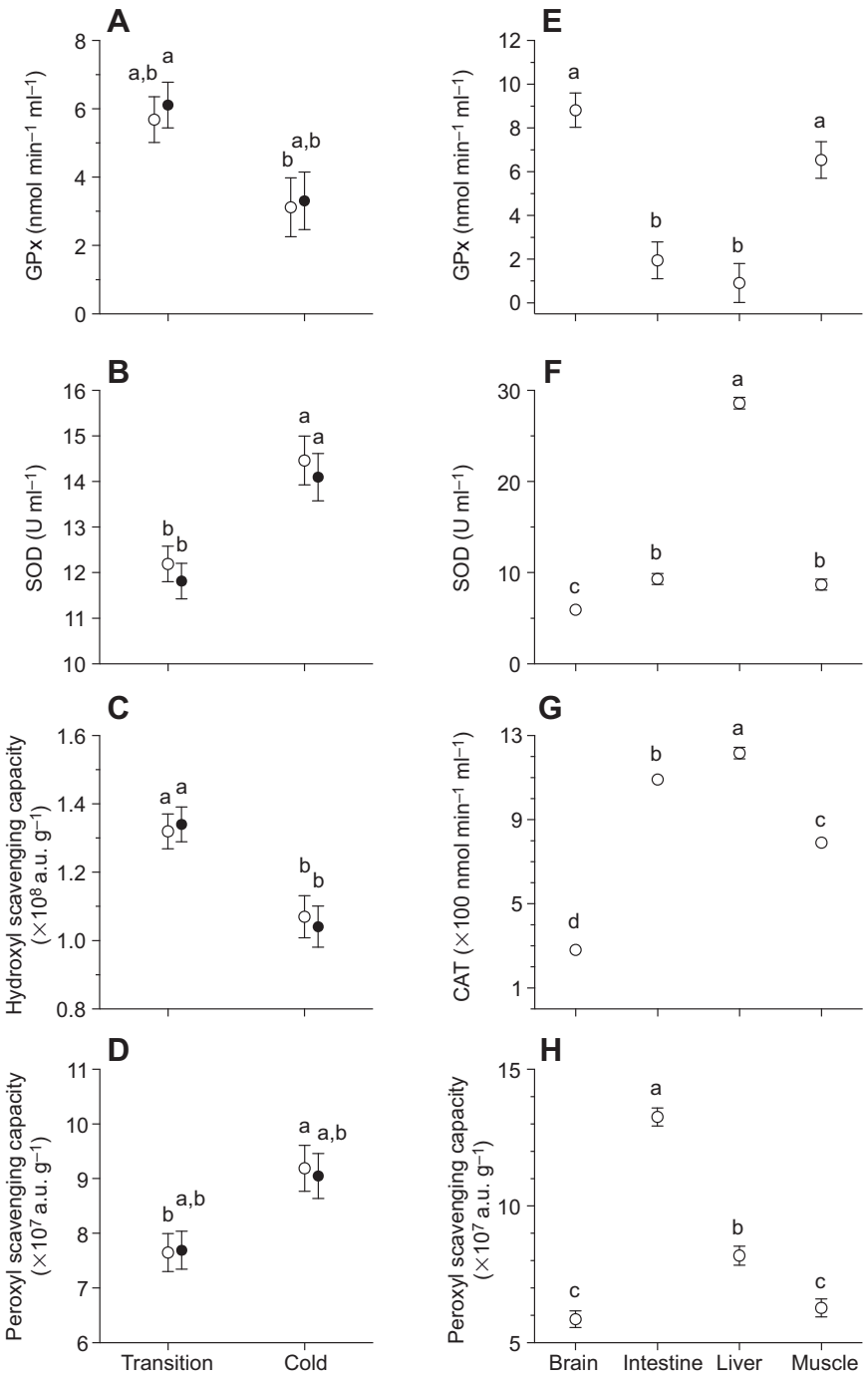

Fig. 1. Enzymatic and non-enzymatic antioxidant measurements in transition and cold phenotypes and in two post-mitotic (muscle, brain) and two mitotic (liver, intestine) tissues. Left: cold versus transition birds. Open circles represent values for birds that experienced a cold drop; filled circles represent values for control individuals. Right: tissue expression. (A,E) Glutathione peroxidase (GPx) activity; (B,F) superoxide dismutase (SOD) activity; (C) hydroxyl scavenging capacity; (D,H) peroxyl scavenging capacity; and (G) catalase (CAT) activity. For the transition birds, final sample sizes were based on *N*=20 individuals (*N*=10 control, *N*=10 cold drop). In the cold group, sample sizes varied between *N*=10 (*N*=5 control, *N*=5 cold drop) and *N*=17 (*N*=8 control, *N*=9 cold drop). Different letters represent significant differences among groups. The data we present are least-square means±s.e.m.; note that in F and G, some symbols are larger than the error bars.

showed a reversed pattern compared with CAT activity, suggesting that the oxidative stress system is conservative and not redundant in function. It should be mentioned here, however, that we did not measure activity through the thioredoxin (TRx) pathway, which has been demonstrated in rat and mouse skeletal muscle or brain to be more active than the GPx pathway (Drechsel and Patel, 2010; Rohrbach et al., 2006).

There were clear differences between phenotypes with, for instance, transition birds showing a lower SOD activity and lower peroxyl scavenging capacity than their cold counterparts. High peroxyl detoxification in the cold phenotype may decrease the need for controlling hydroxyl detoxification as hydroxyl ROS are generated via the Fenton reaction and will attack PUFAs, potentially causing LPOs (Speakman et al., 2015). Though more nuanced, CAT activity was significantly lower in muscle of cold-acclimated birds compared with transition birds and higher in liver (Fig. S1), an organ heavily solicited under cold conditions as a result of high food intake (Devost et al., 2014; Dubois et al., 2016; Barceló et al., 2017; Milbergue et al., 2018). This suggests that there are seasonal changes to antioxidant capacity in response to the generation of differing amounts of ROS, which is consistent with the theory of machinery matching, that organisms equipped to deal with a large seasonal range of temperatures adjust their molecular machinery accordingly in support of their metabolic demands (Hart, 1962; Cooper and Swanson, 1994; Vézina et al., 2006; Olson et al., 2010; Liknes and Swanson, 2011; Swanson and Vézina, 2015).

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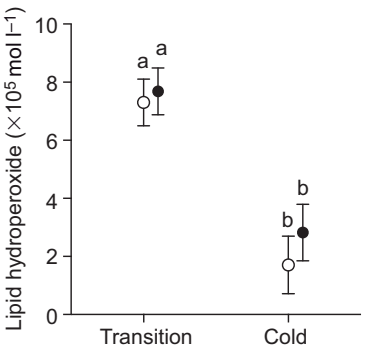

Fig. 2. Lipid peroxidation damage in transition and cold phenotypes. Open circles represent values for birds that experienced a cold drop; filled circles represent values for control individuals. LPO damage was significantly different across treatments, demonstrating a 3.3 times higher concentration of lipid damage in the transition phenotype compared with that in cold-acclimated birds. The data we present are least-square means±s.e.m.

Similar to our results, GPx activity in blood of great tits (*Parus major*) was significantly lower in winter compared with autumn (Norte et al., 2009). However, CAT activity was increased in skeletal muscle, kidney and heart, and GPx activity was increased in heart of voles (*Microtus agrestis*) raised and chronically exposed to cold temperatures compared with voles kept at 22°C (Selman et al., 2000). In barn swallows (*Hirundo rustica*), liver oxidative stress values across the year showed that birds in winter phenotype had higher levels of lipid hydroperoxides, SOD, CAT and GPx activity than individuals measured in summer, although in this specific case winter oxidative stress might have been due to toxic compounds increasing biotransformation activity (Raja-Aho et al., 2012). We saw no effect of a drop in temperature in either phenotype, but did observe a pattern of lower LPO damage and higher lipid protection in the cold (see also Hart, 1962; Cooper and Swanson, 1994; Olson et al., 2010). While others have found that glutathione peroxidase 4 (GPx4) can directly reduce phospholipid hydroperoxides in mammalian cells (Imai and Nakagawa, 2003), we did not measure this specific enzyme. Our results, therefore, suggest that upregulated functions associated with cold acclimatization or acclimation may include some level of lipid membrane protection.

Subjecting birds to a cold drop did not induce changes in the oxidative stress variables in either phenotype or tissue, which suggests that the oxidative stress system might be slow to respond to sudden changes in the environment compared with whole-animal metabolism and/or that oxidative damage might require more time to accumulate to detectable levels than 3–6 h. Alternatively, this finding could also imply that spare capacity for defense against oxidative stress is sufficient to respond to a sudden upregulation of metabolism in these birds, even when maintaining a phenotype matching constant mild temperatures, thus requiring no immediate changes in the variables we measured. Either way, our findings contrast with previous studies on mammals and birds, though none reported data on short-term responses. For example, in chronically cold-stressed rats, a marker of lipid peroxidation damage (MDA content) was increased in the brain, heart, kidney, liver and small intestine, compared with rats kept at 25°C. In parallel, GPx activity was higher in the brain and small intestine and lower in the heart, kidney and liver (Kaushik and Kaur, 2003). In zebra finches, a temperature drop from 24°C to 12°C did not change total antioxidant capacity after 24 h at 12°C, though these birds did accrue significantly more DNA oxidative damage than controls (Stier et al., 2014). After 4 weeks of chronic exposure to 12°C, however, zebra finches showed a decrease in total antioxidant capacity in the blood (Stier et al., 2014). Contrasting with that study, house sparrows (*Passer domesticus*) in summer phenotype that were acutely exposed to cold (2–5°C for 12 h) showed an increase in whole-animal metabolism and total antioxidant capacity (TAC) compared with control birds; whereas gray catbirds (*Dumetella carolinensis*) similarly treated did not show any increase in TAC (Cohen et al., 2008). Therefore, taken together, our results and these studies suggest that the effect of cold exposure on the antioxidant system might be both tissue specific and dependent on the degree and extent of cold exposure encountered, implying that longer durations of cold might be needed to trigger an upregulation of this system (Selman et al., 2000), similar to the seasonal differences we found. They also suggest that upregulation patterns in the antioxidant system may be species specific.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: A.G.J., F.V.; Methodology: A.G.J., E.C.R., K.J.T., K.N.A., A.L.P., L.R., F.V.; Validation: A.G.J., F.V.; Formal analysis: E.C.R., K.J.T., K.N.A., F.V.; Investigation: A.G.J., K.N.A.; Resources: A.G.J., A.L.P., L.R., F.V.; Data curation: K.J.T.; Writing - original draft: A.G.J.; Writing - review & editing: A.G.J., E.C.R., K.J.T., K.N.A., F.V.; Visualization: E.C.R., F.V.; Supervision: A.G.J.; Project administration: A.G.J., F.V.; Funding acquisition: A.G.J.

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Supplementary information
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